Annex I to the CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

International Chemical Identification:

O-Isopropyl ethylthiocarbamate

EC Number: 205-517-7

CAS Number: 141-98-0

Index Number:-

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Not evaluated as part of this dossier.

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Not evaluated as part of this dossier.

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Not evaluated as part of this dossier.

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Not evaluated as part of this dossier.

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Not evaluated as part of this dossier.

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Not evaluated as part of this dossier.

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Not evaluated as part of this dossier.

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Not evaluated as part of this dossier.

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Not evaluated as part of this dossier.

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Not evaluated as part of this dossier.

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3.10.1.1 Study 1 OECD 422: Combined Screening Test

Study reference: Anonymous, 2012a. Combined Repeated Dose Toxicity Study with Reproduction/Developmental toxicity screening test of IPETC (Danafloat™ 262) in Rats by Oral Administration (Unpublished report).

Detailed study summary and results:

Test type:

OECD Guideline 422: Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test. The study deviated from the test guideline in that it used 10 females instead of 12-13, there was no information on oestrous cycle monitoring provided, females were treated until lactation day (LD) 3, testing lasted for 59 days rather than 63, there were no thyroid hormone assessments carried out and there was no historical control data reported. GLP compliant. Unpublished study.

Test substance:

- *Name:* IPETC or Danafloat^{TM}262. Identical to substances identified in CLH dossier.
- *Degree of purity:* 95.7%.
- *Impurities*: Not reported.
- *Batch number:* 0001026602.
- *Test formulation:* The test substance was freshly prepared on each administration day; the vehicle was used as a diluent.

Test animals:

- *Species/strain/sex:* Rat, CD® / Crl: CD(SD), male and female.
- *No. of animals per sex per dose*: 10 animals/sex/group.
- *Age and weight at the study initiation:* 59 days, male body weight range: 271.3 - 295.9g, female body weight range: 200.4 – 233.1g.

Administration/exposure:

- *Route of administration:* Oral; gavage.
- *Duration and frequency of test/exposure period:* Daily administration until at least lactation day (LD) 3.
- *Doses/concentration levels, rationale for dose level selection:* 0, 31, 103 and 309 mg/kg bw/day based on a 14-day dose ranging study in 5 animals/sex/dose. At 103 mg/kg bw/day reduced food consumption in males and at 309 mg/kg bw/day in both sexes; clinical observations (ataxia, pilo-erection, reduced motility and increase salivation) were made and there was a reduction in body weight and food consumption observed in males. There were no test-substance related deaths, macroscopic changes or abnormalities observed in foetuses.
- *Control group and treatment:* 10 animals/sex administered corn oil via oral (gavage).
- *Vehicle and rationale:* Corn oil.
- *Actual doses (mg/kg bw/day):* 0, 31, 103 and 309 mg/kg bw/day.

Description of test design:

- *Details on mating procedure*: Male: female 1:1. The length of cohabitation was up to 14 days. Proof of pregnancy was determined by the presence of a vaginal plug or sperm. If pregnancy was not achieved, females were re-mated with a male with proven fertility in the same treatment group. Procedure was repeated until a minimum of eight pregnant dams were available for each treatment group.
- *Premating exposure period for males and females (P and F1):* Two weeks.
- *Dosing schedules :*
    - Males were treated once daily, treatment commenced 2 weeks pre-mating and continued for at least 28 days.
    - Females were treated once daily, treatment commenced 2 weeks pre-mating and continued until at least lactation day (LD) 3.
- *Parameters assessed for P and F1:*
    - Clinical and mortality observations 1/day and 2/day, respectively.
    - Body weight: Parental male and females were weighed on first day of treatment and monitored weekly and at the termination of study. During gestation females were monitored on days 0, 7, 14 and 20 after mating and on post-natal day (PND) 1 and 4.
    - Food consumption was monitored weekly or daily throughout the study with the exception of mating. Water consumption was monitored daily.
    - Neurological screening was performed in 5 males and 5 females per dose group, 2 hours after dosing before scheduled sacrifice. This was divided into observational screening and functional tests.
    - Haematology, coagulation and clinical chemistry: Blood samples collected at the end of the premating period in 5 males and 5 females per dose group.
    - Thyroid hormones: Not assessed, study pre-dated the current OECD 422 test guideline (2016).

- *Reproductive and development parameters:*
    - Number of pregnant females, pre-coital time and *g*estation was recorded from day 0 of pregnancy
    - Fertility index, gestation index, birth index, live birth index, viability index, pre and post implantation loss.
    - Corpora lutea- number per dam, absolute number per group and mean per group.
    - Implantation sites- number per dam, distribution in the uterine horns, absolute number per group and mean per group.
    - Number of stillbirths.
    - Number of pups per dam at birth and on PND 4.
- *Oestrous cycle length and pattern:* No information available.
- *Sperm examination:* Sperm count, viability and morphology was examined in all adult males during post-mortem examinations.
- *Post mortem examinations:*
    - Adult males were sacrificed after a minimum of 28 days.
    - Adult females who successfully delivered pups were sacrificed on post-natal day (PND) 4.
    - A full post mortem was carried out on all adult animals and the reproductive organs were the focus of the examination. Organs and tissue were weighed and microscopically examined.
    - Pups were sacrificed on PND 4 and examined externally for gross abnormalities.

  - Histopathological examinations were performed on 5 selected males and 5 selected females from the control and high dose treatment group (309 mg/kg bw/day) and on any animals that died prematurely.
- *Parameters assessed for F1:*
  - Number of pups at birth (alive and dead).
  - Live pups were counted and sexed on PND 0 or 1 and on PND 4.
  - Body weight: pups were weighed on PND 1 and 4.
  - Gross abnormalities and any abnormal behaviours were recorded during pup examinations.
- *Post exposure observation period:* None.
- *Historical control data:* None reported. However, there is laboratory background data reported from 8 studies from 2012 - 2013 and common literatures.
- *Statistical methods:* Datasets were compared using Student's t-test, Dunnett' multiple t-test, Bartlett Chi-square and the Fisher exact test.

Results and discussion:

Observations of effects in Parental Generation

- *Mortality:* 1/10 females at 309 mg/kg bw/day died on test day 3. Prone position was observed on the days prior to death and gastric lesion were observed during necropsy. The study authors reported this was a test substance related death, there was no other information on cause of death provided. All other animals survived to scheduled sacrifice.
- *Clinical signs:* There was a reduction in motility observed in both male and females, at ≥ 103 mg/kg bw/day, during the pre-mating period, at 309 mg/kg bw/day during mating and in females at 309 mg/kg bw/day during gestation. Prone position was observed in both sexes at 309 mg/kg bw/day during pre-mating. At 309 mg/kg bw/day, salivation (graded slight to extreme) was observed in males during pre-mating and mating/post-mating period and in 6/9 females during gestation. In females, at 309 mg/kg bw/day, piloerection was observed during premating. There were no abnormalities observed in control animals or treated animals at 31mg/kg bw/day.
- *Body weight:*

  Males*:* At 309 mg/kg bw/day, there was a statistically significant decrease in male mean body weight and mean body weight gain throughout the testing period i.e., from day 8 to day 43. The decrease in mean body weight was, 8.7%, 9.4%, 8.3%, 8.9% and 10.7% on days 8, 15, 22, 29 and 36, respectively. On day 43, the mean body weight (434.36g) in the highest treatment group was 11.9% lower compared to 493.06g in the control group. There was a 24.5%-56.7% decrease in mean body weight gain throughout the testing period. During the last observation period (day 1-43), the mean body weight gain was 55.32g at 309mg/kg bw/day, 27.41% lower compared to 76.21g in the control group. There was also a statistically significant decrease (12%) observed in mean terminal body weight in males at 309 mg/kg bw/day, the mean terminal body weight was 474.98, 471.69, 449.33 and 417.47g at 0, 31, 103 and 309 mg/kg bw/day, respectively.

  Females: In females at ≥ 103 mg/kg bw/day, there was a statistically significant decrease in mean body weight and mean body weight gain during the pre-mating and gestation periods and in terminal body weight.

  There was a statistically significant decrease in mean body weight at ≥ 103 mg/kg bw/day on pre-mating day 8 (5.8% and 8.3% at 103 and at 309 mg/kg respectively) and at 309 mg/kg bw/day on pre-mating day 15 (12.2%). There was a statistically significant decrease in mean body weight gain at ≥ 103 mg/kg bw/day on pre-mating days 1 - 8 (71% and 117% at 103 and at 309 mg/kg, respectively). On pre-mating days 1-15, there was a negative statistically significant decrease in mean body weight

gain (-139%) observed at 309 mg/kg bw/day, the mean body weight gain was 9.95, 8.44, 3.06 and -3.89g at 0, 31, 103 and 309 mg/kg bw/day, respectively.

During the gestation period there was a statistically significant decrease in mean body weight on gestation days (GD) 7 at 309 mg/kg bw/day (9.5%) and at ≥ 103 mg/kg bw/day on GD 14 (8.8% and 16% decrease at 103 and 309 mg/kg bw/day, respectively) and on GD 20 (26% and 33% decrease at 103 and at 309 mg/kg bw/day, respectively). On GD 20, the mean body weight was 396, 382, 291 and 264g at 0, 31, 103 and 309 mg/kg bw/day, respectively. There was a statistically significant decrease in body weight gain at 309 mg/kg bw/day on GD 0-7 (35%) and at ≥ 103 mg/kg bw/day on GD 0-14 (33% and 49% decrease at 103 and at 309 mg/kg, respectively) and on GD 0-20 (68% and 78% decrease at 103 and at 309 mg/kg, respectively). On GD 0-20 the mean body weight gain was 59, 54, 19 and 13g at 0, 31, 103 and 309 mg/kg bw/day, respectively.

The study author reported that the observed increase in post-implantation losses in 7/10 and 9/9 females at 103 mg/kg bw/day and 309 mg/kg bw/day, respectively was the cause of the decrease in body weight gain observed at ≥ 103 mg/kg bw/day.

During the lactation period, due to complete litter loss at 309 mg/kg bw/day, there was no body weight data available for females at this dose. There was no effect on mean body weight or mean body weight gain at ≤ 103 mg/kg bw/day on PND 1 or PND 4. On PND 1, the mean body weight was 303, 304 and 297g and On PND 4, the mean body weight was 308, 308 and 301g at 0, 31 and 103 mg/kg bw/day, respectively. The mean body weight gain from PND 1-4 was 5, 4 and 4g at 0, 31 and 103 mg/kg bw/day, respectively.

There was a statistically significant decrease (9.8% and 18% decrease at 103 and at 309 mg/kg, respectively) observed in female mean terminal body weight (297.76, 292.48, 268.67 and 244.16g at 0, 31, 103 and 309 mg/kg bw/day, respectively).

Table 1: Female mean body weight (g) data measured during the gestation period in the Combined Repeated dose toxicity Study with Reproduction/Developmental toxicity screening test of IPETC (Danafloat™ 262) in Rats by Oral Gavage Administration. (*Anonymous 2012a*).

Dose (mg/kg bw/day)	0	31	103	309
Gestation Period	**Mean body weight (g)**			
GD 0	248.34±12.92	248.26 ±9.96	244.68 ±15.41	233.93 ±16.76
GD 7	279.39±10.99	279.70 ±10.50	267.34 ±15.22	**252.88 ±17.00****
GD 14	317.37±10.70	315.94 ±12.24	**289.94 ±17.76****	**266.87 ±14.96****
GD 20	395.48±19.47	382.08 ±22.26	**291.27 ±30.42****	**263.72 ±17.08****

****p < 0.01**

- *Food consumption:*

  Males: At 309 mg/kg bw/day, there was a statistically significant differences noted in food consumption during the pre-mating period. There was a statistically significant decrease (15%) in consumption on day 1-8 (60g, at 309 mg/kg bw/day compared to 71g in the control group) and a statistically significant increase (8%) in consumption on day 1-15 (63g at 309 mg/kg bw/day compared to 58g in the control group).

  Females: At ≥ 31 mg/kg bw/day, there was a statistically significant decrease in food consumption during the pre-mating period days 1-8. The mean food consumption was 71, 65 (8% reduction), 60 (15% reduction) and 51g (28% reduction) at 0, 31, 103 and 309 mg/kg bw/day, respectively. There were no effects observed in pre-mating days 8-15, during the gestation period for control or treated

females or during the lactation periods for females at ≤ 103 mg/kg bw/day. There was no data collected for females at 309 mg/kg bw/day during the lactation period.

- *Water consumption:* Increased consumption was observed in males at 309 mg/kg bw/day during pre-mating, mating and post-mating periods. At 309 mg/kg bw/day increased consumption was observed in 4/9 females during the pre-mating and mating periods and in 5/9 females during the gestation period. At the same concentration decreased consumption was observed in 1/9 females during the pre-mating period. There were no abnormalities observed in control animals or treated animals at ≤ 103 mg/kg bw/day.
- *Neurological screening*
  - *Observational screening:* No effect observed.
  - *Functional observation battery:* No effect observed.
- *Haematology:*

  Male: There were statistically significant differences observed for haemoglobin content, red blood cells, haematocrit value and mean corpuscular haemoglobin concentration (MCHC) at 103 mg/kg bw/day. The study author reported that these observations were not considered to be test-substance related. In the absence of a dose-response effect and historical control data, the dossier submitter (DS) notes that the toxicological significance of these findings is unclear. There were no effects observed in males at 0, 31 or 309 mg/kg bw/day.

  Female: No effect observed.
- *Clinical Chemistry:*

  Male: At 309 mg/kg bw/day there was a statistically significant increase observed in bile acids, total cholesterol, total protein and a statistically significant decrease in chloride.

  The mean total cholesterol was 1.96, 1.78, 1.96 and 2.48 (27% increase) mmol/L at 0, 31, 103 and 309 mg/kg bw/day, respectively. The mean total cholesterol at the highest dose was outside the background data of the test laboratory (8 studies from 2012 - 2013, n = 80, mean: 1.3 mmol/L, range: 0.68-2.25 mmol/L).

  The mean bile acids were 7.30, 33.14, 28.20 and 79.10 (9.2%) µmol/L at 0, 31, 103 and 309 mg/kg bw/day, respectively. The value at 309 mg/kg bw/day was outside the background data of the test laboratory (8 studies from 2012 - 2013, n = 80, mean: 12.87 µmol/L, range: 2.10-66.5 µmol/L).

  The mean total protein was 59.2, 59.2, 60.4 and 62.6 g/L at 0, 31, 103 and 309 mg/kg bw/day, respectively. The mean total protein at each treatment group was within the background data of the test laboratory (8 studies from 2012 - 2013, n = 80, mean: 58.4 g/L, range: 51-65 g/L).

