DATA EVALUATION RECORD
LGC-35523 (Ethaboxam metabolite)
PC Code: 090205
TXR#: 0056241
MRID#: 48535691

Study Type: 28-Day Oral Toxicity - Rat;
OPPTS 870.3050

Prepared for

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DATA EVALUATION RECORD

STUDY TYPE: 28-Day Oral Toxicity (dietary) - Rat;
OPPTS 870.3050 [§OPP-none] (rodent); OECD 408.

PC CODE: 090205 **DP BARCODE:** D399440

TEST MATERIAL (PURITY): LGC-35523 (92.6%)

SYNONYMS: N-(cyano-thiophen-2-yl-methyl)-oxalamic acid

CITATION: Coleman, D. (2003) LGC-35523; Toxicity study by dietary administration to CD rats for 4 weeks. Huntingdon Life Sciences (Cambridgeshire, England). Laboratory project ID: LKF 122/033024, August 14, 2003. MRID 48535691. Unpublished.

SPONSOR: Valent U.S.A Corporation.

EXECUTIVE SUMMARY:

In a 28-day oral toxicity study (MRID 48535691) ethaboxam metabolite, LGC-35523 (92.6% a.i., Batch #PO30325) was administered to Crl:CD (SD)IGS BR rats (10/sex/dose) in the diet at dose levels of 0, 650, 2000, or 13000 ppm (equivalent to 0, 56.5, 170.9, or1104.1 mg/kg bw/day in males; 0, 56.5, 70.7, or 1155.8 mg/kg bw/day in females).

There were no treatment-related effects on mortality, clinical signs, organ weights, or gross pathology. Histopathology was not evaluated. A statistically significant decrease in mean body weight gain was seen in males in the 13000 ppm group; overall mean food efficiency in this group was reduced 12% (not statistically evaluated). Both sexes in the 13000 ppm group had a significantly increased blood albumin level. Males in the 13000 ppm group had a significantly decreased mean corpuscular volume (MCV); in the absence of other hematological changes, the decreased MCV count in males is unlikely to be treatment-related. Minor hematology and blood chemistry changes were recorded for animals receiving 13000 ppm. Females in the 13000 ppm group showed increases in mean total white blood cell, lymphocyte, monocyte, basophil, and large unstained cell counts, and in blood cholesterol levels. However, the changes in hematology were considered minor. Significant increases in mean motor activity (low and/or high beam) scores were observed at one or more time point in all male treatment groups; mean total low beam scores were significantly increased for all treatment groups. In the absence of a clear dose-response relationship, the findings for motor activity were not considered to be treatment-related.

The NOAEL is 13000 ppm (equivalent to 1104 mg/kg bw/day for males, 1156 mg/kg bw/day

for females) and the LOAEL is not determined.

This 28-day oral toxicity study in the rat is **acceptable/nonguideline** and is appropriate for range-finding purposes.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS:

A. MATERIALS:

1. Test material: LGC-35523 (Ethaboxam metabolite)

Description:	Brown powder
Batch #:	PO30325
Purity:	92.6% a.i.
Compound stability:	Expiration date not provided; stored at 4°C in the dark
CAS # of TGAI:	Not available
Structure:	

2. Vehicle: The basal diet was the vehicle.

3. Test animals:

Species:	Rat	
Strain:	Crl:CD® (SD)IGS BR	
Age/weight at study initiation:	46-50 days; 202.0-263.8 grams (males), 152.5-212.2 grams (females) at initiation of treatment	
Source:	Charles River (UK) Ltd. (Kent, England)	
Housing:	5 of one sex/cage; stainless steel cage with stainless steel mesh lid and floor suspended above absorbent paper	
Diet:	Rat and Mouse No. 1 Maintenance Diet (Special Diets Services Ltd., Witham, Essex, England), *ad libitum* except when urine was being collected and overnight before routine blood sampling	
Water:	Potable water from public supply via polycarbonate bottles with sipper tubes, *ad libitum* except when urine was being collected	
Environmental conditions:	**Temperature:**	20-24°C
	Humidity:	36-62%
	Air changes:	not provided
	Photoperiod:	12 hrs dark/ hrs light
Acclimation period:	13 days	

B. STUDY DESIGN:

1. **In life dates:** Start: May 6, 2003 (initiation of treatment); End: June 4, 2003 (necropsy completed)

2. **Animal assignment:** Upon removal from the transit boxes, animals were placed one at a time in study cages using the sequence of cages in the battery, until each cage held an appropriate number of animals. Animals were assigned to the test groups noted in Table 1.