  The mean chloride was 100.2, 99.44, 98.8 and 98.2 (2%) mmol/L at 0, 31, 103 and 309 mg/kg bw/day, respectively. The mean chloride values at ≥ 103 mg/kg bw/day were outside the background data of the test laboratory (8 studies from 2012 - 2013, n = 80, mean: 102.2 µmol/L, range: 99-105 µmol/L). There were no effects noted for the other treatment groups for these parameters.

  Female: At 309 mg/kg bw/day there was a statistically significant increase observed in total cholesterol and an increase observed in bile acids and alkaline phosphatase.

  The mean total cholesterol was 1.38, 1.55, 1.66 and 2.31 (64%) mmol/L at 0, 31, 103 and 309 mg/kg bw/day, respectively. The mean total cholesterol at 309 mg/kg bw/day was just in range of the background data of the test laboratory (8 studies from 2012 - 2013, n = 80, mean: 1.52 mmol/L, range: 0.99-2.34 mmol/L).

  The mean bile acids were 6.62, 8.94, 10.60 and 37.66 (17.6%) µmol/L at 0, 31, 103 and 309 mg/kg bw/day, respectively. There was no laboratory background data provided for female rats' bile acid values.

  The mean alkaline phosphatase was 138.6, 149.8, 150.2 and 181.4 (32%) U/L at 0, 31, 103 and 309 mg/kg bw/day, respectively. There was no laboratory background data provided for this parameter.

Table 2: Measured Clinical Biochemistry data measured during Combined Repeated dose toxicity Study with Reproduction/Developmental toxicity screening test of IPETC (Danafloat™ 262) in Rats by Oral Administration (*Anonymous 2012a*).

Clinical Biochemistry	Dose (mg/kg bw/day)							
	Males				Females			
	0	31	103	309	0	31	103	309
Alkaline phosphatase (U/L)	185.0± 22.9	201.8±31.6	173.8± 32.3	212.0± 40.0	138.6± 27.3	149.8± 34.8	150.2± 70.1	181.4± 53.5
Total Cholesterol (mmol/L)	1.96± 0.213	1.78± 0.216	1.96± 0.178	**2.48± 0.167****	1.38± 0.252	1.55± 0.358	1.66± 0.044	**2.31± 0.320****
Total protein (g/L)	59.2±1.9	59.2±1.8	60.4±1.1	**62.6±2.1***	62.0± 2.7	61.8± 2.3	64.4± 2.5	65.2 ±4.7
Chloride (mmol/L)	100.2± 0.8	99.44±1.5	98.8±0.8	**98.2±0.8***	102.8±1.3	102.2±1.1	102.4±0.9	102.2±1.3
Bile acids (µmol/L)	7.30± 3.87	33.14± 34.73	28.20± 8.37	**79.10± 55.91****	6.62± 1.96	8.94± 7.04	10.60± 12.88	37.66± 57.95

*** p < 0.05; **p < 0.01**

- *Organ weights:*

  In males, there was an increase in the left and right relative epididymides weight, with statistical significance achieved in the right epididymis at ≥ 31mg/kg bw/day and in the left epididymis at 309 mg/kg bw/day. The left relative epididymides weights were 1.52, 1.62 (7% increase), 1.65 (8% increase) and 1.70 (12% increase) g/kg bw and the right relative epididymides weights were 1.43, 1.53 (7% increase), 1.60 (12% increase) and 1.71 (20% increase) g/kg bw at 0, 31, 103 and 309 mg/kg bw/day, respectively. The mean left and right relative epididymides weights of treated males were within the background data of the test laboratory (8 studies from 2012 - 2013, n = 80; left relative epididymis weight mean: 1.56 g/kg bw, range: 1.12 - 2.18 g/kg bw and right relative epididymis weight mean: 1.58 g/kg bw, range: 0.91 - 2.23 g/kg bw).

  In males, an increase in the right relative gonad weight was observed, with statistical significance at ≥103 mg/kg bw/day. The right relative gonad weight was 4.07, 4.37 (7% increase), 4.62 (14% increase) and 4.85 (19% increase) g/kg bw at 0, 31, 103 and 309 mg/kg bw/day, respectively. The right relative gonad weights of treated males were within the background data of the test laboratory (8 studies from 2012 - 2013, n = 80; mean: 3.92 g/kg bw, range:1.31 - 5.07g/kg bw). There were no effects observed in female relative or absolute gonad weights. There were no histopathological findings reported in the male or female gonads.

  In both sexes there was a statistically significant change in absolute and relative brain weights. In males, at 31 mg/kg bw/day there was a statistically significant 5% increase in absolute brain weight (1.990, 2.098, 2.048 and 1.906g at 0, 31, 103 and 309 mg/kg bw/day, respectively). In males, at 103 mg/kg bw/day there was a statistically significant 10% increase in relative brain weights (4.176, 4.480, 4.594 and 4.426 g/kg bw at 0, 31, 103 and 309 mg/kg bw/day, respectively). There was no background data for male brain weights reported in the study.
  In females, a statistically significant 8% decrease in absolute brain weight was observed at 309 mg/kg bw/day and a statistically significant 13% increase in relative brain weight was observed at ≥103 mg/kg

bw/day. The absolute brain weight at 309 mg/kg bw/day, was 1.754g compared to 1.912g in the control. The relative brain weight was 6.378, 6.486, 7.186 and 7.202g/kg bw at 0, 31, 103 and 309 mg/kg bw/day, respectively. The relative and absolute brain weights at each treatment group were within the background data of the test laboratory (8 studies from 2012 - 2013, n = 80 relative brain weight mean: 6.52 g/kg bw, range: 5.7 - 7.54 g/kg bw and absolute brain weight mean: 1.91g, range: 1.62 - 2.14 g).

In both sexes there was a statistically significant change in absolute and relative liver weights. In males a statistically significant increase was observed in absolute liver weight at 309 mg/kg bw/day and in relative liver weight at ≥103 mg/kg bw/day. The absolute liver weight at 309 mg/kg bw/day was 21.84g (24% increase) compared to 17.64g in the control. The relative liver weight was 37.00, 38.48, 43.64 (19% increase) and 50.54 (37% increase) g/kg bw at 0, 31, 103 and 309 mg/kg bw/day, respectively. There was no background data for male liver weights reported in the study.
In females, there was a statistically significant decrease in absolute liver weight at ≥103 mg/kg bw/day and relative liver weight at 103 mg/kg bw/day. The absolute liver weights were 14.68, 13.86, 10.58 (28% decrease) and 11.96 (19% decrease) g at 0, 31, 103 and 309 mg/kg bw/day, respectively. The relative liver weight was 48.92, 46.88, 39.14 (20% decrease) and 49.02 g/kg bw at 0, 31, 103 and 309 mg/kg bw/day, respectively. The relative and absolute liver weights at each treatment group were within the background data of the test laboratory (8 studies from 2012 - 2013, n = 80, relative liver weight mean: 47.08 g/kg bw, range: 37.30-55.3 g/kg bw and absolute brain weight mean: 13.69g, range: 9.5-17.3g).

In both sexes there was a statistically significant increase in relative heart weights. In males, a statistically significant 14% increase was observed at 103 mg/kg bw/day, the relative heart weights were 2.788, 3.008, 3.180 and 2.910 g/kg bw at 0, 31, 103 and 309 mg/kg bw/day, respectively. There was no background data available for this parameter in males. In females, the relative heart weights were 3.286, 3.578, 3.796 and 3.892 g/kg bw at 0, 31, 103 and 309 mg/kg bw/day, respectively and statistical significance was achieved at 309 mg/kg bw/day (18% increase). The relative heart weights at each treatment group were within the background data of the test laboratory (8 studies from 2012 - 2013, n = 80, relative heart weight mean: 3.39 g/kg bw, range: 2.92 - 4.80 g/kg bw).

In females, there was a statistically significant decrease in the right absolute and relative adrenal weights. At ≥ 103 mg/kg bw/day, there was a statistically significant 40% decrease in the right absolute adrenal weights, (0.045, 0.044, 0.034 and 0.030g at 0, 31, 103 and 309 mg/kg bw/day, respectively). At 309 mg/kg bw/day there was a statistically significant 20% decrease in the right relative adrenal weights (0.122g/kg bw compared to 0.149g/kg bw). There was no background data for adrenal weights reported in the study. There were no effects observed in females left absolute or relative adrenal weights or the males absolute or relative adrenal weights.

In males, there was a statistically significant increase in the right and left absolute and relative kidney weights. The left absolute kidney weights were 1.502, 1.486, 1.690 (13%) and 1.712 (13%) g at 0, 31, 103 and 309 mg/kg bw/day, respectively and statistical significance was achieved at ≥103 mg/kg bw/day. The right absolute kidney weights were 1.580, 1.544, 1.816 (13%) and 1.770g at 0, 31, 103 and 309 mg/kg bw/day, respectively and statistical significance was achieved at 103 mg/kg bw/day. The left relative kidney weights were 3.150, 3.166, 3.790 (24%) and 3.972 (24%) g/kg bw at 0, 31, 103 and 309 mg/kg bw/day, respectively and statistical significance was achieved at ≥103 mg/kg bw/day. The right relative kidney weights were 3.312, 3.288, 4.068 (16%) and 4.104 (22%) g/kg bw at 0, 31, 103 and 309 mg/kg bw/day, respectively, and statistical significance was achieved at ≥103 mg/kg bw/day. There was no background data for male kidney weight reported in the study.

In females, at 309 mg/kg bw/day, there was a statistically significant (15%) increase in the right relative kidney weighs, (3.898 g/kg bw compared to 3.372 g/kg bw in the control). The relative kidney weight at the high dose was within the background data of the test laboratory (8 studies from 2012 - 2013, n = 80, relative kidney weight mean: 3.47 g/kg bw, range: 2.81 - 4.27 g/kg bw).

In females, there was a statistically significant decrease in absolute (34-47%) and relative (30% decrease at all treatment groups) thymus weight at ≥31 mg/kg bw/day. The absolute thymus weights were 0.300, 0.198, 0.200 and 0.164g and the relative thymus weights were 1.002, 0.668, 0.736 and 0.674g/kg bw at 0, 31, 103 and 309 mg/kg bw/day, respectively. The relative and absolute thymus weights at each treatment group were within the background data of the test laboratory (8 studies from 2012 - 2013, n = 80, relative thymus weight mean: 0.98 g/kg bw, range: 0.29 - 1.63 g/kg bw and absolute thymus weight mean: 0.30g, range: 0.09 - 0.49g). The DS notes there is no dose-response relationship, and it is difficult to conclude the toxicological relevance of this observation. There were no effects observed in male animals.

In both sexes at 309 mg/kg bw/day, there was a biologically significant decrease observed in absolute spleen weight (22% in males and 17% in females). At the same dose, males had a biologically significant 11% decrease in relative spleen weights. The males' absolute spleen weights were 0.852, 0.812, 0.806 and 0.706g and the relative spleen weights were 1.786, 1.722, 1.806, 1.644 g/kg bw at 0, 31, 103 and 309 mg/kg bw/day, respectively. The females' absolute spleen weights were 0.634, 0.690, 0.542 and 0.516g at 0, 31, 103 and 309 mg/kg bw/day, respectively. There no effects observed in the females' relative spleen weight. There was no background data for absolute or relative spleen weight reported in the study.

Table 3: Organ weight data measured data measured during Combined Repeated dose toxicity Study with Reproduction/Developmental toxicity screening test of IPETC (Danafloat™ 262) in Rats by Oral Administration *(Anonymous 2012a*).

Organ weights	Dose (mg/kg bw/day)							
	Males				Females			
	0	31	103	309	0	31	103	309
Absolute Epididymides (g) Left	0.721± 0.062	0.762± 0.046	0.733± 0.096	0.709± 0.032	-	-	-	-
Relative Epididymides (g/kg bw) Left	1.519± 0.134	1.618± 0.121	1.646± 0.293	**1.704± 0.140****	-	-	-	-
Absolute Epididymides (g) Right	0.677± 0.054	0.719± 0.049	0.716± 0.073	0.708± 0.056	-	-	-	-
Relative Epididymides (g/kg bw) Right	1.427± 0.118	**1.527± 0.090***	**1.602± 0.213***	**1.708± 0.207****	-	-	-	-
Absolute gonad Left (g)	2.133±0.369	2.064±0.124	2.084±0.106	1.988±0.191	0.071±0.021	0.070±0.013	0.061±0.010	0.053±0.016
Relative Gonad Left (g/kg bw)	4.539±1.095	4.386±0.344	4.661±0.393	4.779 ±0.522	0.237±0.067	0.239±0.053	0.228±0.049	0.220±0.071
Absolute gonad Right (g)	1.924±0.093	2.055±0.141	2.066±0.104	2.018±0.206	0.074±0.017	0.086±0.020	0.063±0.016	0.053±0.015
Relative Gonad Right (g/kg bw)	4.066±0.326	4.368±0.377	**4.619±0.391***	**4.854±0.561****	0.246±0.057	0.293±0.079	0.236±0.074	0.214±0.052
Absolute Brain (g)	1.990±0.048	**2.098±0.055***	2.048±0.040	1.906±.080	1.912±0.049	1.916±0.068	1.944±0.077	**1.754±0.131***
Relative brain (g/kg bw)	4.176±0.174	4.480±0.355	**4.594±0.155***	4.426±0.155	6.378±0.119	6.486±0.418	**7.186±0.375****	**7.202±0.658***
Absolute liver (g)	17.64±1.35	18.08±1.58	19.46±0.56	**21.84±2.20****	14.68±1.05	13.86±0.76	**10.58±0.54****	**11.96±0.85****
Relative liver (g/kg bw)	37.00± 2.43	38.48± 2.40	**43.64± 1.13***	**50.54± 2.07****	48.92±2.81	46.88±2.73	**39.14±3.07****	49.02±2.77
Absolute heart (g)	1.332±0.202	1.416±0.145	1.418±0.027	1.254±0.078	0.986±0.116	1.058±0.046	1.030±0.099	0.950±0.148

Organ weights	Dose (mg/kg bw/day)							
	Males				Females			
	0	31	103	309	0	31	103	309
Relative heart (g/kg bw)	2.788± 0.380	3.008± 0.184	**3.180± 0.110***	2.910± 0.151	3.286±0.364	3.578±0.152	3.796±0.212	**3.892±0.572***
Absolute Adrenal (g) Left	0.036±0.004	0.041±0.02	0.039±0.004	0.038±0.007	0.046±0.008	0.046±0.008	0.039±0.011	0.032±0.006
Relative Adrenal (g/kg bw) Left	0.075±0.007	0.086±0.006	0.088±0.009	0.088±0.019	0.153±0.028	0.156±0.019	0.146±0.051	0.130±0.021
Absolute Adrenal (g) Right	0.037±0.004	0.034±0.06	0.038±0.003	0.038±0.008	0.045±0.003	0.044±0.006	**0.034±0.009***	**0.030±0.006****
Relative Adrenal (g/kg bw) Right	0.077±0.009	0.073±0.013	0.086±0.007	0.088±0.019	0.149±0.009	0.149±0.017	0.127±0.041	**0.122±0.20***
Absolute kidneys (g) Left	1.502±0.094	1.486±0.086	**1.690±0.121***	**1.712±0.086****	0.986±0.116	1.058±0.046	1.030±0.099	0.950±0.148
Relative kidneys (g/kg bw) Left	3.150± 0.174	3.166± 0.185	**3.790± 0.276 ****	**3.972± 0.174****	3.286± 0.364	3.388± 0.280	3.364± 0.403	3.768± 0.170
Absolute kidneys (g) Right	1.580±0.120	1.544±0.175	**1.816±0.098***	1.770±0.124	0.998±0.080	1.002±0.084	0.912±0.126	0.920±0.075
Relative kidneys (g/kg bw) Right	3.312± 0.224	3.288± 0.338	**4.068± 0.217 ****	**4.104± 0.122****	3.372± 0.249	3.522± 0.274	3.420± 0.390	**3.898± 0.218***
Absolute Thymus(g)	0.398±0.172	0.350±0.083	0.258±0.045	0.292±0.082	0.300±0.048	**0.198±0.053****	**0.200±0.028****	**0.164±0.034****
Relative thymus (g/kg bw)	0.836±0.360	0.740±0.161	0.578±0.098	0.682±0.216	1.002±0.169	**0.668±0.160****	**0.736±0.077***	**0.674±0.152****
Absolute spleen (g)	0.852±0.073	0.812±0.159	0.806±0.098	0.706±0.047	0.634±0.074	0.690±0.421	0.542±0.070	0.516±0.176
Relative spleen (g/kg bw)	1.786±0.147	1.722±0.308	1.806±0.200	1.644±0.197	2.114±0.253	2.312±1.353	1.998±0.202	2.122±0.749

*** p < 0.05; **p < 0.01**

- *Macroscopic examination:*
  Two females (1/10 control and 1/10 female at 309 mg/kg bw/day) had clear liquid in the uterine horn. At 31 mg/kg bw/day 1/10 females had yellow content in their stomach and 1/10 females had changes in their spleen (rough surface, adhered to mesenterium and pancreas). 1/10 females at 309 mg/kg bw/day had haemorrhagic foci in stomach and their urinary bladder and colon were tightly packed.
  The study author concluded that none of these changes were test item related. There were no pathological findings in control and treated males.

- *Histopathology examinations:*
  In males at 309 mg/kg bw/day, interstitial lymphocytic infiltrate and mononuclear cell infiltration of the epididymis was observed in 3/5 and 5/5 males respectively compared to 2/5 and 3/5 in the control males. There were incidents of squamous cell hyperplasia of the forestomach in 1/5 males at 309 mg/kg bw/day compared to 0/5 in the control males.

  In females at 309 mg/kg bw/day, there were incidents of peripheral (1/5) and diffuse (1/5) fatty infiltration of the liver compared to 0/5 in control females. At the same dose there were incidents of alveolar histiocytosis (3/5) and haemorrhage (2/5) of the lungs compared to 1/5 incidents in the control. There were incidents of lymphocytic infiltration observed in both thyroids (1/5 each) in females at 309 mg/kg bw/day compared to 0/5 incidents in controls. Lympho-histiocytic infiltration of the urinary bladder was observed in 1/5 females at 309 mg/kg bw/day compared to 0/5 in controls.

  In both sexes at 309 mg/kg bw/day, vacuolisation in the adrenal (I) and (II) cortical was observed in 2/5 (adrenal (I)), 2/5 (adrenal (II)) in males and in 2/5 (adrenal (I)), 2/5 (adrenal (II)) in females compared to no incident in control males and females. At the same dose, there were also incident of lymphoid hyperplasia of the colon. The incidents were 3/5 in males and 2/5 in females compared to 2/5 and 1/5 in control males and females, respectively. There were incidents of lympho-histiocytic infiltrate of the kidneys in high dose males (1/5) and females (1/5) compared to 0/5 in control. In males at 309 mg/kg bw/day, there were incidents of hydronephrosis of the kidneys in 1/5 compared to 0/5 in control. In females at 309 mg/kg bw/day, there were incidents of fatty infiltration in the tubular epithelium of the kidneys in 3/5 compared to 1/5 in control.

  The DS notes that the study author's grading system was difficult to interpret. The DS considers that these histopathological findings were in low numbers, most scenarios were quite close to the background histopathological findings in the control animals and there were no other significant histopathological findings, and thus the biological significance of these findings is unclear.

Table 4: Summary of microscopic finding from the Combined Repeated dose toxicity Study with Reproduction/Developmental toxicity screening test of IPETC (Danafloat™ 262) in Rats by Oral Administration (*Anonymous 2012a*).

Organ **Microscopic finding** Degree of finding	**Dose (mg/kg bw/day)**			
	Males		**Females**	
	0	**309**	**0**	**309**
Epididymis (I)				
Interstitial Lymphocytic infiltrate				
Minimal	2	4	-	-
Interstitial mononuclear cell infiltration				
Minimal	3	5	-	-
Adrenal (I)				
Vacuolisation, cortical				
Minimal	0	2	0	2
Adrenal (II)				
Vacuolisation, cortical				
Minimal	0	2	0	2
Colon				
Lymphoid hyperplasia Peyer's patch				
Minimal	2	3	1	2
Forestomach				
Squamous cell hyperplasia				
Minimal	0	1	3	0
Kidney				
Lympho-histiocytic infiltrate				
Minimal	0	1	0	1
Hydronephrosis				
Minimal	0	1	0	0
Fatty infiltration in the tubular epithelium				
Minimal	0	0	1	3

Organ **Microscopic finding** Degree of finding	**Dose (mg/kg bw/day)**			
	Males		**Females**	
	0	**309**	**0**	**309**
Liver				
Peripheral fatty infiltration				
Minimal	0	0	0	1
Diffuse fatty infiltration				
Minimal	0	0	0	2
Lungs with bronchi/bronchioles				
Alveolar histiocytosis				
Minimal	0	0	1	3
Haemorrhage				
Minimal	0	0	1	2
Thyroid (I)				
Lymphocytic infiltration				
Minimal	0	0	0	1
Thyroid (II)				
Lymphocytic infiltration				
Minimal	0	0	0	1
Urinary bladder				
Lympho-histiocytic infiltration				
Minimal	0	0	0	1

N of animals=5 for control and at 309 mg/kg bw/day both sexes. Female at 309mg/kg bw/day who died prematurely not included in table.

Reproductive parameters

- *Oestrous cycle:* No information available

- *Effects on sperm:* The study author reported that "*spermatogenesis was not affected in the high dose group*," there was no raw data available in the study for the sperm parameters, so the DS could not assess this parameter.
- *Precoital interval:* There was an increase in pre-coital time observed at ≥ 103 mg/kg bw/day (76% and 172% at 103 and 309 mg/kg bw/day, respectively). The pre-coital time was 2.5, 2.7, 4.4 and 6.0 days at 0, 31,103 and 309 mg/kg bw/day, respectively. The pre-coital time range for the individual dams were 1 - 5 days, 1 - 4 days, 1 - 15 days and 1 - 20 days at 0, 31,103 and 309 mg/kg bw/day, respectively. At 103 mg/kg bw/day, 1/10 dams had a pre-coital time of 15 days and at 309 mg/kg bw/day, 2/10 dams had a pre-coital time of 15 and 20 days.

- *Toxic response data including indices of mating, fertility, gestation, live birth, viability, post-implantation survival and lactation, abortions, resorptions; indicate the numbers used in calculating the indices:*

    - There was no effect on the dams' ability to become pregnant. The number of pregnant dams were 9/10,10/10, 10/10 and 9/9 at 0, 31, 103 and 309 mg/kg bw/day, respectively.

    - There was no effect on the fertility index, it was 90%, 100%, 100% and 100% at 0, 31, 103 and 309 mg/kg bw/day, respectively.

    - There was no effect on the pre-implantation loss, it was 3.8, 7.5, 4.0 and 5.1 at 0, 31, 103 and 309 mg/kg bw/day, respectively.

    - There was a statistically significant decrease in the gestation index at ≥ 103mg/kg bw/day, the gestation index was 100%, 90%, 30% and 0% at 0, 31, 103 and 309 mg/kg bw/day. The gestation indices at ≥ 103 mg/kg bw/day were outside the background data of the test laboratory (8 studies from 2012 - 2013, n = 80; mean: 98.6%, range: 89 - 100%). There was no data for mean duration of gestation for females at 309 mg/kg bw/day, due to complete litter loss at this dose. There was no effect observed at ≤ 103 mg/kg bw/day.

    - There were no abortions observed and there was no information reported for resorptions.

    - There was a statistically significant decrease on number of dams with live pups at ≥ 103 mg/kg bw/day, it was 9, 9, 3 and 0 at 0, 31, 103 and 309 mg/kg bw/day, respectively.

    - There was a statistically significant decrease on birth index at ≥ 31 mg/kg bw/day, the birth index was 99.4%, 75.4%, 3% and 0% at 0, 31, 103 and 309 mg/kg bw/day, respectively. The birth indices at ≥ 31 mg/kg bw/day were outside the background data of the test laboratory (8 studies from 2012 - 2013, n = 80, mean: 92.7%, range: 87 - 97%).

    - There were no pups at 309 mg/kg bw/day so there was no live birth index data available. There were no effects observed on live birth index at ≤ 103 mg/kg bw/day. The live birth indices at ≤ 103 mg/kg bw/day (99.4%, 97.8% and 100% at 0, 31 and 103 mg/kg b/day respectively) were within the background data of the test laboratory (8 studies from 2012 - 2013, n = 80, mean: 99.4%, range: 97.8 - 100%).

    - There were no pups at 309 mg/kg bw/day, so there was no data available for viability index. There were no effects observed on viability index at ≤ 103 mg/kg bw/day.

    - There was no information available reported for lactation index.

- *Number of corpora lutea, pre- implantations and implantation sites:* There was no effect on mean corpora lutea, or pre-implantation loss observed. There was no effect observed on total implantation sites or mean

implantation sites. The total implantation sites were 137, 160, 137 and 113 and the mean implantation sites were 15.2, 16.0, 13.7 and 12.6 at 0, 31, 103 and 309 mg/kg bw/day, respectively.

- *Duration of gestation:* There was no mean duration of gestation data available for dams at 309 mg/kg bw/day, due to complete litter loss in the dams. There was no effect observed at ≤ 103 mg/kg bw/day.

- *Parturition and maternal care:* There was no information on issues with parturition or maternal care was reported.

Table 5: Summary of reproductive parameters from the Combined Repeated dose toxicity Study with Reproduction/Developmental toxicity screening test of IPETC (Danafloat™ 262) in Rats by Oral Administration (*Anonymous 2012a*).

Reproductive parameters				
Dose (mg/kg bw/day)	**0**	**31**	**103**	**309**
Number of pregnant dams	9	10	10	9
Pre-coital time	2.5	2.7	4.4	6.0
Fertility index (%)	90	100	100	100
Mean duration of gestation (days)	22.7	23.1	23.3	-
Mean corpora lutea	15.8	17.5	14.2	13.2
Pre-implantation loss (%)	3.8	7.5	4.0	5.1
Total implantation sites	137	160	137	113
Mean implantation sites	15.2	16.0	13.7	12.6

****= p<0.01;** -= No data for litters at 309 mg/kg bw/day

Developmental parameters and observations in offspring

- *Total number of pups born:* There was a statistically significant decrease in the number of pups born at ≥ 103 mg/kg bw/day. The number of pups born was 133, 122, 5 and 0 at 0, 31,103 and 309 mg/kg bw/day, respectively.

- *Number of dams with live pups:* There was a statistically significant decrease in the number of dams with live pups at ≥ 103 mg/kg bw/day. The number of dams with live pups was 9/10, 9/10, 3/10 and 0/9 at 0, 31,103 and 309 mg/kg bw/day, respectively.

- *Post-implantation loss:* There was a statistically significant increase in post-implantation loss at ≥ 31 mg/kg bw/day. The post-implantation loss was 3.7%, 28%, 97% and 100% at 0, 31, 103 and 309 mg/kg bw/day, respectively. The post-implantation loss at ≥ 31 mg/kg bw/day was outside the background data of the test laboratory (8 studies from 2012 - 2013, n = 80, mean: 8%, range: 3.6-12.7%).

- *Birth index and number of live pups:* The birth indices were 99%, 75%, 3% and 0% at 0, 31,103 and 309 mg/kg bw/day, respectively, and the test laboratory background data for birth indices from 8 studies from 2012 – 2013 (n = 80), had a mean of 93%, within a range of 87 - 97%. There was a statistically significant decrease in the total number of pups born (133, 122, 5 and 0 at 0, 31,103 and 309 mg/kg bw/day, respectively).

- *Live birth index:* There was no data available for live birth index at 309 mg/kg bw/day, due to complete litter loss at this concentration. There was no effect at ≤ 103 mg/kg bw/day as the live birth index was

99.4%, 97.8% and 100%. The live birth indices at ≤ 103 mg/kg bw/day were within the background data of the test laboratory (8 studies from 2012 - 2013, n = 80, mean: 99.4%, range: 97.8 - 100%).

- *Viability index:* Due to complete litter loss at 309 mg/kg bw/day, there was no data available for the viability index at this concentration. There was a biologically significant decrease in viability index observed at 103 mg/kg bw/day, the viability index was 98%, 98.4% and 66.7% at 0, 31 and 103 mg/kg bw/day, respectively.

- *Number of stillborn pups:* As there were no pups at 309 mg/kg bw/day, this parameter was reported at 0 for this concentration. There was a no effect observed at ≤ 103 mg/kg bw/day.

- *Number of runts or malformed pups:* As there were no pups at 309 mg/kg bw/day, there was no data available for the number of runts or malformed pups at this concentration. There was no effect seen at ≤ 103 mg/kg bw/day.

- *Sex ratio:* As there were no pups at 309 mg/kg bw/day, there was no data available for sex ratio at this concentration i.e., it was reported as 0:0. There was no effect seen at ≤ 103 mg/kg bw/day.

Table 6: Summary of developmental parameters from the Combined Repeated dose toxicity Study with Reproduction/Developmental toxicity screening test of IPETC (Danafloat™ 262) in Rats by Oral Administration (*Anonymous 2012a*).