TABLE 1. Study design [a]

Test group	Conc. in diet (ppm)	Dose to animal (mg/kg bw/day)	# Males	# Females
Control	0	0 (males) 0 (females)	10	10
Low	650	56.5 (males) 56.5 (females	10	10
Mid	2000	170.9 (males) 170.7 females)	10	10
High	13000	1104.1 (males) 1155.8 (females)	10	10

[a] Data obtained from page 13 (unnumbered table) and page 44 (Table 6) in the study report.

3. **Dose selection rationale:** The dose levels were selected based on previous work with the parent compound (i.e., Ethaboxam) (Huntingdon Life Sciences Report No. LKY 051/962291) and a preliminary 4-week study with LGC-35523 (Huntingdon Life Sciences Report No.LKF 119/030063). In the study with LGC-35523, rats were administered the test substance at dose levels of 650, 2000, or 6500 ppm because LGC-30473 (Ethaboxam) caused marked effects on body weight gain in rats administered 3000 or 5000 ppm for 4 weeks, while 650 or 2000 ppm were well-tolerated. However, LGC-35523 produced no clear effect on body weight gain at 6500 ppm, and other effects observed at this level had a questionable relationship to treatment. The high dose in the current study was selected in order to produce a clear effect on body weight gain. The low and mid dose levels were those used in the preliminary study.

4. **Diet preparation and analysis:** Diets were prepared every 3-4 days until stability was confirmed, and then weekly thereafter (Weeks 2-4). Appropriate amounts of the test substance were mixed with Rat and Mouse No. 1 Maintenance Diet to form two premixes. One premix was used to prepare the low-dose formulation, the other the mid- and high-dose formulations. The premixes were added to basal diet to prepare the final formulations. The storage temperature was not specified. Homogeneity and stability were tested prior to the start of animal treatment on 200 ppm and 20000 ppm formulations. These concentrations bracketed the concentrations used in the current study. Homogeneity was assessed by analyzing samples collected from the top, middle, and bottom of the same formulations. Stability was assessed on samples stored at room temperature (21 °C) for up to 22 days (Day 4, 8, 15, or 22). Samples from the dietary formulations prepared during Weeks 1 and 3 were analyzed for concentration confirmation.

Results:

Homogeneity analysis: Concentrations ranged from 189-213 ppm (mean 204 ppm) for the 200 ppm formulation and from 19200-20400 ppm (mean 19800 ppm) for the 20000 ppm formulation. The mean concentrations were within 2% of the nominal concentration. Precision, measured by the coefficient of variation, was 4.32% and 2.21 % for the 200 and 20000 ppm formulations, respectively.

Stability analysis: Mean (duplicate analyses) concentrations of the 200 ppm formulation ranged from 182-194 ppm during up to 22 days of storage at ambient temperature. The mean

concentrations ranged from –3.0% to –9.1% of the nominal concentration, and from –3.2% to +3.2% of the pre-storage (Time 0) concentration. Mean (duplicate analyses) concentrations of the 20000 ppm formulation ranged from 19000-19700 ppm. The mean concentrations ranged from –1.5% to –5.0% of the nominal concentration, and from 0.0% to -3.6% of the pre-storage (Time 0) concentration.

Concentration analysis: The mean concentrations of the samples collected from the 650, 2000, and 13000 ppm diets during Week 1 were 636, 1910, and 12600 ppm, respectively (-2.2% to –4.5 % of the nominal concentration). The mean concentrations of the samples collected during Week 3 were 652, 2050, and 13000 ppm, respectively (0.0 to +2.5% of the nominal concentration). The test substance was not detected in samples from the untreated basal diet (below the limit of detection of 15 ppm).

The analytical data indicated that the mixing procedure was adequate and that the variance between nominal and actual dosage to the animals was acceptable.