Developmental parameters				
Dose (mg/kg bw/day)	**0**	**31**	**103**	**309**
Total number of pups born	133	122	**5****	**0****
Post-implantation loss (%)	3.7	**27.6****	**97.0****	**100.0****
Gestation index (%)	100	90	**30****	**0****
Number of dams with live pups	9	9	**3****	**0****
Birth index (%)	99.4	**75.4****	**3.0****	**0****
Number of stillborn	1	5	0	0
Number of live pups	132	117	5**	0**
Live birth index (%)	99.4	97.8	100	-
Viability index (%)	98.0	98.4	66.7	-
Number of runts	0	0	0	-
Number of malformed pups	0	0	0	-
Sex ratio M:F on PND 1	69/63	61/56	3/2	0/0

****= p<0.01**; -= No data for litters at 309 mg/kg bw/day.

- *Mean litter or pup weight by sex:* At 309 mg/kg bw/day, there was complete litter loss, so there is no pup body weight data available for this dose group. There were no effects observed for pup mean body weight on PND 1 and 4 at ≤ 103 mg/kg bw/day.

Table 7: Pup mean body weights in both sexes on PND 1 and 4 observed in the Combined Repeated dose toxicity Study with Reproduction/Developmental toxicity screening test of IPETC (Danafloat™ 262) in Rats by Oral Administration (*Anonymous 2012a*).

Dose (mg/kg bw/day)	0	31	103	309
Post-natal development period Sex	Mean body weight (g)			
PND 1				
M	6.31±0.78	5.9±0.42	7.03±0.45	--
F	6.06±0.72	5.67±0.38	6.40±-	--
PND 4				--
M	9.08±1.40	9.03±0.75	7.60±-	
F	9.03±1.20	8.90±0.68	8.90±-	--

--= No data for 309 mg/kg bw/day, - = no Std. Dev for M/F pups at 103 mg/kg bw/day.

- *Anogenital Distance:* Not reported.
- *Areola/Nipple Retention:* Not reported.
- *External examinations:* There was complete litter at 309 mg/kg bw/day and thus no pups available at this concentration for external examination. There were no external visible abnormalities observed at ≤ 103 mg/kg bw/day.

3.10.1.2 Study 2 OECD 414: Prenatal Development Toxicity

Study reference: Anonymous, 2017a, Prenatal Development Toxicity Study after repeated oral administration in Wistar Rats with IPETC. (Unpublished report).

Detailed study summary and results:

Test type

OECD Guideline 414: Prenatal developmental toxicity. The study predates the current OECD 414 test guideline and deviated from the test guideline in that; there were no thyroid parameters i.e., weight, hormone and/or histopathology data reported and no anogenital distance reported.

GLP compliant. Unpublished study.

Test substance:

Name: *O*-Isopropyl ethylthiocarbamate or IPETC. Identical to substance identified in CLH dossier.

Degree of purity: 95.7% w/w.

Impurities: Not reported.

Batch number: 0001026602.

Test substance formulation: The test item and control formulations were freshly prepared daily on administration days.

Test animals:

Species/strain/sex: Rat Wistar Crl:WI (Han), male and female.

No. of animals per sex per dose: 156 animals in total; 52 males and 104 females. 24 females in control and 3 mg/kg bw/day treatment group and 25 females in the 10 and 30 mg/kg bw/day treatment group.

Age and weight at the study initiation: Males: 12-17 weeks; 320-350g. Females: 11-12 weeks; 203-242g.

Administration/exposure:

Route of administration: Oral; gavage

Duration and frequency of test/exposure period: Daily administration from gestation day (GD) 5 until GD 19.

Doses and rationale for dose level selection: 0, 3, 10 and 30 mg/kg bw/day. Dose selection was based on the results of an OECD 414 dose range finding study and the OECD 422 study outlined in section 3.10.1.

Dose-ranging finding study:

Females, Wistar Crl:WI (Hans) rats aged 11-12 weeks old were paired 2:1 with males Wistar Crl:WI (Hans) rats aged 19-20 weeks old prior to treatment. Eight female rats were treated via oral gavage with control (corn oil) and four female rats per dose group were treated with test substance (300 and 500 mg/kg bw/day) daily from GD 5 until GD 19. Male rats were not treated. Observations including body weight, food consumption and clinical signs were made during the testing period and on GD 20 sperm positive females were sacrificed and examined macroscopically for any structurally abnormalities or pathological changes that may have affected pregnancy. The uterine contents of pregnant and non-pregnant females were examined. Foetal examinations were carried out from foetuses in one dam. The foetuses were examined for external abnormalities.

On GD 6 and 7, females at 500 mg/kg bw/day had increased weight loss. Two dams lost 21g (8.5%) and 22g (9.2%) in body weight respectively between day 1 and 2 (GD 5 and GD 6) of dosing. The remaining two dams lost 9g (3.75%) and 15g (5.69%), respectively between day 1 and 3 (GD 5 and GD 7) of dosing. These dams also had significant clinical signs. As a result of these findings, the dosing concentration for these 4 dams was reduced to 300 mg/kg bw/day for rest of the study i.e., from GD 8-19 and the dams from the two dose groups were combined as one group indicated as “treated dams” below.

There was no mortality observed in either treatment group. Dams in the test substance treatment groups showed signs of weight loss (7/8) piloerection (8/8), disturbance of bedding (8/8), dehydration (1/8) and salivation (2/8).

The mean body weight and body weight gain was lower in the treated dams throughout the treatment period. On GD 20, the mean body weight (terminal body weight including gravid uterine weight) was 235.17g in the treated dams, 29% lower compared to 332.17g in the control group. During the gestation period (GD 0-20), there was an 85% decrease observed in the mean body weight gain (15.50g) in the treated dams compared to the control group (108.50g). The adjusted mean terminal maternal weight of the treated dams was slightly lower (10% decrease), it was 234.70g compared to 261.63g in controls.

Mean food consumption was lower in the treated dams compared to the control dams throughout most of the treatment period, the mean food consumption from GD 0-20 was 24% lower in the treated dams (332.33g) compared in the control dams (435.17g).

Biologically significant observations were noted during examinations of the reproductive parameters in the treated dams. The mean uterine weight was biologically significantly lower in the treated females, it was 0.97g (1.4% lower) compared to 70.53g in the control group. There was 100% post-implantation loss compared to 5.42% in control dams, there were no mean live foetuses in treated dams compared to 13 in the control and the mean number of early resorptions was 10.50 in treated dams compared to 0.5 control dams. The mean number of corpora lutea and implantations sites were slightly decreased in treated dams (11.00 and 10.50 respectively) compared to the control dams (14.50 and 13.67 respectively). Due to complete implantation loss in the treated dams, it was not possible to make a comparison for the following parameters mean number of late resorptions and number of dead foetuses. There was no difference observed in the mean pre-implantation loss (5.84% in control and 6.75% in treated dams).

There were no foetuses in the treated dams' group and thus there was no data available for comparison for the following parameters: sex ratio, mean foetuses' weight, mean total litter weight and external examinations.

There were no external abnormalities reported for the control foetuses.

The authors of the dose range finding study concluded that based on the findings in the present study, and increased loss of implantations at 31 mg/kg bw/day in the OECD 422 study (*Anonymous, 2012a*), the dosing schedule for the main OECD 414 study (*Anonymous, 2017a*) was set at 3, 10 and 30 mg/kg bw/day.

Control group and treatment: 24 female rats administered corn oil via oral (gavage)

Vehicle: Corn oil

Actual doses (mg/kg bw/day): 0, 3, 10 and 30 mg/kg bw/day

Description of test design:

- *Details on mating procedure:* Male: female 1:2. Proof of pregnancy was determined by sperm in vaginal smear.
- *Dosing schedule:* Females were treated once daily, 7 days/week from GD 5 until GD 19
- *Parameters assessed for parental animals:*
  - Clinical and mortality observations: 1/day and 2/day, respectively
  - Body weight: All animals were weighed before pairing. Females were monitored on GD 0, 5, 8, 11, 14, 17 and 20.
  - Food consumption: Females' consumption was monitored on GD 0, 5, 8, 11, 14, 17 and 20. Food consumption for males during the complete study and females during the mating period was not measured.
  - Post-mortem examinations: Females were sacrificed on GD 20 and examined macroscopically for any structural abnormalities or pathological changes. Uteri were removed and pregnancy status of each female was determined. Non-gravid uteri were further examined to confirm non-pregnant status. Gravid uterus with cervix were weighed, corpora lutea were counted and the uterine contents were examined for embryonic or foetal deaths, viable foetuses, and the degree

of late and early resorptions. Each uterine horn was also examined to determine the number and position of foetuses.

- *Foetal evaluations:* All foetuses were weighed and sexed based on their anogenital distance (AGD). Half of each litter were examined for soft tissue anomalies and the first 20 litters per group were examined for skeletal alterations.
- *Historical control:* The study report provides historical control data (HCD) for Wistar rat for a study data range from 2011 - 2015.
- *Statistical methods:* Datasets were analysed using one-way ANOVA, post-hoc Dunnett Test or a Fisher's exact test using GraphPad Prism V.6.01 software and data was considered statistically significant if $p<0.05$.

Results and discussion

Observations of effects in Parental Generation

Mortality: None observed.

Clinical signs: Alopecia was observed on various areas of the body; hindlimb, forelimb, abdomen and dorsal back in 1/24, 2/24, 1/25 and 3/25 females at 0, 3, 10 and 30 mg/kg bw/day, respectively. There were no other clinical signs reported.

Body weight and body weight gain: During the gestation period, there was a statistically significant increase in mean body weight at 3 mg/kg bw/day and 30 mg/kg bw/day. At 3 mg/kg bw/day, there was a statistically significant 3.5%-4.6% increase from GD 0-14 and at 30 mg/kg bw/day there was a statistically significant 4%-4.4% increase from GD 0-8. There was no difference in mean body weight observed at 10 mg/kg bw/day throughout the gestation period. The mean body weights were within the historical control range of the test laboratory (Rat Wistar, (2011 - 2015), n = 416, mean: 324.18g, range: 245 - 384g). The study author reported that the increase in mean body weight was not dose-dependent and was not considered test item related. The DS agrees with the original study author, the increase in mean body weight was not test substance related. There were no effects on mean body weight gain observed.

The study author calculated the adjusted maternal weight (271.06, 278.11, 274.23 and 278.80g at 0, 3, 10 and 300 mg/kg bw/day, respectively) and there were no effects observed. The adjusted maternal weights were within the historical control range of the test laboratory (Rat Wistar, (2011 - 2015), n = 416, mean: 265.79g, range: 222 - 316g).

There were no effects on terminal body weight (335.14, 348.78, 336.75 and 338.81g at 0, 3,10 and 300 mg/kg bw/day, respectively) observed on GD 20.

Table 8: Maternal Mean body weight measured during the Prenatal Development Toxicity Study after repeated oral gavage administration in Wistar Rats with IPETC. (*Anonymous 2017a*).

Dose (mg/kg bw/day)	0	3	10	30
Gestation Period	**Mean body weight (g)**			
GD 0	225.90±9.44	**233.88±11.76***	232.29 ±11.59	**235.05±10.99***
GD 5	243.95 ±10.34	**253.04±12.64***	249.46 ±9.77	**254.24±11.26****
GD 8	246.90 ±14.84	**258.17±10.04****	253.92 ±11.29	**257.81±10.72****
GD 11	260.67 ±12.70	**271.96±12.74****	265.75±10.90	269.29±12.75
GD 14	271.24 ±15.23	**282.88±14.36***	275.04±12.10	279.95 ±13.14
GD 17	297.57 ±17.79	309.42±14.88	300.29±15.95	302.05 ±15.01
GD 20	335.14±23.94	348.75±17.26	336.75±20.63	338.81 ±18.01

*** p < 0.05; **p < 0.01**. N= 21, 24, 24 and 21 at 0, 3, 10 and 30 mg/kg bw/day, respectively.

Food consumption: There were no effects observed.
Water consumption: Not reported.
Thyroid hormones: Not reported.
Macroscopic finding: 1/25 females at 10 mg/kg bw/day had a high volume of fat tissue attached to the uterus, the study author reported that this finding "*was spontaneous in nature as the finding occurred in a single isolated female"*. There were no other macroscopic findings reported.
Organ weights: There was no effect on the gravid uterine weight, the mean uterine weights were 64, 71, 63 and 60g at 0, 3, 10 and 30 mg/kg bw/day, respectively. The mean uterine weight was also within the historical control range of the test laboratory (Rat Wistar, (2011 – 2015), n = 416, mean: 58.4g, range: 0.5 - 109.5g). Other organ data was not reported.
Histopathology examinations: Not reported.

Reproductive parameters
Number of pregnancies: There were no effects observed on the number of pregnancies in the treated females. The number of pregnant females was 21/21, 24/24, 24/24 and 21/21 at 0, 3, 10 and 30 mg/kg bw/day, respectively.
Resorptions: There was a slight increase in mean percentage of early resorptions at 30 mg/kg bw/day, the mean early resorptions were 0.76%, 0.46%, 0.67% and 1.05% at 0, 3, 10 and 30 mg/kg bw/day, respectively. However, there was no statistical significance noted and the value was within the historical control range of the test laboratory (Rat Wistar, (2011 - 2015), n = 416, mean: 0.65%, range: 0% - 8%). There were no effects observed on the mean percentage of late resorptions (0%, 0%, 0.04% and 0% at 0, 3, 10 and 30 mg/kg bw/day, respectively) in the treated females.
Number of implantations: There was no effect observed on the number of implantation sites in treated females. The mean number of implantation sites were in 12.14, 13.13, 11.79 and 12.38 at 0, 3, 10 and 30 mg/kg bw/day, respectively.
Pre and post implantation loss: There was no effect observed in pre -implantation loss, the mean percentage pre-implantation loss per litter was 12.96%, 5.17%, 11.83% and 7.45% at 0, 3, 10 and 30 mg/kg bw/day, respectively.
There was a slight increase in post-implantation loss at ≥10 mg/kg bw/day, the mean post-implantation loss per litter was 5.7%, 3.5%, 7.1% and 8.2% at 0, 3, 10 and 30 mg/kg bw/day, respectively. There was no statistical significance noted and the value was within the historical control (HCD) range of the test laboratory (Rat Wistar, (2011 – 2015), n= 416, mean: 6.96%, range: 0% - 100%). The DS queries the validity of this

historical control data range as there was 100% post-implantation loss in the absence of treatment. The mean value of the HCD indicates the 100% value is an outlier, or there is some problem with the animal test facility controls and is not representative of the whole HCD (if the HCD was normally distributed between 7 – 100% then a mean value of approximately 50% would be expected and the measurement of post-implantation loss in this instance would be meaningless). Comparing the historical control mean value (6.96%) to the concurrent control and treated dams, the DS concludes there is little evidence of a substance mediated effect at the doses tested. However, the DS acknowledges that this mean value for the HCD could be skewed high if it incorporated the high and unlikely value of 100%.

Corpora lutea: There was no effect on corpora lutea. The mean number of corpora lutea were 14.0, 13.88, 13.33 and 13.38 at 0, 3, 10 and 30 mg/kg bw/day treated females, respectively.

Table 9: Maternal reproduction parameters examined during the Prenatal Development Toxicity Study after repeated oral administration in Wistar Rats with IPETC. (*Anonymous 2017a*).

Reproductive Parameter					
Dose (mg/kg bw/day)	**0**	**3**	**10**	**30**	**HCD**
Number of pregnant females (%)	21	24	24	21	Not reported
Mean % of early resorptions per litter	0.76±1.41	0.46±0.78	0.67±1.05	1.05±1.20	Mean=0.65 Range= 0-8
Mean % of late resorptions per litter	0.00±0.00	0.00±0.00	0.04±0.20	0.00±0.00	Mean=0.02 Range= 0-1
Mean number of implantation sites	12.14±2.90	13.13±1.57	11.79±2.47	12.38±1.86	Mean=11.11 Range= 1-17
Mean % pre-implantation loss	12.96±17.54	5.17±5.01	11.83±15.79	7.45±9.61	Mean=14.82% Range= 0-85.71%
Mean % post-implantation loss	5.68±9.66	3.47±5.95	7.14±13.39	8.23±8.68	Unreliable
Mean number of corpora lutea	14.00±2.93	13.88±1.83	13.33±1.37	13.38±1.47	Mean= 12.98 Range=1-20

N= 21, 24, 24 and 21 at 0, 3, 10 and 30 mg/kg bw/day, respectively. HCD; Rat Wistar, Study Date Range; 2011 – 2015, Number of animals in historical control group=416

Foetal observations

Litter size: There was no effect on litter size observed in the treated females, the mean number of foetuses were 11.38, 12.67, 11.08 and 11.29 at 0, 3, 10 and 30 mg/kg bw/day, respectively.

Number of viable:

Viable foetuses: There was no effect on the number of viable or live foetuses observed in the treated females, the mean number of foetuses were 11.38, 12.67, 11.08 and 11.29 at 0, 3, 10 and 30 mg/kg bw/day, respectively.

Dead foetuses: There was no effect on dead foetuses observed in the treated females, the mean number of foetuses were 0, 0, 0 and 0.5 at 0, 3, 10 and 30 mg/kg bw/day, respectively.

Sex ratio: There was an apparent statistically significant increase observed in the number of males born at 10 mg/kg bw/day. The number of males were 5.5, 7.0, 5.5 and 5.4 at 0, 3, 10 and 30 mg/kg bw/day, respectively. The number of males born at 10 mg/kg bw/day was within the historical control range of the test laboratory (Rat Wistar, (2011 – 2015), n= 416, mean: 5.24%, range: 0 - 12%). The relevance is of little concern here as there is no apparent dose response. There were no effects observed on the number of females born (5.9, 5.7, 5.5 and 5.9 at 0, 3, 10 and 30 mg/kg bw/day, respectively). There was no statistically significant difference noted in the male to female ratio for the control and treatment groups (1.1, 1.6, 1.1 and 1.1 at 0, 3, 10 and 30 mg/kg bw/day, respectively).
Mean foetal weight: There was a statistically significant decrease (8.6%) in mean foetal weight at 30 mg/kg bw/day. The mean foetal weights were 3.7, 3.7, 3. 7 and 3.4g at 0, 3, 10 and 30 mg/kg bw/day, respectively and all values were within the historical control range of the test laboratory (Rat Wistar, (2011 – 2015), n = 416, mean: 3.67g, range: 2.85 - 5.69g).
Litter weight: There were no effects observed in total litter weight (41.92, 46.33, 40.67, 37.99g at 0, 3, 10 and 30 mg/kg bw/day, respectively), and the values were within the historical control range of the test laboratory (Rat Wistar, (2011 - 2015), n = 416, mean: 37.92 g, range: 0 - 85.30g). There was a significant increase on male litter weight at 10 mg/kg bw/day, the male litter weight was 20.80, 26.23, 20.80 and 18.64g at 0, 3, 10 and 30 mg/kg bw/day, respectively. The male litter weights in the control and treatment groups were within the historical control range of the test laboratory (Rat Wistar, (2011 - 2015), n = 416, mean: 19.52g, range: 0 - 58.30g). There were no effects observed in female litter weight (21.12, 20.10, 19.86 and 19.35g at 0, 3, 10 and 30 mg/kg bw/day, respectively), and the values were within the historical control range of the test laboratory (Rat Wistar, (2011 - 2015), n = 416, mean: 18.40 g, range: 0 - 44.20g).
Anogenital Distance (AGD): Not reported.

Table 10: Foetal parameters examined during the Prenatal Development Toxicity Study after repeated oral administration in Wistar Rats with IPETC. (*Anonymous 2017a*).

Foetal Parameter					
Dose (mg/kg bw/day)	**0**	**3**	**10**	**30**	**HCD**
Mean number of foetuses	11.38±2.84	12.67±1.66	11.08±2.80	11.29±1.55	Not reported
Live foetuses per litter	11.38±2.84	12.67±1.66	11.08±2.80	11.29±1.55	Mean=10.43 Range= 0-17
Dead foetuses per litter	0.00±0.00	0.00±0.00	0.00±0.00	0.05±0.00	Mean=0.01 Range= 0-1
No. of male foetuses	5.52±2.36	**7.00±1.14***	5.54±2.21	5.38±1.32	Mean=5.24 Range= 0-12
No. of female foetuses	5.86±2.06	5.67±1.86	5.54±1.91	5.90±1.92	Mean=5.22 Range= 0-12
Sex ratio	1.12±0.70	1.63±1.40	1.13±0.66	1.13±0.90	Mean=1.30 Range= 0-11
Mean foetal weight (g)	3.70±0.20	3.66±0.21	3.67±0.16	**3.38±0.28*****	Mean=3.67 g Range= 2.85-5.69 g
Total litter weight (g)	41.92±10.35	46.33±6.62	40.67±10.34	37.99±5.08	Mean=37.92 g Range= 0-85.30g
Male litter weight (g)	20.80±8.97	**26.23±5.67***	20.80±8.20	18.64±3.84	Mean=19.52 g Range= 0-58.30 g
Female litter weight (g)	21.12±7.41	20.10±6.75	19.86±6.96	19.35±6.19	Mean=18.40 g Range= 0-44.20 g

p<0.05; **p<0.01;p<0.001;** N= 21, 24, 24 and 21 at 0, 3, 10 and 30 mg/kg bw/day, respectively. HCD; Rat Wistar, Study Date Range; 2011 – 2015, Number of animals in historical control group=416

External examinations: There were incidence of various external malformations and variations reported for individual pups in the control and treated groups. Variations included haematoma at the left and right forelimb, back and left scapula region. Malformations included one foetus with an absent left eye and misshapen snout and one foetus with an umbilical hernia. There was no statistical significance observed and the biological

significance of any of these findings is unclear, as all findings occurred in individual pups and either fall within the historical control data or there is no historical control data for that parameter.

Visceral examination: There were incidence of various visceral observations reported for pups in the control and treated groups. There were incidences of fluid in abdomen, discolouration in the adrenal gland, kidney, liver, thymus and lung, dilated renal and ureter, fluid in the peritoneal cavity, cervical thymus remnant, transposed umbilical artery, convoluted ureter and spotted urinary bladder wall. There was no statistical significance observed and the biological significance of any of these findings is unclear, as all findings occurred in low numbers of treated pups and often in lower numbers than that observed in the control pups. The findings also either fall within the historical control data or there is no historical control data for that parameter reported.

Skeletal examination:

Anomalies or malformations

There was an increase in the foetal incidence, litter incidence and number of affected litters with incidence of rudimentary seventh right and bilateral cervical rib (grey zone anomaly). The foetal incidence data for both parameters are outlined in Table 11. The litter incidence of rudimentary seventh right cervical rib was 0%, 18%, 10% and 25% and the number of affected litters was 0, 4, 2 and 5 at 0, 3, 10 and 30 mg/kg bw/day, respectively. Statistical significance was achieved at 30 mg/kg bw/day for number of affected litters. The litter incidence and the number of affected litters of rudimentary seventh right cervical rib at 3 and 30 mg/kg bw/day were outside the historical control range of the test laboratory (Rat Wistar, (2011 – 2015), number of foetuses/litters examined = 416, litter incidence mean: 1.45%, litter incidence range: 0.0% - 14.3%, number of affected litters range: 0 - 2). The study author considered these incidences at 30 mg/kg bw/day '*adverse effects of treatment'* and, the DS considers the biological relevance of this finding indicative of a substance related effect even in the absence of a clear dose response. The incidences in the low dose group of 3 mg/kg bw/day are considered similar to normal background levels.

The litter incidence of rudimentary seventh bilateral cervical rib was 0%, 0%, 0% and 15% and the number of affected litters was 0, 0, 0 and 3 at 0, 3, 10 and 30 mg/kg bw/day, respectively. The number of affected litters at 30 mg/kg bw/day was outside the historical control range of the test laboratory (Rat Wistar, (2011 – 2015), number of foetuses/litters examined = 416, litter incidence mean: 1.02%, litter incidence range: 0.0% - 18.2%, number of affected litters range: 0 - 2). The DS, considers these incidences biologically relevant.

There was an increase in the foetal and litter incidence of misshapen humeri (malformation), of the forelimb and there was a statistical increase in the number of affected litters with incidence at 30 mg/kg bw/day. The foetal incidence data is outlined in Table 11. The litter incidence was 0%, 0%, 5% and 25% and the number of affected litters was 0, 0, 1 and 5 at 0, 3, 10 and 30 mg/kg bw/day, respectively. There was no historical control data for this parameter reported. The study author considered these incidences at 30 mg/kg bw/day as '*adverse effects of treatment',* the DS considers that this increase in incidences may be indicative of a treatment related effect.

There was an increase in the foetal incidence, litter incidence and the number of affected litters with incidence of short bilateral scapula (variation), at 30 mg/kg bw/day. The foetal incidence data is outlined in Table 11. The litter incidence was 0%, 0%, 0% and 10% and the number of affected litters was 0, 0, 0 and 2 at 0, 3, 10 and 30 mg/kg bw/day, respectively. There was no statistical difference reported and the parameters were within the historical control range of the test laboratory (Rat Wistar, (2011 – 2015), number of foetuses/litters examined = 416, litter incidence mean: 0.57%, litter incidence range: 0.0%-12.5%, the number of affected

litters range: 0 - 2). The biological relevance of this finding is somewhat unclear but in general bent or shortened long bones in the rat are considered variations rather than malformations. Since the rat skeletal system undergoes further development and ossification postnatally these effects are typically characterised as being transient in nature with no long-term adverse effects on function or survival.

Table 11: Foetal skeletal anomalies or malformations observed during the Prenatal Development Toxicity Study after repeated oral administration in Wistar Rats with IPETC. (*Anonymous 2017a*).

Anomaly		Dose (mg/kg bw/day)				
		0	**3**	**10**	**30**	**HCD Litter incidence**
Scapula (B) short (variation)	Foetal incidence	0	0	0	2	Mean=0.57
	Litter incidence (%)	0.00	0.00	0.00	10.00	% Range=0-12.50%
	No of Litters with at least 1 Incidence	0	0	0	2	Range= 0-3
Cervical rib (7th) (B) rudimentary (grey zone anomaly)	Foetal incidence	0	0	0	4	% Mean=1.02%
	Litter incidence (%)	0.00	0.00	0.00	15.00	% Range=0-18.18%
	No of Litters with at least 1 Incidence	0	0	0	3	Range= 0-2
Cervical rib (7th) (R) rudimentary (grey zone anomaly)	Foetal incidence	0	4	2	6	% Mean=1.45%
	Litter incidence (%)	0.00	18.18	10.00	25.00	% Range=0-14.29%
	No of Litters with at least 1 Incidence	0	4	2	**5***	Range= 0-2
Forelimb humeri - misshapen (malformation)	Foetal incidence	0	0	1	9	No data
	Litter incidence (%)	0.00	0.00	5.00	25.00	
	No of Litters with at least 1 Incidence	0	0	1	**5***	

***p<0.05;** N= 20, 22, 20 and 20 at 0, 3, 10 and 30 mg/kg bw/day, respectively. HCD; Rat Wistar, Study Date Range: 2011 – 2015, Number of animals in historical control group=416. (B)= bilateral, (L)=left and (R)= right.

Variations

There was an increase in the foetal incidence, litter incidence and the number of affected litters in skull and bone variations; incomplete ossification in the frontal bilateral skull, hyoid body, interparietal bone in the skull, parietal bone in the bilateral skull, supraoccipital bone in the skull, and zygomatic arch in the right skull.

The foetal incidence data of incomplete ossification in the frontal bone bilateral skull is outlined in Table 12. The litter incidence of incomplete ossification in the frontal bone bilateral skull was 0%, 0%, 20% and 35% and the number of affected litters was 0, 0, 4 and 7 at 0, 3, 10 and 30 mg/kg bw/day, respectively, with statistical significance achieved at 30 mg/kg bw/day for the number of affected litters. Both parameters were within the historical control range of the test laboratory (Rat Wistar, (2011 – 2015), number of foetuses/litters examined

= 416, litter incidence mean: 12%, litter incidence range: 0.0% - 47.4%, number of affected litters range: 0 - 9).

The foetal incidence data of incomplete ossification of the hyoid body is outlined in Table 12. The litter incidence of incomplete ossification of the hyoid body was 5%, 9%, 5% and 25% and the number of affected litters was 1, 2, 1 and 5 at 0, 3, 10 and 30 mg/kg bw/day, respectively. There was no statistical significance achieved however, both parameters at 30mg/kg bw/day were outside the historical control range of the test laboratory (Rat Wistar, (2011 – 2015), number of foetuses/litters examined = 416, litter incidence mean: 2%, litter incidence range: 0% - 20%, number of affected litters range: 0 - 2).

The foetal incidence data of incomplete ossification of the interparietal bone in the skull is outlined in Table 12. The litter incidence of incomplete ossification of the interparietal bone in the skull was 55%, 55%, 75% and 85% and the number of affected litters was 11, 12, 15 and 17 at 0, 3, 10 and 30 mg/kg bw/day, respectively. Statical significance was not achieved, and both parameters were within the historical control range of the test laboratory (Rat Wistar, (2011 – 2015), number of foetuses/litters examined = 416, litter incidence mean: 65%, litter incidence range: 10% - 91%, number of affected litters range: 0 - 20).