5. **Statistics:** For continuous data, Bartlett's test was first applied to test for homogeneity of variance between the groups. Treated groups were then compared with the control group, depending on the results of Bartlett's test.

The following sequence of statistical tests was used for grip strength, motor activity, body weight gain, organ weight, and clinical pathology data:

If 75% of the data across all groups were the same, a frequency analysis was applied. Treatment groups were compared using a Mantel test for a trend of proportions. Pairwise Fisher's Exact tests were used to compare each dose group with the control, as applicable.

If Bartlett's test was not significant at the 1% level, parametric analysis was applied. If the F1 test for monotonicity of dose-response was not significant at the 1% level, Williams' test for a monotonic trend was performed. If the F1 test was significant, Dunnett's test was used. If Bartlett's test was significant at the 1% level, logarithmic and square-root transformations were tried. If Bartlett's test was still significant, non-parametric tests were applied. If the H1 test for monotonicity of dose-response was not significant at the 1% level, Shirley's test for a monotonic trend was performed. If the H1 test was significant, Steel's test was performed.

For organ weight data, analysis of covariance was performed using terminal body weight as the covariate when the within group relationship between organ weight and body weight was significant at the 10% level.

The Reviewer considers the analyses used to be appropriate.

Mean body weight, food consumption, and food efficiency data were not analyzed for statistical significance.

C. METHODS:

1. Observations:

1a. Cageside observations: Animals were inspected at least twice daily for signs of poor health or reaction to treatment.

1b. Clinical examinations: A detailed physical examination and arena observations were performed on all animals before initiation of treatment and weekly during the treatment period. Particular attention was paid to possible signs of neurotoxicity (e.g., convulsions, tremors, abnormal gait or behavior).

1c. Neurological evaluations: The following evaluations (measurements) were performed during Week 4 of treatment; all animals were not necessarily tested on the same day, but the numbers of animals and time of testing were balanced across the groups.

- Sensory reactivity: approach response (blunt probe), touch response (blunt probe on nape of neck), auditory startle response (sudden loud noise), tail pinch response (forceps)
- Grip strength: forelimb and hindlimb strength using Mecmesin Portable Force Indicators
- Motor activity using a Rodent Activity Monitoring System (infrared high and low beam breaks)

2. **Body weight:** Animals were weighed one week before initiation of treatment, on the day of treatment initiation (Week 0), weekly throughout treatment, and before necropsy.

3. **Food consumption and compound intake:** Food consumption for each cage was determined for the week before the initiation of treatment and weekly during the treatment period. Mean weekly diet consumption was calculated as g food/animal/week. Food efficiency [body weight gain (g)/food consumption (g/animal) x 100] values were calculated weekly for each cage; overall group mean values were calculated as overall group mean body weight gain, divided by total food consumption, expressed as a percentage. Compound intake (mg/kg bw/day) values were calculated as time-weighted averages from the nominal test substance concentration, food consumption, and body weight data. The weekly body weight values were based on the mean of the mid-week body weights.

4. **Ophthalmoscopic examination:** The eyes of all animals were examined before the initiation of treatment using a binocular indirect ophthalmoscope. During Week 4, the eyes of animals in the control and 13000 ppm groups were similarly examined; in the absence of treatment-related findings in these animals, examinations were not performed on animals in the 650 and 2000 ppm groups.

5. **Hematology and clinical chemistry:** During Week 4, blood was collected from all animals (fasted overnight) for hematology and clinical chemistry analyses. Blood was withdrawn from the retro-orbital sinus while the animals were under light general anesthesia with isoflurane. The CHECKED (X) parameters were examined.

a. Hematology:

X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)*
X	Leukocyte count (WBC)*	X	Mean corpusc. HGB conc. (MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpusc. volume (MCV)
X	Platelet count*		Reticulocyte count
X	Blood clotting measurements*		
X	Activated partial thromboplastin time (APTT)		
	Clotting time		
X	Prothrombin time (PT)		

* Recommended for 28-day oral rodent studies based on Guideline 870.3050

b. Clinical chemistry:

X	ELECTROLYTES	X	OTHER
X	Calcium	X	Albumin*
X	Chloride	X	Creatinine*
	Magnesium	X	Urea*
X	Phosphorus	X	Total cholesterol*
X	Potassium*		Globulins [a]
X	Sodium*	X	Glucose*
X	**ENZYMES** (more than 2 hepatic enzymes e.g., *)	X	Total bilirubin
X	Alkaline phosphatase (ALK)*	X	Total protein (TP)*
	Cholinesterase (ChE)	X	Triglycerides
	Creatine phosphokinase		Serum protein electrophoresis
	Lactic acid dehydrogenase (LDH)		
X	Alanine aminotransferase (ALT/also SGPT)*		
X	Aspartate aminotransferase (AST/also SGOT)*		
	Sorbitol dehydrogenase*		
X	Gamma glutamyltransferase (GGT)*		
	Glutamate dehydrogenase		

* Recommended for 28-day oral rodent studies based on Guideline 870.3050
[a] To determine the albumin/globulin (A/G) ratio, the globulin concentration was calculated by subtracting the albumin concentration from the total protein concentration.

6. **Urinalysis*:** Overnight urine samples were collected from fasted animals during Week 4. The CHECKED (X) parameters were examined.

X	Appearance*	X	Glucose*
X	Volume*	X	Ketones
X	Specific gravity/osmolality*	X	Bilirubin
X	pH*	X	Blood/blood cells*
X	Sediment (microscopic)		Nitrate
X	Protein		Urobilinogen

* Optional for 28-day oral rodent studies

7. **Sacrifice and pathology**: All animals were sacrificed on schedule by carbon dioxide asphyxiation and subjected to a detailed gross pathological examination. All external features and orifices were examined. The cranial roof was removed for observation of the brain, pituitary gland, and cranial nerves. A ventral mid-line incision was made and the neck with associated tissues, and the thoracic, abdominal, and pelvic cavities and their viscera were examined *in situ*. The CHECKED (X) tissues were collected and fixed in 10% neutral

buffered formalin, with the exception of the testes and epididymides, which were fixed in Bouin's solution, and the eyes, which were fixed in Davidson's fluid. The (XX) organs, in addition, were weighed. None of the tissues were examined histologically.

X	DIGESTIVE SYSTEM	X	CARDIOVASC./HEMAT.	X	NEUROLOGIC
	Tongue	X	Aorta	XX	Brain*+ (cerebellum, cerebrum, midbrain)
X	Salivary glands	XX	Heart*+	X	Peripheral nerve* (sciatic)
X	Esophagus		Bone marrow*	X	Spinal cord*
X	Stomach*	X	Lymph nodes*	X	Pituitary*
X	Small intestine (Peyers patch)	XX	Spleen*+	X	Eyes (optic nerve)*
X	Large intestine	XX	Thymus*+	X	**GLANDULAR**
XX	Liver*+			XX	Adrenal gland*+
	Gall bladder (not rat)*	X	**UROGENITAL**	X	Lacrimal gland
	Bile duct (rat)	XX	Kidneys*+	XX	Parathyroid*[a]
X	Pancreas*	X	Urinary bladder*	XX	Thyroid*
X	Cecum	XX	Testes*+	X	**OTHER**
X	Rectum	XX	Epididymides*+	X	Bone (sternum and femur)
		X	Prostate*	X	Skeletal muscle (thigh)
		X	Seminal vesicles*	X	Skin*
X	**RESPIRATORY**	XX	Ovaries*	X	All gross lesions and masses*
X	Trachea*	XX	Uterus*(with cervix)		
X	Lung*	X	Mammary gland		
X	Nose				
X	Pharynx				
X	Larynx				

* Recommended for 28-day oral rodent studies based on Guideline 870.3050
\+ Organ weights required for rodent studies.
[a] weighed with thyroid

II. RESULTS:

A. OBSERVATIONS:

1. **Clinical signs of toxicity:** There were no treatment-related clinical findings. A low incidence (1-2 animals) of hair loss was observed in all test groups, including the control. Other findings (vocalization, lack of grooming) occurred in 1-2 animals, with no dose-response trend.

2. **Mortality:** There were no unscheduled mortalities.