The foetal incidence data of incomplete ossification of the parietal bone in the bilateral skull is outlined in Table 12. The litter incidence of incomplete ossification of the parietal bone in the bilateral skull was 25%, 36%, 65% and 60% and the number of affected litters was 5, 8, 13 and 12 at 0, 3, 10 and 30 mg/kg bw/day, respectively. Statical significance was not achieved, and both parameters were within the historical control range of the test laboratory (Rat Wistar, (2011 – 2015), number of foetuses/litters examined = 416, litter incidence mean: 49%, litter incidence range: 5% - 87.50%, number of affected litters range: 1-15).

The foetal incidence data of incomplete ossification of the supraoccipital bone in the skull is outlined in Table 12. The litter incidence of incomplete ossification of the supraoccipital bone in the skull was 35%, 59%, 55% and 80% and the number of affected litters was 7, 13, 11 and 16 at 0, 3, 10 and 30 mg/kg bw/day, respectively. Statical significance was achieved for the number of affected litters at 30 mg/kg bw/day. The test laboratory HCD for this parameter in 416 Wistar rat foetuses per litter during a time range of 2011 – 2015, was reported as a mean litter incidence of 39%, a litter incidence range of 14% - 100% and number of affected litters range of 1 - 23. The DS, has concerns related to the reliability of this HCD and there was no HCD raw data available to confirm the data's reliability.

The foetal incidence data of incomplete ossification of the zygomatic arch in the right skull is outlined in Table 12. The litter incidence of incomplete ossification of the zygomatic arch in the right skull was 5%, 0%, 5% and 35% and the number of affected litters was 1, 0, 1 and 7 at 0, 3, 10 and 30 mg/kg bw/day, respectively. At 30 mg/kg bw/day, statistical significance was achieved for the number of affected litters and both parameters were outside the historical control range of the test laboratory (Rat Wistar, (2011 – 2015), number of foetuses/litters examined = 416, litter incidence mean: 1.4%, litter incidence range: 0% -13%, number of affected litters range: 0 - 1).

The DS considers most of these effects as variations and indicative of an adverse substance-related effect.

There was an increase in foetal incidence, litter incidence and the number of affected litters in scapula variations; bent bilateral and right scapula and spine, bent bilateral and right scapula and incomplete ossification of the bilateral scapula (outlined in table 12). There was no statistical significance achieved for any of these variations. The litter incidences of bent bilateral and right scapula and spine and incomplete ossification of the bilateral scapula was relatively low (5-15%), there was no historical data for these variations.

The foetal incidence data of both scapula parameters are outlined in Table 12. The litter incidence of bent bilateral scapula was 0%, 0%, 5% and 20% and the number of affected litters was 0, 0, 1 and 4 at 0, 3, 10 and 30 mg/kg bw/day, respectively. The litter incidence of bent right scapula was 5%, 4.6%, 10% and 25% and the

number of affected litters was 1, 1, 2 and 5 at 0, 3, 10 and 30 mg/kg bw/day, respectively. The litter incidence and number of affected litters for the right scapula at ≥ 10 mg/kg bw/day and for the bilateral scapula at 30 mg/kg bw/day were outside the historical control range of the test laboratory (Rat Wistar, (2011 – 2015), number of foetuses/litters examined = 416, litter incidence mean: 1.08%, litter incidence range: 0% - 5.3%, number of affected litters range: 0 - 1). These findings are indicative of treatment related effects.

At 30mg/kg bw/day, there was an increase in foetal incidence, litter incidence and the number of affected litters in sternebrae variations; incomplete ossification of the first, second, third and fourth sternebrae, unossified fourth sternebrae and misaligned third sternebrae (outlined in table 12). None of these incidences were observed in the concurrent control or other treatment groups. There was a low litter incidence (10-15%) and there was no statistical significance achieved. When comparing the findings to the historical control range of the test laboratory (Rat Wistar, (2011 – 2015), number of foetuses/litters examined = 416), only the litter incidence (10%) and the number of affected litters (2) of misaligned third sternebrae were outside the historical control range of the test laboratory (litter incidence mean: 0.21%, litter incidence range: 0% - 4.6%, number of affected litters range: 0 - 1).

There was an increase in foetal incidence, litter incidence and the number of affected litters with rib anomalies such as, full right fourteenth rib and wavy ribs. The foetal incidence of full right fourteenth rib is outlined in Table 12. The litter incidence of full right fourteenth rib was 0%, 4.6%, 10% and 15% and the number of affected litters was 0, 1, 2 and 3 at 0, 3, 10 and 30 mg/kg bw/day, respectively. The litter incidence and number of affected litters were both within the historical control range of the test laboratory (Rat Wistar, (2011 – 2015), number of foetuses/litters examined = 416, litter incidence mean: 4.8%, litter incidence range: 0%-18%, number of affected litters range: 0 - 4). Although statistical significance was not achieved and the findings are within the historical control range, the DS considers that the increase in incidence with increasing concentration, albeit minor indicative of a dose-related effect.

The foetal incidence of wavy rib is outlined in Table 12. The litter incidence of wavy ribs was 45%, 32%, 60% and 80% and the number of affected litters was 9, 7, 12 and 16 at 0, 3, 10 and 30 mg/kg bw/day, respectively. At 30 mg/kg bw/day, statistical significance was achieved for the number of affected litters. At the same dose, litter incidence of wavy ribs was outside the historical control range of the test laboratory (Rat Wistar, (2011 – 2015), number of foetuses/litters examined = 416, litter incidence mean: 35%, litter incidence range: 0% - 73%, number of affected litters range: 0 - 16). This finding is indicative of a treatment effect at 30 mg/kg bw/day.

There was an increase in the foetal incidence, litter incidence and the number of affected litters in forelimb variations such as incomplete ossification of the humeri and unossified metacarpals. The foetal incidence of both parameters is outlined in Table 12.

At 30 mg/kg bw/day the litter incidence of incomplete ossification of the humeri was 10% and the number of affected litters was 4, compared to 0% and 0, respectively in the control and other treatment groups.

The litter incidence of unossified metacarpals was 10%, 36%, 20% and 60% and the number of affected litters was 2, 8, 4 and 12 at 0, 3, 10 and 30 mg/kg bw/day, respectively. At 30 mg/kg bw/day, statistical significance was achieved for the number of affected litters. Both parameters were within the historical control range of the test laboratory (Rat Wistar, (2011 – 2015), number of foetuses/litters examined = 416, litter incidence mean: 14%, litter incidence range: 0% - 63%, number of affected litters range: 0 - 15). The DS considers the statistical increase of the number of affected litters indicative of a treatment effect at 30 mg/kg bw/day.

Table 12: Foetal skeletal variations observed during the Prenatal Development Toxicity Study after repeated oral administration in Wistar Rats with IPETC. (*Anonymous 2017a*).

Variations		Dose (mg/kg bw/day)				
		0	**3**	**10**	**30**	**HCD Litter incidence**
Skull frontal (B) Incomplete ossification	Foetal incidence	0	0	5	9	% Mean=11.96
	Litter incidence (%)	0.00	0.00	20.00	35.00	% Range=0-47.37%
	Litters with at least 1 Incidence	0	0	4	**7****	Range= 0-9
Hyoid body Incomplete ossification	Foetal incidence	1	2	1	7	% Mean=2.13
	Litter incidence (%)	5.00	9.09	5.00	25.00	% Range=0-20%
	Litters with at least 1 Incidence	1	2	1	5	Range= 0-2
Skull interparietal Incomplete ossification	Foetal incidence	28	24	43	62	% Mean=64.65
	Litter incidence (%)	55.00	54.55	75.00	85.00	% Range=10-90.91%
	Litters with at least 1 Incidence	11	12	15	17	Range= 0-20
Skull parietal (B) incomplete ossification	Foetal incidence	14	13	27	31	% Mean=49.31
	Litter incidence (%)	25.00	36.36	65.00	60.00	% Range=5-87.50%
	Litters with at least 1 Incidence	5	8	**13***	12	Range= 1-15
Skull supraoccipital incomplete ossification	Foetal incidence	12	23	19	34	Unreliable
	Litter incidence (%)	35.00	59.09	55.00	80.00	
	Litters with at least 1 Incidence	7	13	11	**16****	
Skull zygomatic arch (R) incomplete ossification	Foetal incidence	1	0	1	9	% Mean=1.42
	Litter incidence (%)	5.00	0.00	5.00	35.00	% Range=0-12.50%
	Litters with at least 1 Incidence	1	0	1	**7***	Range= 0-1
Scapula (and spine) (B) bent	Foetal incidence	0	1	1	2	no data
	Litter incidence (%)	0.00	4.55	5.00	10.00	
	Litters with at least 1 Incidence	0	1	1	2	
Scapula (and spine) (R) bent	Foetal incidence	0	0	0	5	no data
	Litter incidence (%)	0.00	0.00	0.00	15.00	
	Litters with at least 1 Incidence	0	0	0	3	
Scapula (B) bent	Foetal incidence	0	0	1	4	% Mean=1.08
	Litter incidence (%)	0.00	0.00	5.00	20.00	% Range=0-5.26%

Variations		Dose (mg/kg bw/day)				
		0	**3**	**10**	**30**	**HCD Litter incidence**
	Litters with at least 1 Incidence	0	0	1	4	Range= 0-1
Scapula (R) bent	Foetal incidence	1	1	2	6	% Mean=1.08
	Litter incidence (%)	5.00	4.55	10.00	25.00	% Range=0-5.26%
	Litters with at least 1 Incidence	1	1	2	5	Range= 0-1
Scapula (B) incomplete ossification	Foetal incidence	0	0	0	2	no data
	Litter incidence (%)	0.00	0.00	0.00	10.00	
	Litters with at least 1 Incidence	0	0	0	2	
Sternebrae (1st) incomplete ossification	Foetal incidence	0	0	0	2	% Mean=6.90
	Litter incidence (%)	0.00	0.00	0.00	10.00	% Range=0-87.50%
	Litters with at least 1 Incidence	0	0	0	2	Range= 0-7
Sternebrae (2nd) incomplete ossification	Foetal incidence	0	0	0	4	% Mean=19.06
	Litter incidence (%)	0.00	0.00	0.00	15.00	% Range=0-65.22%
	Litters with at least 1 Incidence	0	0	0	3	Range= 0-15
Sternebrae (3rd) incomplete ossification	Foetal incidence	0	0	0	4	% Mean=5.38
	Litter incidence (%)	0.00	0.00	0.00	15.00	% Range=0-40.00%
	Litters with at least 1 Incidence	0	0	0	3	Range= 0-3
Sternebrae (4th) incomplete ossification	Foetal incidence	0	0	0	3	% Mean=10.53
	Litter incidence (%)	0.00	0.00	0.00	10.00	% Range=0-58.33%
	Litters with at least 1 Incidence	0	0	0	2	Range= 0-14
Sternebrae (4th) unossified	Foetal incidence	0	0	0	2	% Mean=1.33
	Litter incidence (%)	0.00	0.00	0.00	10.00	% Range=0-11.11%
	Litters with at least 1 Incidence	0	0	0	2	Range= 0-2
Sternebrae (3rd) misaligned	Foetal incidence	0	0	0	2	% Mean=0.21
	Litter incidence (%)	0.00	0.00	0.00	10.00	% Range=0-4.55%
	Litters with at least 1 Incidence	0	0	0	2	Range= 0-1
Rib (14th) (R) Full	Foetal incidence	0	1	2	3	% Mean=4.80
	Litter incidence (%)	0.00	4.55	10.00	15.00	% Range=0-18.18%

Variations		Dose (mg/kg bw/day)				
		0	**3**	**10**	**30**	**HCD Litter incidence**
	Litters with at least 1 Incidence	0	1	2	3	Range= 0-4
Ribs wavy	Foetal incidence	18	13	23	62	% Mean=34.86
	Litter incidence (%)	45.00	31.82	60.00	80.00	% Range=0-72.73%
	Litters with at least 1 Incidence	9	7	12	**16***	Range= 0-16
Forelimb humeri incomplete ossification	Foetal incidence	0	0	0	7	No data
	Litter incidence (%)	0.00	0.00	0.00	10.00	
	Litters with at least 1 Incidence	0	0	0	4	
Forelimb metacarpal(s) unossified	Foetal incidence	2	14	7	21	% Mean=13.80
	Litter incidence (%)	10.00	36.36	20.00	60.00	% Range=0-62.50%
	Litters with at least 1 Incidence	2	8	4	**12***	Range= 0-15

p<0.05; **p<0.01;p<0.001;** N= 20, 22, 20 and 20 at 0, 3, 10 and 30 mg/kg bw/day, respectively. HCD; Rat Wistar, Study Date Range: 2011 – 2015, Number of litters in historical control group=392. (B)= bilateral, (L)=left and (R)= right.

Craniofacial findings

There was an increase in foetal incidence, litter incidence and the number of affected litters of dilated third ventricle at 30 mg/kg bw/day. The foetal incidence are outlined in Table 13. The litter incidence was 9.5%, 8.3%, 8.3% and 23.8% and the number of affected litters was 2, 2, 2 and 5 at 0, 3, 10 and 30 mg/kg bw/day, respectively. Both parameters were within the historical control range of the test laboratory (Rat Wistar, (2011 – 2015), number of foetuses/litters examined = 416, litter incidence mean: 24%, litter incidence range: 0% - 86%, number of affected litters range: 0 - 18).

There was an increase in foetal incidence, litter incidence and a statistically significant increase in the number of affected litters of small pituitary gland at 10 mg/kg bw/day. The foetal incidence are outlined in Table 13. The litter incidence was 0%, 8.3%, 33.3% and 4.8% and the number of affected litters was 0, 2, 8 and 1 at 0, 3, 10 and 30 mg/kg bw/day, respectively. Both parameters were outside the historical control range of the test laboratory (Rat Wistar, (2011 – 2015), number of foetuses/litters examined = 416, litter incidence mean: 1%, litter incidence range: 0% - 13%, number of affected litters range: 0 - 3). The biological significance of this finding is uncertain.

Table 13: Foetal craniofacial findings observed during the Prenatal Development Toxicity Study after repeated oral administration in Wistar Rats with IPETC. (*Anonymous 2017a*).

Craniofacial findings		Dose (mg/kg bw/day)				
		0	**3**	**10**	**30**	**HCD Litter incidence**
Ventricle (3rd) dilated	Foetal incidence	2	2	4	6	% Mean=28.80 % Range=0-85.71% Range= 0-18
	Litter incidence (%)	9.52	8.33	8.33	23.81	
	Litters with at least 1 Incidence	2	2	2	5	
Pituitary gland small	Foetal incidence	0	2	8	1	% Mean=0.96 % Range=0-13.04% Range= 0-3
	Litter incidence (%)	0.00	8.33	33.33	4.76	
	Litters with at least 1 Incidence	0	2	**8****	1	

p<0.05; **p<0.01;p<0.001;** N= 21, 24, 24 and 21 at 0, 3, 10 and 30 mg/kg bw/day, respectively. HCD; Rat Wistar, Study Date Range; 2011 – 2015, Number of litters in historical control group=392. (B)= bilateral, (L)=left and (R)= right.

3.10.2 Human data

No information available

3.10.3 Other data

No information available

3.11 Specific target organ toxicity – single exposure

Not evaluated as part of this dossier.

3.12 Specific target organ toxicity – repeated exposure

Not evaluated as part of this dossier.

3.13 Aspiration hazard

Not evaluated as part of this dossier.

3.14 Endocrine Disruption for Human Health

Not evaluated as part of this dossier.

4 ENVIRONMENTAL HAZARDS

4.1 Degradation

4.1.1 Ready biodegradability (screening studies)

Study reference:

Anonymous (2012b), Biodegradability of Danafloat™ 262 (IPETC) in the Closed Bottle Test (OECD 301D) (Unpublished report).

Detailed study summary and results:

Test type

OECD Test Guideline Ready Biodegradability Closed Bottle Test D (OECD 301D). No deviations reported and the test is GLP compliant.

Test substance

The product Danafloat™ 262 (IPETC) was used in this study, the product is considered to be equivalent to the substance *O*-isopropyl ethylthiocarbamate identified in the CLH dossier. No information on impurities provided.

Materials and methods

The inoculum used in this 28-day study was secondary effluent collected from a municipal wastewater treatment plant in Usserød, Denmark, which predominantly receives domestic sewage. The medium was aerated to an initial oxygen concentration of approximately 9 mg O_2 per litre and inoculated with 0.5 mL secondary effluent per litre. The test mixtures were dispensed to 300 ml biochemical oxygen demand (BOD) bottles. The bottles were completely filled, closed with glass stoppers and incubated in the dark. The test was conducted at a temperature of 20.0 ± 0.3 °C. The pH at the start of the test was 7.3 - 7.5 and was 7.1 - 7.5 at the end of the test. The inoculated medium was divided into four parts for the test mixtures, test mixtures employed in this study included 15 flasks containing test medium alone (inoculum control), 15 flasks containing test medium with test product, 2.2 mg/L (test suspension), 15 flasks containing test medium with reference substance, 2.0 mg/L (activity control), and 15 flasks containing test medium with test product and reference substance (toxicity control), 2.2 mg/L and 2.0 mg/L, respectively.

An aqueous stock solution (110.4 mg/L) of the test product was prepared in Milli-Q water and then added to one part of the test medium to achieve a nominal concentration of approx. 2.2 mg/L.

At the start of the test and on days 7, 14, 21 and 28, three bottles of each test mixture were harvested and the oxygen concentration in these bottles was measured by use of an oxygen electrode. The oxygen electrode was placed in the BOD bottle immediately after the stopper had been removed. The test mixture was gently stirred, and the oxygen concentration recorded when the value was stable. The theoretical oxygen demand (ThOD) of Danafloat™ 262 (IPETC) was quantified based on a measurement of the chemical oxygen demand (COD).

At the start of the test, the pH was measured to be 7.3 - 7.5 and therefore no adjustment was necessary.

The reference substance was added to another part of the inoculated medium to a final concentration of 2.0 mg/L corresponding to a ThOD of 3.3 mg O_2/L.

As a toxicity control, the test product and reference substance were added to the inoculated medium at the same test concentrations as when tested individually, 2.2 mg/L and 2.0 mg/L, respectively.

Results:

The oxygen concentration measurements in the test mixtures during the 28-day test period are presented in the table below.

Table 14: oxygen measurements.

Time (days)	Inoculum controls (mg O_2/L)			Test product "Danafloat™ 262 (IPETC)" (mg O_2/L)			Activity control (sodium benzoate) (mg O_2/L)			Toxicity controls "Danafloat™ 262 (IPETC)" + sodium benzoate (mg O_2/L)		
	1	2	3	1	2	3	1	2	3	1	2	3
0	9.09	9.05	9.13	9.19	9.16	9.25	9.13	9.19	9.06	9.23	9.22	9.12
7	9.07	9.10	9.06	9.03	9.06	9.07	6.95	6.89	6.97	6.84	7.02	6.85
14	8.85	8.82	8.91	8.89	8.90	8.83	6.76	6.41	6.25	6.44	6.45	6.19
21	8.82	8.90	8.71	8.62	8.75	8.59	6.34	6.43	6.06	6.24	6.40	6.06
28	8.56	8.51	8.52	8.51	8.57	8.59	6.30	6.05	6.22	6.12	6.06	5.99

An overview of the 28 day biodegradability of the test product, the reference substance (sodium benzoate) and the toxicity control are presented in the table below.

The degradation of the test product was expressed as the percentage BOD of the ThOD. The biodegradability of the test product and the reference substance was calculated by the following formula:

$$\%\ degraded = \frac{BOD\ (test - inoculum\ control)}{ThOD} \text{ X } 100$$

where BOD and ThOD are expressed in mg O_2/L.

Table 15: Biodegradability of test product, the reference substance, and the toxicity control (average of triplicate values).

Time (days)	Test product "Danafloat™ 262 (IPETC)" (% of ThOD)	Activity control (sodium benzoate) (% of ThOD)	Toxicity control "Danafloat™ 262 (IPETC)" + sodium benzoate (% of ThOD)
	Average	Average	Average
7	3.3	65.2	31.0
14	2.4	72.6	35.4
21	6.7	76.9	36.5
28	2.1	71.2	35.1

Very low concentrations of nitrite and nitrate - nitrogen (NO_x-N) were detected at the beginning and end of the test, this indicates that the nitrification level had no significant effect on oxygen consumption and therefore correction of the measured BOD for oxygen consumption due to nitrification was not necessary.

The validity criteria as per the OECD test guideline were fulfilled:

- The total oxygen consumption in the inoculum control was 0.56 mg O_2/L after 28 days, which is below the threshold of 1.5 mg O_2/L and the oxygen concentration in the test bottles was not at any time below 0.5 mg O_2/L.
- The difference between the extremes of replicate values of the biodegradability of the test product at the end of the test was 2%, which is less than the maximum difference between replicates of 20% for valid tests.
- The BOD of the reference substance reached 72.6% of ThOD after 14 days, which is higher than the required 60% after 14 days.

- The test product was not inhibitory to the inoculum. Biodegradation was 35.4% of the total ThOD in the toxicity controls, which is higher than the required 25% after 14 days.

On the basis of this result the test substance cannot be considered as readily biodegradable as biodegradation was less than 60% of ThOD for the duration of this 28-day test.

4.1.2 BOD_5/COD

No data available

4.1.3 Aquatic simulation tests

No data available

4.1.4 Other degradability studies

No data available

4.2 Bioaccumulation

No data available

4.3 Acute toxicity

4.3.1 Short-term toxicity to fish

Study reference:

Anonymous (2013a): Acute Toxicity of Danafloat™ 262 (IPETC) to zebra fish *(Danio rerio)* under static renewal conditions. (Unpublished report).

Detailed study summary and results:

Test type

This test was conducted according to test guideline OECD No. 203 (Fish Acute Toxicity Test) in a static renewal 96-hour test. The study is GLP compliant, and no deviations were reported.

Test substance

• The product Danafloat™ 262 (IPETC) was used in this study, the product is considered equivalent to the substance *O*-isopropyl ethylthiocarbamate as identified in the CLH dossier.
• Information on impurities not reported

Materials and methods

The test was performed with zebra fish commercially purchased in Denmark. The fish were of the length specified in the OECD guideline. The test fish were randomly chosen from the stock population and transferred from the stock population to the test aquaria within 30 minutes, making up 10 fish per concentration. The test product was tested at the following nominal concentrations: 0 (control), 7.5, 15, 30, 60 and 120 mg/L.

Freshly produced synthetic medium was used in the test. The medium was prepared from Millipore water according to the ISO 7346. Two stock solutions of 120 mg/L were prepared by weighing out 240 mg of the test

product and dissolving it in 2 L of zebra fish test medium. The test solutions were prepared by diluting the stock solution in the zebra fish test medium. A fresh stock solution was prepared for the renewal of the test solutions after 48 hours.

The test was performed as a static renewal test, i.e., all test media were renewed after 48 hours. The test was carried out at 23.4 ± 0.2 °C in a climate room with normal laboratory light having a daily light/dark period of 14:10 hours. The acute test was run in 4 L glass aquaria, each containing 2 L of test solution.

Temperature, pH and dissolved oxygen were measured daily, before and after renewal of test medium and at the start and at the end of the test. Room temperature was recorded continuously by a thermologger.

To verify the tested concentrations, samples were collected at test start, before and after renewal of the test solutions and at the end of the test for chemical analysis of the test product. Duplicate samples of 10 mL were collected in 20 mL plastic vials for later analysis. The samples were stored at -20 ± 2.0 °C until the end of the study. The samples taken were anonymized with unique sample numbers. Although duplicate samples were collected from each of the test concentrations, only one of these samples was analysed. The other samples were stored as spare samples and are discarded after sponsor's approval of the report. Furthermore, only samples relevant for the calculation of the LC_{50} values were analysed. The chemical analyses were not performed in compliance with the OECD GLP principles.

A 48-hour test with the reference substance potassium dichromate ($K_2Cr_2O_7$) was performed in order to verify the sensitivity of the test organisms. The reference test was performed at the following concentrations: 0 (control), 50, 100, 200, 300 and 400 mg/L. Ten fish were exposed to each concentration.

Mortality was recorded after 2, 24, 48, 72 and 96 hours. Records were kept of visible abnormalities, i.e., loss of equilibrium, changes in swimming behaviour, respiratory function and pigmentation, these effects were recorded daily.

Results:

The data on the chemical analysis of a sample of the test concentrations are presented in the table below.

Table 16: **Results of the chemical analysis of subsamples of the test solution.**

Sampling time	Nominal "Danafloat™ 262 (IPETC)" concentration [mg /L]	Estimated nominal IPETC concentration [mg /L]	Measured IPETC concentration [mg/L]	Measured IPETC concentration in % of the estimated nominal concentration [%]
0 hours	30	28.7	27.6	96.1
48 hours (old)	30	28.7	20.7	72.1
48 hours (new)	30	28.7	26.7	93.0
96 hours	30	28.7	23.3	81.2
0 hours	60	57.4	55.1	96.0
48 hours (old)	60	57.4	48.4	84.3
48 hours (new)	60	57.4	54.9	95.6
96 hours	60	57.4	48.5	84.5
0 hours	120	114.8	108.7	94.7

The chemical analysis of *O*-isopropyl ethylthiocarbamate in samples collected during the test confirmed that the measured concentrations of the test substance were 72 - 96% of the estimated concentrations. As, in

average, the measured *O*-isopropyl ethylthiocarbamate concentrations in percentage of the estimated nominal concentrations were 89% and thus within ± 20% of the estimated nominal *O*-isopropyl ethylthiocarbamate concentrations, the statistical calculation was made based on the estimated nominal concentrations.

Based on the mortality observed during the study, the effect concentrations lethal to 50% (LC_{50}) of the test organisms at each of the recommended observation times (24h, 48h, 72h and 96h) were estimated. An overview of the estimated effect concentrations is presented in the table below, 95% confidence intervals could not be calculated.

Table 17: Effect concentrations obtained in acute toxicity tests with the test product "Danafloat™ 262 (IPETC)", the active ingredient *O*-isopropyl ethylthiocarbamate and $K_2Cr_2O_7$ (48 hours).

Product	Nominal concentration (mg/L)			
	LC50 (24 h)	LC50 (48h)	LC50 (72 h)	LC50 (96h)
"Danafloat™ 262 (IPETC)"	73	73	73	73
IPETC	70	70	70	70
$K_2Cr_2O_7$	-	189	-	-

The validation criteria of the test according to the OECD test guideline were fulfilled:

- The mortality in the controls did not exceed 10% at the end of the test period.
- The dissolved oxygen concentration was ≥ 60% throughout the test period.

The average fish loading of 0.69 g/L met the recommendations in the OECD test guideline and the sensitivity of the fish was verified by testing the mortality of the reference substance ($K_2Cr_2O_7$). The LC_{50} was determined to be 189 mg/L, which is below the normal range found at DHI (200 - 400 mg/L). This result indicates a slightly higher sensitivity than normally observed.

The study summary reported the 96-hour LC_{50} for *O*-isopropyl ethylthiocarbamate as 70 mg/L, confidence intervals could not be determined.

4.3.2 Short-term toxicity to aquatic invertebrates

Study reference:
Anonymous (2013b): Acute toxicity of Danafloat™ 262 (IPETC) in *Daphnia magna.* (Unpublished report).

Detailed study summary and results:

Test type:
This semi-static 48-hour test was conducted according to test guideline OECD No. 202.

Test substance
• The product Danafloat™ 262 (IPETC) was used in this study, the product is considered equivalent to the substance *O*-isopropyl ethylthiocarbamate as identified in the CLH dossier.
• Information on impurities not reported.

Materials and methods

The test was carried out in 50 mL glass beakers with 25 mL of test solution. The test was carried out as a 48-hour semi-static test, i.e., all test solutions were renewed after 24 hours. The number of immobile animals was recorded after 24 hours and 48 hours.

Danafloat™ 262 (IPETC) was tested at the following nominal concentrations of the product: 0 (control), 2, 5, 10, 20, 50 and 100 mg/L. To verify the actual test concentrations, samples of the test solutions were collected for chemical analysis. The chemical analysis of the test substance showed a measured *O*-isopropyl ethylthiocarbamate concentration of 80 - 95% of the estimated nominal concentration of *O*-isopropyl ethylthiocarbamate. As the tested concentrations were maintained within ± 20% of the nominal concentrations, the statistical calculation was made using the nominal concentrations.

A stock solution of 100 mg/L was prepared by weighing out 100 mg of the test product and dissolving it in 1 L of test medium. The pH in the stock solutions were 7.9 (t = 0h) and 8.0 (t = 24h) and was therefore not adjusted. The test solutions were prepared by diluting the appropriate amounts of stock solution in test medium. The test product was tested at the following nominal concentrations: 0 (control), 2, 5[, 10, 20, 50 and 100 mg/L.