3. **Neurological evaluations:** There were no apparent treatment-related effects on sensory reactivity, grip strength, or motor activity. There were no statistically significant differences in sensory reactivity or grip strength parameters between the treatment groups and the control. The mean high and low beam scores for all male treatment groups were higher than the controls for most of the 1-hour recording period. The mean high beam scores were statistically significant for the 650 ppm group at the 18 minute time point and for the 2000 and 13000 ppm groups at the 6 and 18 minute time points when compared with the control. The mean low beam scores for males in all treatment groups were significantly higher than the control at the 12 and 18 minute time points. The mean total low beam scores for all

treatment groups also were significantly greater than the control, but there was no dose-response trend. In the absence of clear dose-response trends in males, the findings for the beam break scores were not considered treatment-related. For females, the mean high and low beam scores were not statistically different from the control for any treatment group at any time period. Selected motor activity data are summarized in Table 2.

TABLE 2. Motor activity (mean beam breaks) during Week 4 of treatment [a]

Conc. of LGC-35523 in diet (ppm)	Beam Level	No. of beam breaks (mean±SD)			
		Time (minutes)			Total (60 minutes)
		6	12	18	
Males (n=10)					
0 (control)	High	125±36.9	64.1±38.5	25.7±19.1	329.1±102.0
	Low	280.1±85.8	133.6±59.1	100.2±65.9	912.1±363.7
650	High	136.3±31.9	87.3±12.2	74.1*±32.5	470.3±164.8
	Low	301.9±53.7	212.7**±36.0	163.4*±56.1	1232.7*±256.9
2000	High	157.5*±30.9	86.0±32.2	57.5*±29.6	449.8±240.9
	Low	351.6±32.6	236.1**±45.8	168.7*±65.7	1322.4*±440.5
13000	High	154.5*±22.1	83.7±11.1	55.0**±30.9	482.7±165.6
	Low	316.3±44.6	205.0**±25.7	141.8*±39.1	1201.0*±303.7
Females (n=10)					
0 (control)	High	167.0±24.1	73.9±25.4	53.8±29.4	481.4±150.3
	Low	331.8±59.4	214.1±40.3	156.0±58.9	1289.0±286.1
650	High	161.1±20.0	95.0±37.9	46.7±37.8	500.9±178.5
	Low	336.9±39.1	229.6±56.4	127.2±94.0	1301.7±458.7
2000	High	141.3±39.5	78.9±42.0	62.7±39.6	490.5±195.7
	Low	303.9±64.9	159.0±63.4	139.4±58.1	1159.7±345.7
13000	High	150.8±33.9	92.8±27.9	58.2±38.5	482.0±161.8
	Low	310.5±61.2	172.8±61.3	137.3±69.7	1175.2±263.4

[a] Data obtained from pages 39-40 (Table 2b) in the study report.
* Statistically different (p <0.05) from the control.
** Statistically different (p <0.01) from the control.

B. <u>BODY WEIGHT AND WEIGHT GAIN</u>: Body weight was statistically evaluated as mean body weight gain; mean body weights were not statistically compared with the control. The mean total body weight gain for males in the 13000 ppm group was statistically significantly less than the control (reduction of 14%). Mean body weight gains for females in the 2000 and 13000 ppm groups were less than the control group (reduction of 16% and 10%, respectively), but the differences were not statistically significant. Mean absolute body weights were comparable to controls for all treatment groups. Body weight and body weight gain data for the treatment period are summarized in Table 3.

TABLE 3. Average body weights and body weight gains during 28 days of treatment [a]

Conc. of LGC-35523 (ppm)	Mean body weight (g±SD [b])					Total body weight gain	
	Week 0	Week 1	Week 2	Week 3	Week 4	g/animal (mean±SD)	% of control
Males (n=10)							
0 (control)	231.8±11.12	281.5±12.71	327.0±15.54	364.1±19.51	378.8±22.55	146.9±15.49	-
650	237.5±13.01	293.4±16.70	342.7±22.41	384.1±28,42	399.4±30.05	161.8±19.89	110
2000	242.8±11.47	292.3±14.84	345.9±22.17	383.5±27.31	397.9±28.21	155.1±21.62	106
13000	234.5±17.44	269.8±19.79	315.6±23.10	345.2±27.18	360.1±29.86	125.6*±15.55	86
Females (n=10)							
0 (control)	178.2±12.85	197.0±13.29	218.9±13.54	235.7±12.31	234.4±11.94	56.2±6.17	-
650	182.4±14.13	203.1±13.61	225.7±14.73	239.5±13.55	239.2±18.60	56.8±8.43	101
2000	179.7±11.65	202.4±10.66	220.2±13.98	226.9±11.26	227.1±15.98	47.4±7.96	84

Conc. of LGC-35523 (ppm)	Mean body weight (g±SD [b])					Total body weight gain	
	Week 0	Week 1	Week 2	Week 3	Week 4	g/animal (mean±SD)	% of control
13000	180.4±13.44	193.6±14.38	212.2±14.92	229.4±17.19	231.0±15.91	50.6±9.40	90

[a] Data obtained from page 41 (Table 3) in the study report.
[b] The standard deviations were calculated by the Reviewer from data on pages 87-90 (Appendix 3) in the study report.
* Statistically different (p <0.05) from the control.

C. FOOD CONSUMPTION AND COMPOUND INTAKE:

1. **Food consumption:** Food consumption data were not statistically analyzed. Males in the 13000 ppm group showed a decrease in mean food consumption during Week 1 compared with the control (approx. 11%); mean food consumption during the following weeks was comparable to the control. There were no other differences in mean food consumption that appeared to be potentially treatment-related.

2. **Compound consumption:** The time-weighted average test substance consumption values are summarized above in Table 1.

3. **Food efficiency:** Mean food conversion efficiency during the 4-week treatment period was reduced in females in the 2000 ppm group (13%) and in both sexes in the 13000 ppm group (reduction of 12% for males, 9% for females) when compared with the respective controls. These differences are consistent with the reduced body weight gains seen in these groups.

D. OPHTHALMOSCOPIC EXAMINATION: The ophthalmological examinations performed during Week 4 revealed no apparent findings related to test substance treatment.

E. BLOOD ANALYSES:

1. **Hematology:** A statistically significant increase in the mean total white blood cell count (37%), with associated significant increases in mean lymphocyte (43%), basophil (100%), monocyte (73%), and large unstained cell counts (67%), was found in females in the 13000 ppm group. However, the changes in hematology were considered minor. A small, but statistically significant decrease (3%) in mean corpuscular volume (MCV) was observed in males in the 13000 ppm group. Because the decrease in MCV was small and there were no other significant differences in hematological parameters for the male 13000 ppm group, including the number of erythrocytes, that would suggest a treatment-related effect on red blood cell production, it is unlikely that the MCV effect seen in this group was treatment-related. Data for selected hematological parameters are summarized in Table 4.

TABLE 4. Selected hematological parameters (mean±SD) during Week 4 of treatment [a]

Parameter (mean±SD)	Concentration of LGC-35523 in diet (ppm)			
	0 (control)	650	2000	13000
Males (n=10)				
MCV (fL)	58.7±1.65	58.0±1.67	58.9±1.56	57.1*±0.63
WBC count (x 10^9/L)	11.26±1.688	11.04±1.484	11.76±2.713	12.25±3.281
Lymphocytes (x 10^9/L)	9.08±1.662	8.87±1.267	9.74±2.420	9.72±2.522
Basophils (x 10^9/L)	0.04±0.013	0.03±0.010	0.04±0.016	0.05±0.019
Monocytes (x 10^9/L)	0.26±0.052	0.28±0.095	0.29±0.118	0.28±0.106
Large unstained cells (x 10^9/L)	0.24±0.061	0.27±0.104	0.24±0.085	0.25±0.110

Parameter (mean±SD)	Concentration of LGC-35523 in diet (ppm)			
	0 (control)	650	2000	13000
Females (n=10)				
MCV (fL)	57.3±1.09	56.0±0.83	56.7±0.98	56.7±1.19
WBC (total) (x 10^9/L)	8.94±1.791	9.24±2.992	9.40±3.001	12.29**±2.628
Lymphocytes (x 10^9/L)	7.31±1.398	8.00±2.855	8.29±2.652	10.48**±2.348
Basophils (x 10^9/L)	0.02±0.008	0.03±0.020	0.03±0.014	0.04*±0.018
Monocytes (x 10^9/L)	0.15±0.044	0.11±0.027	0.15±0.061	0.26**±0.071
Large unstained cells (x 10^9/L)	0.12±0.053	0.15±0.058	0.14±0.058	0.20**±0.071

[a] Data obtained from pages 45-48 (Table 7) in the study report.
* Statistically different (p <0.05) from the control.
** Statistically different (p <0.01) from the control.