A strain of *Daphnia magna* Straus (*Cladocera, Crustacea*), which has been cultured at DHI since 1979 was used in this test. The culture is fed with *Pseudokirchneriella subcapitata* three times a day by a peristaltic pump system. Young animals (less than 24 hours old) from this culture were exposed to a dilution series of the test product. Freshly produced ISO-medium was used in the test.

The test was run as a semi-static test, twenty animals were exposed at each concentration. Five animals less than 24 hours old were transferred to each of 4 test beakers per concentration by use of a nylon net. The control group consisted of 30 animals, 6 beakers each containing 5 animals. After 24 hours of exposure, all test solutions were renewed. The number of immobile animals was recorded after 24 hours and after 48 hours. On this basis, the effect concentrations (EC) were determined.

Dissolved oxygen and pH were measured at 0 hours, at 24 hours before and after renewal of test solutions and at 48 hours. The test was run in a climate room at 20.1 ± 0.2 °C, in total darkness. The temperature was recorded continuously during the test by means of a thermologger.

In accordance with the test guideline, a 24-hour acute toxicity test on the reference substance potassium dichromate ($K_2Cr_2O_7$) was performed to check the sensitivity of the test animals. The reference substance was tested at the following concentrations: 0.2, 0.4, 0.7, 1.0, 1.4 and 2.0 mg $K_2Cr_2O_7$/L.

Results:

Results of the chemical analyses of samples covering 0-100% effect are presented in the table below.

Table 18: Results from chemical analyses of IPETC. Recovery is calculated in percent of the estimated nominal concentration of *O*-isopropyl ethylthiocarbamate.

Sampling time	Nominal "Danafloat™ 262 (IPETC)" concentration [mg /L]	Estimated nominal IPETC concentration [mg /L]	Measured IPETC concentration [mg/L]	Measured IPETC concentration in % of the estimated nominal concentration [%]
0 hours	10	9.6	8.2	85
24 hours (old)	10	9.6	8.6	90
24 hours (new)	10	9.6	8.5	89
48 hours	10	9.6	8.2	85
0 hours	20	19.1	16.7	87
24 hours (old)	20	19.1	15.2	80
24 hours (new)	20	19.1	16.6	87
48 hours	20	19.1	16.4	86
0 hours	50	47.9	45	94
24 hours (old)	50	47.9	40.7	85
24 hours (new)	50	47.9	43.7	91
48 hours	50	47.9	44.4	93
0 hours	100	95.7	88.5	93
24 hours (old)	100	95.7	84.8	89
24 hours (new)	100	95.7	91.3	95
48 hours	100	95.7	84	88

The chemical analysis of the test substance showed a measured *O*-isopropyl ethylthiocarbamate concentration of 80-95% of the estimated nominal concentration. As the tested concentrations were maintained within ± 20% of the nominal concentrations, the statistical calculation was made using nominal concentrations.

The EC_{10} and EC_{50} values for the test product, IPETC and for $K_2Cr_2O_7$ (24 h only) are given in the table below.

Table 19: Effect concentrations obtained in *Daphnia magna* acute immobilization test of "Danafloat™ 262 (IPETC)" expressed as the concentrations of "Danafloat™ 262" and *O*-isopropyl ethylthiocarbamate, respectively.

Product	Concentration (mg/L)			
	EC10 (24 h)	EC50 (24h)	EC10 (48 h)	EC50 (48h)
"Danafloat™ 262 "	75 (• - 90)	91(• - 99)	30 (18-40)	63 (54-76)
IPETC	72 (• - 86)	87(• - 95)	29 (17-38)	60 (52-73)
$K_2Cr_2O_7$	1.1 (0.90-1.3)	1.6 (1.4-1.7)	-	-

Values in brackets are 95% confidence intervals; • = no confidence interval could be calculated.

The validity criteria as specified in the methods are considered fulfilled:

- Less than 10% of the control animals were immobilized.
- The dissolved oxygen saturation at the end of the test was 98% in all tested concentrations and the control.
- The 24h EC_{50} value of the reference substance potassium dichromate was 1.6 (1.4 - 1.7) mg/L, which is within the range specified in ISO 6341 (0.6 - 2.1 mg/L).

- Chemical analysis of the test substance conducted showed a measured *O*-isopropyl ethylthiocarbamate concentration of 80-95% of the estimated nominal concentration in the test solutions. As the tested concentrations were maintained within ± 20% of the nominal concentrations, the statistical calculation was made based on the nominal concentrations.

The study summary reported the 48-hour EC_{50} for *O*-isopropyl ethylthiocarbamate as 60 mg/L, with 95% confidence intervals of 52 – 73 mg/L.

4.3.3 Algal growth inhibition tests

Study reference:
Anonymous (2013c), Algal growth inhibition test with "Danafloat™ 262 (IPETC)". (Unpublished report).

Detailed study summary and results:

Test type
The product Danafloat™ 262 (IPETC) was tested for inhibitory effects on the growth of the algae *Pseudokirchneriella subcapitata* according to OECD 201. The test was GLP compliant, and no deviations were reported.

Test substance
• The product Danafloat™ 262 (IPETC) was used in this study, the product is considered to be equivalent to the substance *O*-isopropyl ethylthiocarbamate identified in the CLH dossier.
• Information on impurities not reported.

Materials and methods
The toxicity of the test product (Danafloat^{TM}262 (IPETC)) was determined as the growth inhibition of the freshwater algae *Pseudokirchneriella subcapitata* (clone: NIVA, CHL 1). The algae were cultured at DHI under test conditions prior to the start of the test.

"Danafloat™ 262 (IPETC)" was tested at the following nominal concentrations: 0 (control), 1.0, 2.0, 5.0, 10, 20, 50 and 100 mg/L.

At the start of the test the cell density was approx. 8,300 cells/mL. The test was performed in 250 mL wide neck glass flasks, each containing 100 mL of test solution consisting of algal growth medium, algae and test product. The test design consisted of triplicate test flasks of each concentration, six controls (flasks with algae but no test product) and a blank of each concentration (flasks with test product but no algae). Furthermore, one flask of the control and one flask of each test concentration without algae were used to collect samples for chemical analysis.

The algae were incubated for approximately 72 hours under continuous shaking at 23.1 ± 0.1 °C and constant illumination from a panel of fluorescent light with an intensity of approx. 60-120 µmol x m^{-2} x sec^{-1}.

At the start of the test the pH was measured to be 7.9 - 8.0 and 7.9 - 8.8 at termination.

The algal biomass is expressed as the *in-vivo* fluorescence. For the measuring range used in the test, the correlation between cell counts, using a Beckman Coulter Multisizer, and *in-vivo* fluorescence, using a Turner TD-700 Laboratory Fluorometer, is validated twice a year. The identity and normal appearance of *Pseudokirchneriella*

subcapitata in the control was confirmed at the end of the test by microscopy. At the beginning of the test and at 24, 48 and 72 ± 2 hours of incubation, the algal growth was measured in the triplicate test flasks, the blanks and the six controls.

To verify the tested concentrations, duplicate samples of each test concentration were taken at the initiation of the test, every 24 hours and at the termination of the test.

Results

The primary data on the chemical analysis from the test are presented in the table below.

Table 20: Results of the chemical analyses of IPETC from the test solutions without algae.

Sampling time (hours)	Nominal "Danafloat™ 262 (IPETC)" concentration (mg /L)	Estimated nominal IPETC concentration (mg /L)	Measured IPETC concentration (mg/L)	Measured IPETC concentration in % of the estimated concentration (%)	Average measured IPETC concentration in % of the estimated concentration (%)
0	Control	0	0*	-	-
72	Control	0	0*	-	
0	1.0	0.96	0.7*	-	-
24	1.0	0.96	0.1*	-	
48	1.0	0.96	0.0*	-	
72	1.0	0.96	0.7*	-	
0	2.0	1.91	1.5*	-	-
24	2.0	1.91	2.0*	-	
48	2.0	1.91	1.0*	-	
72	2.0	1.91	0.8*	-	
0	5.0	4.79	4.9	102	88
24	5.0	4.79	4.0	83.6	
48	5.0	4.79	3.7	77.3	
72	5.0	4.79	4.3	89.9	
0	10	9.57	8.3	86.7	91
24	10	9.57	8.7	90.9	
48	10	9.57	9.0	94.0	
72	10	9.57	8.8	92.0	
0	20	19.1	17.4	90.9	92
24	20	19.1	18.0	94.0	
48	20	19.1	17.2	89.9	
72	20	19.1	17.5	91.4	
0	50	47.9	46.1	96.3	95
24	50	47.9	45.4	94.9	
48	50	47.9	45.1	94.3	
72	50	47.9	44.5	93.0	
0	100	95.7	91.9	96.0	96
24	100	95.7	91.2	95.3	
48	100	95.7	91.6	95.7	
72	100	95.7	90.8	94.9	

* = According to the analytical test report from Cheminova A/S, non-valid results close to the limit of detection.

According to the analytical test report, the chemical analysis of the test substance on test solutions with less than 2 mg/L *O*-isopropyl ethylthiocarbamate cannot be considered valid.

The results from the test substance analysis of the nominal test concentrations 5.0 - 100 mg/L showed a measured *O*-isopropyl ethylthiocarbamate concentration of 88 - 96% of the estimated nominal concentration of *O*-isopropyl ethylthiocarbamate. As the test concentrations 5.0 - 100 mg/L were maintained within ± 20%

of the nominal concentrations, the same thing was assumed to apply for test concentrations below 5.0 mg/L, and the statistical calculation was thus based on the nominal concentrations.
The effect on the yield of the algae was calculated in addition to the effect on growth rate as the effect on yield may be needed to fulfil specific regulatory requirement in some countries. The algal biomass is expressed as the *in-vivo* fluorescence.

The NOEC and EC_x values for the test product are presented below and calculated as the concentrations of "Danafloat™ 262" and *O*-isopropyl ethylthiocarbamate, respectively. The calculated values for the reference substance, $K_2Cr_2O_7$, are given in Table 8.3. Figure 8.1 shows the growth inhibition of *Pseudokirchneriella subcapitata* at the various nominal test concentrations of "Danafloat™ 262 (IPETC)".

Table 21: Effect concentrations obtained in the algal growth inhibition test with "Danafloat™ 262 (IPETC)" expressed as the concentration of "Danafloat™ 262" and *O*-isopropyl ethylthiocarbamate, respectively.

Response variables	Product	Nominal concentration (mg/L)			
Growth rate		**NOEC**	E_rC_{10}	E_rC_{20}	**E_rC_{50}**
	"Danafloat™ 262"	1.0	1.5 (1.2-1.8)	3.7 (3.2-4.2)	21.7 (20.2-23.3)
	IPETC	1.0	1.4 (1.2-1.7)	3.6 (3.1-4.0)	20.7 (19.3-22.3)
Yield		**NOEC**	E_yC_{10}	E_yC_{20}	**E_yC_{50}**
	"Danafloat™ 262"	1.0	<1.0	0.97 (0.74-1.2)	3.4 (2.9-3.8)
	IPETC	1.0	<1.0	0.93 (0.71-1.2)	3.2 (2.8-3.7)

Values in parentheses are 95% confidence intervals.

Table 22: Effect concentrations obtained in the algal growth inhibition test with potassium dichromate.

Response variable	Concentration (mg/L)		
Growth rate	NOEC	EC_{10}	EC_{50}
	0.4	0.61 (0.58-0.63)	1.09 (1.06-1.11)

Values in parentheses are 95% confidence intervals.

Growth and growth inhibition were calculated for each test concentration relative to the control. The concentrations inhibiting the growth at 10%, 20% and 50% were calculated for two different response variables, the growth rate (E_rC_{10}, E_rC_{20} and E_rC_{50}) and the yield (E_yC_{10}, E_yC_{20} and E_yC_{50}) by use of the computer program TOXEDO. The yield is defined as the biomass at the end of the exposure period minus the biomass at the start of the exposure period. The NOEC value was determined by use of the computer program Dunnett's procedure as the highest tested concentration, at which no significant inhibition was observed on growth rate or yield compared with the control.

The validation criteria of the test according to the OECD guideline were fulfilled. The control growth rate exceeded 1.4 per day (growth rate: 0.064 per hour ~ 1.5 per day), the variation coefficient of the control growth rates did not exceed 5% (variation coefficient: 4.9%). The control pH did not increase more than 1.5 during

the test and the mean value of the variation coefficient of the growth rates in the control replicates, calculated in sections of 24 hours during the 72-hour test period, did not exceed 35% (mean variation coefficient: 14.0%). The sensitivity of the algae was verified by testing the inhibitory effect of the reference substance potassium dichromate. The EC_{50} value of 1.09 (1.06-1.11) mg/L showed that the sensitivity of the algae was not significantly different from the results obtained in an international ring test in 1981 (EC_{50} = 1.19 (0.65-1.73) mg/L).

Exposure of the algae to the test substance resulted in a 70.25-hour *O*-isopropyl ethylthiocarbamate E_rC_{50} of 20.7 mg/L, with a 95% confidence interval of 19.3 to 22.3 mg/L, and a 70.25-hour *O*-isopropyl ethylthiocarbamate E_yC_{50} of 3.2 mg/L, with a 95% confidence interval of 2.9 to 3.8 mg/L.

4.3.4 *Lemna* sp. growth inhibition test

No data available.

4.4 Chronic toxicity

No data available.

4.4.1 Fish short-term toxicity test on embryo and sac-fry stages

No data available.

4.4.2 Aquatic Toxicity – Fish, juvenile growth test

No data available.

4.4.3 Chronic toxicity to aquatic invertebrates

No data available.

4.4.4 Chronic toxicity to algae or aquatic plants

See section 4.3.3 for full study details.

Exposure of the algae to the test substance resulted in a 70.25-hour NOEC for *O*-isopropyl ethylthiocarbamate of 1 mg/L and an E_rC_{10} of 1.4 mg/L (95% C.I. of 1.2 – 1.7 mg/L).

4.5 Acute and/or chronic toxicity to other aquatic organisms

No data available.

4.6 ENDOCRINE DISRUPTION FOR THE ENVIRONMENT

No data available.

5 PERSISTENT, BIOACCUMULATIVE AND TOXIC (PBT) OR VERY PERSISTENT, VERY BIOACCUMULATIVE (VPVB) PROPERTIES UNDER CLP ANNEX I, 4.3

Not evaluated as part of this dossier.

6 PERSISTENT, MOBILE AND TOXIC (PMT) OR VERY PERSISTENT, VERY MOBILE (vPvM) PROPERTIES UNDER CLP ANNEX I, 4.4

Not evaluated as part of this dossier.

7 ADDITIONAL HAZARDS: HAZARDOUS TO THE OZONE LAYER

Not evaluated as part of this dossier.