2. **Clinical chemistry:** The mean albumin concentration for both sexes in the 13000 ppm group was statistically significantly higher than the control. Males in all treatment groups and females in the 13000 ppm group had a significantly higher mean albumin/globulin (A/G) ratio; a clear dose-response relationship was not seen in males. Males in the 13000 ppm group also had a significantly higher mean bilirubin level, and females in the 13000 ppm group had a significantly higher mean cholesterol level. In the absence of a dose-response relationship, the increase in the bilirubin level in males was not considered to be treatment-related. Females in the 650 ppm group had a significantly lower mean calcium level; in the absence of a dose-response, this finding was not considered treatment-related. Selected clinical chemistry (blood) parameter values are summarized in Table 5.

TABLE 5. Selected clinical chemistry parameters (mean±SD) during Week 4 of treatment [a]

Parameter (mean±SD)	Concentration of LGC-35523 in diet (ppm)			
	0 (control)	650	2000	13000
Males (n)				
Bilirubin (µmol/L)	1±0.5 (9)	2±0.5 (10)	1±0.5 (10)	2**±0.3 (9)
Cholesterol (mmol/L)	1.84±0.272 (10)	1.98±0.292 (10)	1.95±0.383 (10)	1.94±0.265 (10)
Calcium (mmol/L)	2.67±0.042 (10)	2.65±0.075 (10)	2.74±0.105 (10)	2.71±0.075 (10)
Albumin (g/L)	34±0.9 (9)	34±1.3 (10)	35±1.0 (10)	36**±1.2 (9)
A/G ratio	1.09±0.110 (9)	1.19*±0.081 (10)	1.18*±0.073 (10)	1.20**±0.055 (9)
Females (n)				
Bilirubin (µmol/L)	2±0.5 (10)	2±0.5 (10)	2±0.9 (10)	2±0.7 (10)
Cholesterol (mmol/L)	1.96±0.164 (10)	2.10±0.506 (10)	2.18±0.273 (10)	2.42**±0.329 (10)
Calcium (mmol/L)	2.81±0.085 (10)	2.71*±0.057 (10)	2.79±0.058 (10)	2.83±0.082 (10)
Albumin (g/L)	38±1.6 (10)	38±1.4 (10)	40±1.3 (10)	41*±2.5 (10)
A/G ratio	1.21±0.057 (10)	1.22±0.079 (10)	1.24±0.084 (10)	1.27**±0.068 (10)

[a] Data obtained from pages 49-54 (Table 8) in the study report.
* Statistically different (p <0.05) from the control.
** Statistically different (p <0.01) from the control.

F. **URINALYSIS:** There were no apparent effects of test substance treatment on urinalysis parameters. The mean pH of the urine for females in the 650 ppm group (7.0±0.25, p <0.01) was significantly higher than that of the control group (6.6±0.31); the mean pH for the 13000 ppm group (6.4±0.19, p <0.05) was significantly lower. In the absence of a dose-response trend, these findings were not considered treatment-related.

G. **SACRIFICE AND PATHOLOGY:**

1. **Organ weight:** There were no apparent effects of test substance treatment on organ weights. There were no statistically significant differences between the organ weights of the treatment

groups and those of the controls. Although statistically significant increases in mean white blood cell counts were found in females in the 13000 ppm group, there were no statistically significant increases in the weight of lymphoid organs (thymus, spleen). The mean spleen weight for females in the 13000 ppm group was increased approx. 10% when compared with the control; the mean thymus weight was decreased approx. 10%, with no dose-response trend seen for either organ.

2. **Gross pathology:** There were no apparent effects of test substance treatment on macroscopic findings. Findings were limited to 1-2 animals in a group and/or did not show a dose-response trend.

3. **Microscopic pathology:** Histopathological evaluations were not performed.

III. DISCUSSION AND CONCLUSIONS:

A. INVESTIGATORS' CONCLUSIONS: Treatment-related findings were observed at 13000 ppm.

In males, high and low beam motor activity scores were generally higher than the control values throughout the recording period. Statistical analysis showed inter-group differences at some early time points and with some total scores. However, comparison of the data with historical control data (*Note: historical control data were not provided in the report*) indicated that the control scores in the current study tended to be low or below previously recorded ranges. In the absence of a clear dose-response relationship or similar findings in females in any of the parameters examined (clinical signs, detailed physical examination, arena observations, sensory reactivity, or grip strength), the differences are not considered to be treatment-related.

Lower body weight gain observed for females in the 200 ppm (-16%) and 13000 ppm (-10%) groups was not considered adverse because there was no dose-response trend, the differences did not achieve statistical significance, and the range of individual body weights was similar for all groups. The overall body weight gain for males in the 13000 ppm group (-14%) achieved statistical significance; food consumption for this group was lower during the first week of dosing. Minor hematology and blood chemistry changes were recorded for animals receiving 13000 ppm. Although there was a dubious reduction in weight gain at 2000 ppm for females, in the absence of hematological and blood chemistry changes, it is considered that 2000 ppm (171 mg/kg/day for both sexes) represents the NOAEL in this study.

B. REVIEWER COMMENTS:

A statistically significant decrease in mean body weight gain (14%) was seen in males in the 13000 ppm group compared with the control. Reduced mean body weight gains for females in the 2000 and 13000 ppm groups (16% and 10%, respectively) were not statistically significant. However, mean absolute body weights were comparable to controls for all treatment groups. Therefore, the decreases in body weight gains were not considered adverse. Overall mean food efficiency was reduced 12% for males in the 13000 ppm group, and 13% and 9% for females in the 2000 and 13000 ppm groups, respectively (not statistically analyzed). Motor activity mean total low beam scores, and low beam scores at the 12 and 18

minute time points were significantly increased for males in all treatment groups. Mean high beam scores were significantly increased for all male treatment groups at the 18 minute time point and for the 2000 and 13000 ppm groups at the 6 minute time point. Because the findings for the mean beam scores in males did not show a clear dose-response relationship, they are not considered to be treatment-related. Although the report states that the beam scores for the controls, when compared with historical control data, were low or below the historical range, the historical control data were not provided in the report. These data should be submitted to strengthen the conclusion that the differences in motor activity were not treatment-related.

A statistically significant increase in the mean total white blood cell count (37%), with associated significant increases in mean lymphocyte (43%), basophil (100%), monocyte (73%), and large unstained cell (67%) counts, was found in females in the 13000 ppm group. However, these changes in hematology were considered minor. There was no evidence of treatment-related macropathology in the lymphoid organs (spleen, thymus, lymph nodes) or statistically significant increases in the weight of lymphoid organs (thymus, spleen). The mean spleen weight for females in the 13000 ppm group was increased approx. 10% when compared with the control; the mean thymus weight was decreased approx. 10%, with no dose-response trend seen for either organ. A statistically significant decrease (3%) in mean corpuscular volume observed in males in the 13000 ppm group, in the absence of other significant differences in hematological parameters, is not likely treatment-related. Both sexes in the 13000 ppm group had statistically significant higher mean blood albumin concentrations compared with the control. Males in all treatment groups and females in the 13000 ppm group had significantly higher mean A/G ratios. Females in the 13000 ppm group had a significantly higher mean cholesterol level. Females in the 650 ppm group had a significantly lower blood calcium level and males in the 13000 ppm group had a significantly higher bilirubin level; in the absence of a consistent dose-response relationship, these findings are not considered treatment-related.

Histopathological examinations were not performed. OPPTS 870.3050 guidelines state that, minimally, full histopathology should be performed on the preserved organs and tissues of the control and high dose groups.

The NOAEL is 13000 ppm (equivalent to 1104 mg/kg bw/day for males, 1156 mg/kg bw/day for females) and the LOAEL is not determined.

This 28-day oral toxicity study in the rat is **acceptable/nonguideline** and is appropriate for range-finding purposes.

C. STUDY DEFICIENCIES: