



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

005732

MEMORANDUM

FEB 27 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Review of Toxicity Studies for Isoxaben/
1471-EUP-TN for Wheat and Barley

TO: Richard Mountfort
PM 23
Registration Division (TS-767)

FROM: Margaret Jones *Margaret Jones 2/19/87*
Review Section III
Toxicology Branch (TS-769)

THROUGH: Marcia Van Gemert, Ph.D., Head
Review Section III
Toxicology Branch (TS-769) *Marcia Van Gemert 2/12/87*

and Theodore M. Farber, Ph.D., Chief
Toxicology Branch (TS-769) *Theodore M. Farber 2/19/87*

Chemical: Isoxaben (EL-107) Caswell No.: 419F

Record Numbers: 144029, 144031, 155426, 154868, 184264, 184267

Accession Numbers: 073292-073299, 073441, 265729, 265731-265740

Petition Number: 1471-EUP-TN, 5G3216

Action Requested: Review toxicity data for the proposed EUP
for use of Isoxaben on wheat and barley for broadleaf weeds.
Discuss company response to EPA review of mutagenicity studies.

Conclusion: Data Evaluation Reports are attached for each of
the studies in the data package. These are briefly summarized
below. A decision on the EUP for this chemical will be made
following consideration of all the data ("weight of evidence
approach") by the Toxicology Branch Peer Review committee.
Before the committee can consider Isoxaben the petitioner will
need to provide contemporaneous historical control data from
the laboratory performing the chronic mouse and rat studies.
The Peer Review committee will look at the findings which
appear positive. For example, in the rat study the committee
will look at the kidney (progressive glomerulonephrosis), the
liver (hepatocellular adenomas), and the adrenal cortex
(pheochromocytomas). In the mouse, the committee will look at
the liver (hepatocellular adenomas and carcinomas and other
hepatocellular pathology).

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Data required for the EUP which are outstanding:

1. Historical control data as indicated above. This information will be necessary to present to the Toxicology Branch Peer Review Committee as it considers all the data in making a decision on EL-107.
2. Acute oral LD₅₀ for the technical chemical. No information on this testing requirement appears in Toxicology Branch files.
3. Concerning the category of mutagenicity testing, the Agency has adequate studies to fulfill the categories of gene mutation and other genotoxic effects. However, the category of structural chromosome aberration testing needs to be satisfied. The dominant lethal study in the rat is provisionally acceptable. Positive control data will be necessary to remove this qualification.

The sister chromatid exchange study using bone marrow of Chinese hamsters was reviewed and found to be unacceptable. The petitioner has submitted additional information and the points in question are now adequately explained. However, there is now a question whether cytotoxicity has been adequately demonstrated. There is apparently no evidence that the compound reached the bone marrow in sufficient concentration to produce cytotoxicity.

As soon as one of the above (or both) questions are satisfied the category of structural chromosome aberration will be fulfilled.

Summary of Data Reviewed for EL-107 Technical:

1. Acute Dermal LD₅₀: >2000 mg/kg bodyweight
Toxicity Category III
2. Subchronic Mouse/ 3 months with one month reversibility phase:

Dose levels tested by diet: 0, 10, 100, 1400, 12500 ppm
Males-dose related increases in liver hypertrophy; males and females- increased liver weight at 1400 and 12500 ppm; males and females- liver microsomal enzyme induction at 1400 and 12500 ppm NOEL: 100 ppm (14-17 mg/kg/day), LEL: 1400 ppm (200-249 mg/kg/day)

Core grade: Minimum

3. Subchronic Dog/ 3 months

Dose levels tested by capsules: 0, 25, 110, 500 mg/kg/day
Males- increases in liver and liver/bodyweight at 500 mg/kg/day;
Females- slight increase in liver/bodyweight at 500 mg/kg/day
NOEL: 110 mg/kg/day, LEL: 500 mg/kg/day

Core grade: Minimum

4. Subchronic Rat/ 3 months with one month reversibility phase:

Dose levels tested by diet: 0, 0.05, 0.14, 0.42, 1.25% (0, 500, 1400, 4200, and 12500 ppm)

At 3 months:

Females: increased liver weight and liver/bodyweight ratio at all doses, Males: increased liver/bodyweight at 0.42 and 1.25%. Elevated liver microsomal enzyme activity at 0.42 in males and at 0.14, 0.42, and 1.25%.

At 4 months:

Liver weights were normal, except for males at 1.25%, enzyme activity was normal.

Core grade: Guideline

5. Subchronic liver toxicity in Mice/ 3 months

Dose levels tested by diet: 0, 100, 1000, 12500 ppm
Males and females- elevated liver weights at 1000 ppm and elevated liver microsomal enzyme activity at 12500 ppm
NOEL: 100 ppm (14-17 mg/kg/day), LEL: 1000 ppm (139-169 mg/kg/day)

Core grade: Supplementary (not a guideline requirement)

6. Chronic/oncogenicity study in Rats/ 2 years

Dose levels tested by diet: 0, 125, 1250, 12500 ppm
Males and females- body weight and body weight gains were decreased at the high dose; clinical chemistry parameters associated with kidney and liver toxicity (BUN, creatinine, alkaline phosphatase) were affected at the mid and high doses; Males and females- liver/bodyweight, kidneys/bodyweight, heart/bodyweight, brain/bodyweight were increased at the high dose; males- prostate/brain weight decreased at the high dose
NOEL: 125 ppm (5-6 mg/kg/day); LEL: 1250 ppm (51-62 mg/kg/day)

Core grade: Minimum

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7. Chronic/oncogenicity in Mice/ 24 months:

Dose levels tested by diet: 0, 100, 1000, 12500 ppm
Males and females: increase in hepatocellular adenomas and in combined hepatocellular adenomas and carcinomas at the high dose; males and females- increases in hepatocytomegaly and hepatocellular hyperplasia at the high dose and increases in incidence and severity of hepatocellular vacuolation at the mid and high doses; males and females- increase in liver and liver/bodyweight at the high dose;
NOEL: 100 ppm (12 mg/kg/day); LEL: 1000 ppm (114-124 mg/kg/day)

Core grade: Minimum

8. Three-generation Reproduction in Rats:

Dose levels tested by diet: 0, 500, 2500, 12500 ppm
Parental NOEL: 500 ppm
Parental LEL: 2500 ppm
Lowered bodyweight and bodyweight gain, lowered efficiency of food utilization during growth, gestational and lactational periods of parental females at 12500 ppm, decreased bodyweight of parental males during growth period at 12500 ppm, increased relative liver weight of parental males and females at 12500 ppm and parental females at 2500 ppm
Reproductive NOEL: 2500 ppm
Reproductive LEL: 12500 ppm
Reduced numbers of viable pups in F_{2a} and F_{2b}, lowered bodyweight in progeny on postpartum day 21, and increased microphthalmia at 12500 ppm
Embryo/fetotoxicity NOEL: 2500 ppm
Embryo/fetotoxicity LEL: 12500 ppm
Decreases in viable fetuses/litter, increases in resorptions and postimplantation losses, increases in hydroureter at 12500 ppm for both generations; increases in microphthalmia at 12500 ppm

Core grade: Minimum

9. Developmental Toxicity/ Rat

Dose levels tested by gavage on gestational days 6-15:
0, 100, 320, 1000 mg/kg/day
Maternal toxicity NOEL: 320 mg/kg/day
Maternal toxicity LEL: 1000 mg/kg/day
Decreased bodyweight gain during dosing
Embryo/fetotoxicity NOEL: 320 mg/kg/day
Embryo/fetotoxicity LEL: 1000 mg/kg/day
Increased preimplantation loss, increased resorptions, smaller litter size, increases in runt fetuses

Core grade: Minimum

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10. Developmental toxicity/ Rabbit

Dose levels tested by gavage on gestational days 6-13:
0, 100, 320, 1000 mg/kg/day
Maternal toxicity NOEL: >1000 mg/kg/day
Embryo/fetotoxicity NOEL: >1000 mg/kg/day

Core grade: Minimum

11. Mutagenicity studies.

- a. Chromosome aberrations in vivo (dominant lethal in rats)
Dose levels tested by diet: 0, 500, 2500, 12500 ppm
No dominant lethality at dietary concentrations up to
12500 ppm (932 mg/kg/day)

Provisionally Acceptable: Positive Control data are
necessary

- b. Reverse mutation in Salmonella Typhimurium using the
Ames test
Negative induction of revertants in 5 histidine
auxotrophic tester strains of S. Typhimurium

Acceptable

- c. Chromosome aberrations in vivo (mouse micronucleus)

Results inconclusive - presumptively positive in males;
Elevated percent polychromatic erythrocytes (PCE) over
controls (5.54 v. 0.10) when tested at 5000 mg/kg

Inconclusive:

- 1) Equal numbers of females (and males) should be
tested
- 2) Cytotoxicity should be demonstrated with an MTD
(greater than 5000 mg/kg and less than 10000 mg/kg)
- 3) Sample bone marrow starting at 12 hours post-dosing,
and at 24, 36, and 48 hours.

12. Percutaneous absorption/ Rhesus monkeys

Dose levels tested: 2 mg/kg intravenously and topically
Eleven (11) percent of the topical dose was absorbed
through the skin. Topical absorption was about 7.5 percent
of the intravenous dose (administered one month prior to
topical administration)

Acceptable

13. Metabolic Studies

a. Rat Study

Single oral doses of 250 mg/kg were almost entirely excreted by 48 hours. After 72 hours 90 percent of the original dose was recovered in the feces. Majority of fecal recovery portion was unmetabolized EL-107. About 20 percent of the dose was absorbed and one half excreted as metabolites in urine and one half as metabolites in feces.

Acceptable

b. Excretion pattern in Mice

Single doses administered by gavage in polyethylene glycol: 1, 15, 100, 200 mg/kg in 10 ml/kg bodyweight. More than 89% of the radioactivity was excreted in the first day. As doses increased, proportionately less of the compound was excreted in urine and more in the feces (as percent dose administered).

Core: Supplementary; this was a range finding study only

c. Excretion in expired air/ Rat

Doses tested: single oral dose of 250 mg/kg. Radioactivity recovered at 48 hours was 85% for males and 92.6% for females (of the administered dose). Expired air accounted for 2.4% for males and 2.8% for females (of the administered dose). Thus expired air accounts for a small fraction of the excretion route of the test substance and males do not show complete recovery of radioactivity at 48 hours. There is the possibility of residual radioactivity in the carcass and therefore some concern for bioaccumulation.

Core: Acceptable

d. Distribution in Rats

Doses administered by gavage: 4 hour groups (5/sex) received 1.0 ml and 24 hour groups (5/sex) received 1.1 ml/100 gm. bodyweight which is roughly equivalent to 1000 mg/kg as the final dose. The majority of radioactivity remains in the intestinal tract. Tissue to plasma ratios indicate some accumulation in tissues at levels above plasma levels.

Supplementary: The observations should have been carried out beyond 24 hours since significant amounts of radioactivity were found at 24 hours and Section F

Guidelines specify elimination of 90% of radioactivity or 7 days of observation, whichever arrives first.

- e. Repeated dose distribution/ Rat
Dose administered by gavage: 250 mg/kg for 14 days (cold treatment) then one single oral dose of 250 mg/kg radiolabeled EL-107. Predosing animals did not alter the pattern of excretion of EL-107. Males excreted 80.59% in 7 days and females excreted 96.1% in a similar period (combined urinary and fecal elimination).

Acceptable

- f. Absorption and disappearance in plasma of Mice

Doses administered by gavage: 1, 15, 100, 200 mg/kg in 10 ml/kg polyethylene glycol.
Plasma half-life was 8.3-8.9 hours with the majority of radioactivity eliminated from the plasma by 72 hours.
Absorption from the gut is apparently limited above doses of 100 mg/kg but elimination from plasma is not affected by increasing doses.

Supplementary- range- finding study to determine doses for mouse oncogenicity study

14. One year Dog Study

Doses tested in gelatin capsules: 0, 10, 100, and 1000 mg/kg
Mid and high dose animals showed increased alkaline phosphatase levels. Liver/brain weight was elevated in high dose males and females, and liver/bodyweight was elevated in females. High dose males showed evidence of liver microsomal enzyme induction.

NOEL: 10 mg/kg

LEL: 100 mg/kg

Core grade: Minimum

15. One year Rat Study

Doses tested in the diet: 0, 125, 1250, 12500 ppm
High dose females showed decreases in body weights, mean weight gains and efficiency of food utilization. High dose males and females and mid dose females at 6 and 12 months and mid dose males at 3 and 6 months showed increased activity of liver microsomal enzymes.

NOEL: 1250 ppm

LEL: 12500 ppm

Core grade: Supplementary; the study was designed as a preliminary chronic toxicity study in rats. The full chronic toxicity study is summarized above and the DER attached.

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16. Chronic/oncogenicity study/ Rats/ INTERIM REPORT

Doses tested in the diet: 0, 125, 1250, 12500 ppm
High dose females showed decreased weight gain at 3, 6 and 12
months. High dose females showed increases in platelet
counts at 6 and 12 months.
NOEL: 1250 ppm, LEL: 12500 ppm

Core grade: Supplementary

ATTACHMENTS

Study/Lab/Study #/Date	Material	Accession No.	Results: LD50, LC50, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
3- generation reproduction- rat; Lilly Research Lab; # R 15382, R 03783, R 14183; 12/83	EL-107 tech.	251939	Interim report- 2nd generation data Reproductive NOEL- insufficient data Systemic NOEL = 0.05 ppm Systemic LEL = 0.25 ppm (depressed body weight) Feto-toxic NOEL = 0.25 ppm Feto-toxic LEL = 1.25 ppm (exencephally and microphthalmia) Levels tested by diet- 0, 500, 2500 and 12500 ppm		Minimum 003773
21 Day dermal - rabbit; Eli Lilly Res. Lab.; no. B01783; 12/83	EL - 107 technical and formulation FN - 7033	252915	NOEL (technical EL - 107) : < 1055 mg/kg/body wt (only level tested) NOEL (formulation FN - 7033) : < 500 mg/kg/body wt (increases in thyroid weights per 100 grams of body weight. This study is not sufficient for regulatory purposes		Minimum 004034
90 Day oral - dog; Eli Lilly & Co.; #D33582; 12/82	EL-107	250791	NOEL < 0.25 g/kg (LDT)(relative increase of SAP, induction of hepatic microsomal enzymes). Levels tested = 0, 0.25, 0.5, 1.0 g/kg		Minimum 003434
90 Day feeding - rat; Eli Lilly & Co.; #R00182; 12/82	EL-107 92.4%	250792	NOEL < 1.25% EL-107 (LDT) (absolute and relative liver weight increases, relative kidney weight increases, and induction of hepatic enzymes.) Levels tested - 0, 1.25, 2.5, and 5.0% of EL - 107		Minimum 003434

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Study/Lab/Study #/Date	Material	ACCESSION No.	LD50, LC50, PIS, NOEL, LEL	Category	Doc. No.
90 Day feeding/recovery-rat; Eli Lilly & Co.; #R12582; 3/83	EL-107 (78%)	250792	Interim Report NOEL < 0.05% EL - 107 (LDT)(increased absolute and relative liver weight, induction of hepatic microsomal enzymes). Levels tested = 0, 0.05, 0.14, 0.42 1.25% of EL - 107	Supplementary	003434
Biliary excretion - rat; Eli Lilly & Co.; #R10562 & R11482; 12/81	EL-107 92.4% spiked with 14C-EL-107 100%	250791	Doses: 10 and 250 mg/kg/day (single dosing only) in F-344 strain 24 hr. excretion of bile was measured. There were indications that biliary excretion rates were related to rate-limiting gastrointestinal absorption. Sex differences were noted. Unexplained sex differences were noted.	Acceptable	003434
Excretion - rat; Eli Lilly & Co.; #R07262 & R08882; 12/81	EL-107 92.4% spiked with 14C-EL-101 100%	250791	Doses: 10, 100, 250, 500 and 1000 mg/kg (single dosing only). Collections were made of urine and feces during 1st, 2nd, and 3rd 24 hr. periods. Decreasing ratios of urinary to fecal excretion indicates that gastrointestinal absorption is rate-limiting. Most excretion in first hour. After 72 hrs., mean percent of label excreted ranged from 70.8% to 94.9% (male) and 88.2% to 106.3% (female).	Acceptable	003434
Tissue distribution - rat; Eli Lilly & Co.; #R-0-5082 & R-0-6182; 12/82	EL-107 92.4% spiked with 14C-EL-107 100%	250791	Some animals were killed after 4 hrs., some after 24 hrs. Build up in some tissues was quite large at 24 hrs. Tissue distribution was shown but distribution and possible storage could be better elucidated by a longer-term study.	Acceptable	003434

003434

Study/Lab/Study #/Date	Material	No.	LD50, LC50, PIS, MUEL, LEL	Category	Doc. no.
Dermal sensitization - guinea pig; Eli Lilly Res. Labs.; no G00183; 12/83	EL - 107 technical and 1:1 aqueous dilution of suspensor concentrate formulation (FN7033, 50% EL - 107)	252915	Not sensitizing		Minimum 004034
Primary dermal irritation - rabbit; Eli Lilly and Co.; #B-D-58-81; 9/81	EL-107	250791	no irritation from 200 mg/kg.	IV	Minimum 003434
Acute dermal LD50 - rabbit; Eli Lilly & Co.; #B-D- 58-81; 9/81	EL-107	250791	LD50 > 200 mg/kg (only dose tested)	Supplementary 003434	
Primary eye irritation - rabbit; Eli Lilly & Co.; #B-E-61-81; 9/81	EL-107	250791	Corneal dullness, mild iritis, and slight conjunctivitis. All treated eyes normal within 72 hrs.	III	Guideline 003434
Acute inhalation LC50- rat; Eli Lilly & Co.; #R-H-37-81; 9/81	EL-107	250791	LC50 > 1.99 + 0.199 mg/L (11.5 mg/L nominal)	II	Minimum 003434
Acute oral LD50 - mice; Eli Lilly & Co.; #M-O-49-81 & M-O-50-81; 4/81	EL-107 25%	250791	LD50 > 10,000 mg/kg (only dose tested)	IV	Guideline 003434
Acute intraperitoneal LD50 - mice; Eli Lilly & Co.; #M-P-18-81 & M-P-19-81; 4/81	EL-107 25%	250791	LD50 > 5000 mg/kg	Acceptable 003434	

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Study/Lab/Study #/Date	Material	No.	LD50, LD50, P10, MUEL, LEL	Category	Vol. No.
Acute intraperitoneal LD50 - rat; Eli Lilly & Co.; #R-P-4-81 & R-D-5-81; 4/81	EL-107 25%	250791	LD50 > 2000 mg/kg (only level tested)		Acceptable 003434
Acute oral LD50 - rat; Eli Lilly and Co.; #R-545-79 & R-546-79; 4/81	formulation- 50% EL-107 (50W)	250791	Oral LD50 > 500 mg/kg (HDT)	III	Minimum 003434
Acute dermal LD50 - rabbit; Eli Lilly & Co.; BD-169-79; 4/81	formulation 50% EL-107 (50W)	250791	Dermal LD50 > 2000 mg/kg only dose tested	III	Minimum 003434
Primary dermal irritation - rabbit; Eli Lilly and Co.; #B-D-169-79; 4/81	formulation 50% EL-107 (50W)	250791	Dermal irritation slight.	IV	Minimum 003434
Primary eye irritation - rabbit; Eli Lilly & Co.; #U-E-186-79; 4/81	formulation 50% EL-107 (50W)	250791	Slight to moderate conjunctivitis, corneal dullness, mild iritis. One eye positive sodium fluorescein response at 24 hrs. All clear within 7 days.	II	Guideline 003434
Acute inhalation LC50 - rat; Eli Lilly and Co.; #R-H-88-79; 4/81	formulation 50% EL-107 (50W)	250791	LC50 > 2.42 mg/L (only level tested)	III	Minimum 003434
Acute oral LD50 - rat; Eli Lilly & Co.; #R-O-48-81 & R-O-49-81; 4/81	formulation 50% EL-107 (50W)	250791	LD50 > 10,000 mg/kg (only level tested)	IV	Guideline 003434

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CONFIDENTIAL BUSINESS INFORMATION
DOES NOT CONTAIN
NATIONAL SECURITY INFORMATION (EO 12065)

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EPA: 68-02-4225
DYNAMAC NO. 261-81
January 20, 1987

DATA EVALUATION RECORD

EL-107

Acute Dermal Toxicity Study in Rabbits

STUDY IDENTIFICATION: Lake, S. G., and Negilski, D. S. The acute dermal toxicity of Technical EL-107 in the New Zealand white rabbit. (Unpublished project No. B-D-124-84 prepared by Lilly Research Laboratories, Division of Eli Lilly and Co., Greenfield, IN, for Elanco Products Company, Indianapolis, IN; dated November 6, 1984.) Accession No. 073293.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 1-20-87

1. CHEMICAL: EL-107 (85.0% compound No. 121607, N-[3-(1-ethyl-1-methyl-propyl)-5-isoxazolyl]-2,6-dimethoxybenzamide; [REDACTED])

2. TEST MATERIAL: EL-107, technical grade, contained 85 percent compound No. 121607; test sample was lot No. Z-10025. See CBI appendix for percent and composition of Technical and other components.

3. STUDY/ACTION TYPE: Acute dermal toxicity study in rabbits.

4. STUDY IDENTIFICATION: Lake, S. G., Negilski, D. S. The acute dermal toxicity of technical EL-107 in the New Zealand white rabbit. (Unpublished project No. B-D-124-84 prepared by Lilly Research Laboratories, Division of Eli Lilly and Co., Greenfield, IN, for Elanco Products Company, Indianapolis, IN; dated November 6, 1984.) Accession No. 073293.

5. REVIEWED BY:

William M. Butler, Jr., M.S.
Principal Author
Dynamac Corporation

Signature: William M. Butler Jr.
Date: 1-20-87

Paul Wennerberg, D.V.M., M.S.
Independent Reviewer
Dynamac Corporation

Signature: Paul Wennerberg
Date: 1-20-87

6. APPROVED BY:

Finis L. Cavender, Ph.D.
Acute Toxicity
Technical Quality Control
Dynamac Corporation

Signature: Finis L. Cavender
Date: 1/20/87

Margaret L. Jones
EPA Reviewer

Signature: Margaret L. Jones
Date: 1-21-87

Marcia Van Gemert, Ph.D.
EPA Section Head

Signature: Marcia Van Gemert
Date: 1/21/87

7. SUMMARY:

Five male and five female New Zealand white rabbits, 12-18 weeks old, were obtained from Langshaw Farms, Augusta, Michigan, with initial body weights of 2.56 ± 0.352 kg (males) and 2.78 ± 0.235 kg (females). The animals were acclimated to the laboratory for 2 weeks, and during this period they were treated prophylactically with a miticide. The backs of all animals were clipped free of hair 24 hours before dosing. Tap water and Purina Certified High Fiber Rabbit Chow No. 5325 were administered ad libitum.

The test material, EL-107, technical grade, lot No. Z-10025, was administered topically to the shaved intact skin at a dose of 2000 mg/kg body weight.

The test material was applied directly to a dampened gauze pad (source of dampness not identified) placed on the treatment site and covered by a nonocclusive dressing, which was held in place by an elastic sleeve. Twenty-four hours after applications, the bandages were removed and the site washed with warm water. Restriction collars were worn by each rabbit for 48 hours following treatment to prevent ingestion of the test material.

One hour after the dressing was removed and daily thereafter for 13 days, all animals were observed for signs of toxicity, mortality, and dermal irritation. Individual body weights were recorded prior to exposure and weekly thereafter. A gross necropsy was performed on all of the animals at termination.

All of the rabbits survived the 14-day study. The dermal LD₅₀ was greater than 2000 mg/kg body weight. There were no overt signs of systemic toxicity, and all animals gained weight during the study. Very slight to slight dermal irritation was observed in all males and in three female rabbits. In two female rabbits no dermal effects were observed. Barely perceptible to slight edema was observed in three males and two females. The signs of irritation were not manifested until observation day 4, and the duration of these effects lasted through day 11.

8. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

The general design, conduct, and reporting of the study were acceptable. The use of a single dose level in an acute dermal study is in line with the EPA guidelines for a limit test. The acute dermal LD₅₀ for EL-107 is greater than 2000 mg/kg, which corresponds to Toxicity Category III.

A quality assurance statement was present, signed, and dated.

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9. CLASSIFICATION: Core minimum.

Dermal LD₅₀: >2000 mg/kg body weight.

Toxicity Category: III.

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Reviewed by: Margaret L. Jones *M. L. Jones 1/2/87*
Section III, Tox. Branch (TS-769C)
Secondary reviewer: Marcia Van Gemert, Ph.D. *M. Van Gemert 1/6/87*
Section III, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Subchronic Mouse/3 months with one month reversibility phase TOX. CHEM. NO.: 419F

ACCESSION NUMBER: 265731 MRID NO.:

TEST MATERIAL: Isoxaben Technical

SYNONYMS: EL-107

STUDY NUMBER(S): M02782

SPONSOR: ELANCO Products Co., Eli Lilly and Co.

TESTING FACILITY: Toxicology Division, Lilly Research Labs,
Greenfield, Indiana

TITLE OF REPORT: A Subchronic Toxicity Study of EL-107 Administered
in the Diet to B6C3F₁ Mice For Three Months
with a One Month Reversibility Phase

AUTHOR(S): S.G. Lake, R.W. Usher, B.L. Hawkins

REPORT ISSUED: May, 1985 (Study period: 10/29/82-2/27/83)

CONCLUSIONS: B6C3F₁ mice (25/sex/dose) were administered 0.001, 0.01, 0.14, or 1.25 % EL-107 for three months. Ten from each dose group were held for a one month recovery period after the sacrifice of 15 per group. No mortalities occurred during the study period and alopecia was the toxic sign observed most frequently. Alopecia persisted to the end of the recovery period, however the incidence was not dose related.

At three month sacrifice (15/sex/dose), males showed a dose related increase in minimal liver hypertrophy at 3 months accompanied by significantly elevated liver weights at doses of 0.14 and 1.25% EL-107. Females showed significantly elevated liver weights at doses of 0.14 and 1.25% EL-107 without concurrent hypertrophy. The no observed effect level (NOEL) for liver hypertrophy and increased liver weight was 0.01% EL-107. The NOEL for enzyme induction was 0.01% EL-107.

After one month recovery (10/sex/dose), males showed a dose-related increase in MCHC at doses of 0.01 through 1.25% EL-107 and dose related decreases in leukocyte counts which were significant from 0.01 through 1.25% EL-107.

Classification: core- Supplementary. Food consumption was not reported and compound intake can only be approximated from the information given. Initial values of hematology and clinical chemistry parameters were apparently not measured.

Special Review Criteria (40 CFR 154.7)

A. MATERIALS:

1. Test compound: EL-107, a mixture of isomers including Lilly compound 121607, N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide, Lilly compound N-[3-(1,1-dimethylbutyl)5-isoxazolyl]-2,6-dimethoxybenzamide, and Lilly compound 173490, N-[3-(1,1,2-trimethylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide); Combined purity at study initiation was 94.2% [redacted] and combined purity at termination was 93.7% [redacted].

[redacted] Isomer 173490 was found at the final analysis of the test compound due to a change in analytical methodology. Appropriate pages from Appendix D are attached which show the percentages of active ingredients and impurities. Description none given Batch # H02-2G6-118 (Dry).

2. Test animals: Species: mouse, Strain: B6C3F₁, Age: 5-6 weeks at study initiation (after 7 days acclimation), Weight: 18.5+ 0.2 g. (males), 15.5+ 0.1 g. (females), Source: Harlan-Sprague Dawley, Inc., Indianapolis, Indiana.

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned randomly to the following test groups:

Test Group	Dose in diet (ppm)	Main Study		Sacrifice		One Month Reversibility	
		3 months male	3 months female	3 months male	3 months female	male	female
00 (Cont.)	0	25	25	15	15	10	10
01 (0.001%)	10	25	25	15	15	10	10
02 (0.01%)	100	25	25	15	13	10	10
03 (0.14%)	1400	25	25	15	15	10	10
04 (1.25%)	12500	25	25	15	15	10	10

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

2. Diet preparation

Diet was prepared every two weeks and stored at room temperature. Samples of treated food were analyzed for stability at 0, 1, 2, and 4 weeks (1.25% preparation) and homogeneity of concentration (0.001% preparation) at start (10/28/82), middle (12/9/82), and the end (1/20/83) of the study.

Results- Test article was stable at 25°C and 37°C in the 1.25% dose preparation over 4 weeks. Results of stability for other concentrations were not reported. Homogeneity was demonstrated for the 0.001% concentration in 5 samples tested. Results of homogeneity assays for other concentrations were not reported.

3. Animals received standard mash diet (Purina Certified Chow No. 5002) and water ad libitum.

4. Statistics - The following procedures were utilized in analyzing the numerical data:

Dunnett's test (Dunnett, 1964) was used to analyze differences between controls and treated groups for body weight, weight gain, hematology, clinical chemistry, and organ weight data. Bartlett's method (Steel and Torrie, 1960) was used to test homogeneity of variances. Statistical significance was considered to be demonstrated at $p < 0.05$. Several of the numerical values were analysed by EPA statisticians.

5. Quality assurance was reported for 4 inspections between 10/82 and 5/83. Diet preparation, sample collection, physical/ocular examination, body weight, gross necropsy, organ weight, and slide staining were several procedures inspected.

C. METHODS AND RESULTS:

1. Observations

Animals were inspected daily for general physical condition and behavior and weekly for signs of toxicity and mortality.

Toxicity/Mortality (survival)

Two females from group 03 (0.14%) were missing and supposed escaped at 42 days. All remaining animals survived until scheduled sacrifice. Alopecia was the only observed toxic sign and occurred in forty percent (99/250 animals) during the course of the study. At sacrifice the number with alopecia was minimal-one female of 147 examined at 90 day sacrifice and 12 animals of 100 examined at the 120 day sacrifice. Incidence was not dose related.

2. Body weight

Animals were weighed weekly for the entire 90 day study and the additional 30 day recovery period.

Results: Significantly lower weight gain occurred in males receiving 0.001% EL-107 from day 0-7 ($p < 0.01$), and in males receiving 0.14% EL-107 from day 0-14 ($p < 0.05$), and significantly higher body weight and weight gain occurred in males receiving 0.01% EL-107 from day 0-77. Significantly lower weight gain occurred in females receiving 0.001% EL-107 from day 0-14 ($p < 0.05$) and from day 0-21 ($p < 0.01$). Females receiving 0.14 and 1.25% EL-107 had significantly lower body weights compared to controls for the 0-21 day period. The table on page 4b shows the weights discussed above.

At the end of the recovery period, there was lower body weight and lower weight gain in males from group 03 (0.14% EL-107) as compared to controls during days 0-121 although the differences were apparently not statistically significant. No significant differences were noted in females after the recovery period.

Mean Body Weight and Body Weight Gain in 90 day Mouse Study with EL-107 (g)
Interval Days

Males	% EL-107			
	0-7	0-14	0-77	0-121
0	21.4 (3.1)	23.4 (5.1)	29.5 (11.2)	32.1 (14.7)
0.001	20.8 (1.8**)	23.2 (4.2)	29.8 (10.8)	31.1 (12.8)
0.01	21.4 (3.3)	22.9 (4.8)	31.5*(13.4**a)	32.6 (15.1)
0.14	21.5 (2.9)	22.6 (4.0*)	28.6 (10.0)	27.2 (9.3)
1.25	21.7 (3.2)	23.4 (5.0)	30.3 (11.8)	31.1 (13.3)
Females	0-14	0-21		
0	18.8 (3.2)	19.8	(4.1)	
0.001	18.2 (2.0*)	19.2	(3.0*)	
0.01	18.2 (2.9)	19.4	(4.0)	
0.14	18.3 (3.6)	18.6**	(3.8)	
1.25	18.6 (3.0)	18.8*	(3.2)	

* Statistically different from controls ($p < 0.05$), Dunnett's T, two-tailed.

** Statistically different from controls ($p < 0.01$), Dunnett's T, two-tailed.

a. Both mean body weight and mean body weight gain were significantly greater than controls at this dose.

3. Food consumption and compound intake

Food efficiency and compound intake were calculated using the food consumption value for in-house controls (Study MO 3381) and body weight gain data from the present study (MO 2782). Actual compound intake was calculated at 1, 2, and 3 months from "in-house control mouse food consumption and actual body weight data from the study animals" (p. 681, Appendix H, Study No. M02782). Mean intake was the average of 3 measurements.

Food consumption/ Food Efficiency/Compound Intake

Appendix H shows food consumption reported as a mean value for 3 months for a total of 30 male and female controls from Study No. M03381. Males: 4.0 g/day, Females: 3.9 g/day.

Mean Intake of EL-107 (mg/kg/day)

Group	01	02	03	04
Males	1.4	14.2	200.0	1783.4
Females	1.7	17.2	249.1	2203.2

Food efficiency cannot be determined from the reported information since actual food consumption was not reported for each dose group for the present study (No. M02782). Further comments are found in part D.1., p. 14, of this Data Evaluation Report.

The effect of EL-107 on food consumption and food efficiency cannot be determined from the given information. Intake of EL-107 can only be considered approximate since in-house control values for food consumption were used to calculate compound intake.

4. Ophthalmological examinations

Performed weekly on animals. Study procedures indicate "color and appearance of eyes" were noted weekly.

Results- No abnormal ophthalmological signs were reported.

5. Blood was collected at the termination of treatment (3 months) and at the end of the reversibility phase (1 month additional). for hematology and clinical analysis from all animals. 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 corpuscular HGB conc.(MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpuscular volume (MCV)
	Platelet count*		Reticulocyte count
	Blood Clotting Measurements		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic and chronic studies

Results-Hematology- Males and Females sacrificed at 90 days showed hematology values similar to controls except for males receiving 0.001% EL-107 which showed significantly increased MCHC (p<0.05) and females receiving 0.01% EL-107 which showed significantly lower leukocyte counts.

Males sacrificed at 121 days showed a dose related decrease in leukocyte count as shown in appended Table 7 from Test Report M02782. There also appears to be a possible dose related increase in MCHC in males at 121 days. Other values significantly lower than controls at 121 days were RBC and PCV in the 0.01% group. Females previously receiving 0.14% EL-107 sacrificed at 121 days showed significantly lower leukocyte counts (p<0.05).

The no observed effect level for hematology effects appears to be 0.001% EL-107 as demonstrated by lower leukocyte counts and increased MCHC in males at 121 days.

b. Clinical Chemistry

<p>X Electrolytes:</p> <ul style="list-style-type: none"> Calcium* Chloride* Magnesium* Phosphorous* Potassium* Sodium* <p>Enzymes</p> <ul style="list-style-type: none"> x Alkaline phosphatase x Cholinesterase# x Creatinine phosphokinase*° x Lactic acid dehydrogenase x Serum alanine aminotransferase (also SGPT)* x Serum aspartate aminotransferase (also SGOT)* gamma glutamyl transferase glutamate dehydrogenase 	<p>X Other:</p> <ul style="list-style-type: none"> Albumin* x Blood creatinine* x Blood urea nitrogen* Cholesterol* Globulins x Glucose* x Total Bilirubin* Total Serum Protein* Triglycerides Serum protein electrophoresis
--	--

- * Required for subchronic and chronic studies
- # Should be required for OP
- ° Not required for subchronic studies

Results- Males at the 90 day sacrifice- showed significantly lower glucose at 0.001% EL-107 (p<0.05), showed significantly lower blood urea nitrogen at 0.14% and 1.25% EL-107 (p<0.05), and showed significantly lower total bilirubin at 0.01 and 0.14% EL-107 (p<0.05) These values are shown in appended Table 8 from Test Report M02782.

Females at the 90 day sacrifice- showed significantly lower total bilirubin at 0.001 and 0.01% EL-107 (p<0.05).

Males at the 121 day sacrifice- showed significantly higher glucose at 1.25% EL-107 (p<0.05).

Females at the 121 day sacrifice- showed significantly higher total bilirubin at 0.01 and 0.14% EL-107 (p<0.05).

EL-107 administration produced contradictory effects on glucose levels (lower in males, higher in females) during three months of treatment. EL-107 lowered total bilirubin at low doses in males and females during three months of treatment. Bilirubin values were similar to controls in males after recovery, but were increased over control values in females after recovery, apparently caused by withdrawal of the test compound. There is weak statistical evidence that Total Bilirubin values were increased after withdrawal of EL-107 according to EPA statistical analysis.

6. Urinalysis^o

Urinalysis was not performed, and is not required for a subchronic study on a routine basis.

<input checked="" type="checkbox"/>	Appearance*	<input checked="" type="checkbox"/>	Glucose*
<input type="checkbox"/>	Volume*	<input type="checkbox"/>	Ketones*
<input type="checkbox"/>	Specific gravity*	<input type="checkbox"/>	Bilirubin*
<input type="checkbox"/>	pH	<input type="checkbox"/>	Blood*
<input type="checkbox"/>	Sediment (microscopic)*	<input type="checkbox"/>	Nitrate
<input type="checkbox"/>	Protein*	<input type="checkbox"/>	Urobilinogen

- * Required for chronic studies
- ^o Not required for subchronic studies

7. Sacrifice and Pathology -

All animals that died and that were sacrifice schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

<u>X</u>	<u>X</u>	<u>X</u>
Digestive system	Cardiovasc./Hemat.	Neurologic
Tongue	.Aorta*	x .Brain*tcerebrum,cerebellum, brain stem
x .Salivary glands*	xx .Heart*	Periph. nerve*#
x .Esophagus*	x .Bone marrow*	Spinal cord (3 levels)*#
x .Stomach*	x .Lymph nodes*	x .Pituitary*
x .Duodenum*	xx .Spleen*	x Eyes (optic n.)*#
x .Jejunum*	x .Thymus*	Glandular
x .Ileum*	Urogenital	xx .Adrenals* w. kidneys
x .Cecum*	xx .Kidneys* tw. adrenals	Lacrimal gland#
x .Colon*	x .Urinary bladder*	x Mammary gland*#
x .Rectum*	xx .Testes*†	x .Parathyroids*††
xx .Liver*†	Epididymides	x .Thyroids*††
Gall bladder*#	xx Prostate	Other
x .Pancreas*	Seminal vesicle	x Bone*#
Respiratory	xx Ovaries*† w. uterus	x Skeletal muscle*#
.Trachea*	xx .Uterus* w. ovaries	x Skin*#
x .Lung*		x All gross lesions and masses*
Nose°		
Pharynx°		
Larynx°		

* Required for subchronic and chronic studies

° Required for chronic inhalation

In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement

† Organ weights required in subchronic and chronic studies

†† Organ weight required for non-rodent studies

Enzyme Induction- Enzyme induction was measured by determining hepatic-p-nitroanisole 0-demethylase activity from liver homogenates prepared using 2 g. of liver from 5 animals/sex/dose taken at necropsy.

Results of Enzyme Induction Measurements- At the three month sacrifice there was significant stimulation of enzyme in males and females receiving 0.14 and 1.25% EL-107 as indicated by increases in paranitrophenol (PNP) produced per milligram of protein per hour. The values are found in appended Table 10 from Study M02782. The levels of PNP were slightly elevated in males and slightly lowered in females at one month recovery, however, none of the differences was statistically significant. Enzyme activity returned to normal after withdrawal of the test compound. The no observed effects level for enzyme induction was 0.01% EL-107.

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Results-

a. Organ weight

Absolute organ weights-At 90 day sacrifice- mean liver weights in males were significantly elevated at 0.14% (p<0.05) and 1.25% (p<0.01) EL-107. Females showed mean uterine weights (with ovaries) that were respectively 16%, 13%, 13.5%, and 13% lower at 1.25, 0.14, 0.01, and 0.001% EL-107. There is weak statistical evidence that uterine weights were lower than controls at the 90 day sacrifice according to analysis by EPA statisticians.

-At 121 day sacrifice- males showed no significantly different mean absolute organ weights. Females showed significantly lower mean kidney weights (with adrenals) after withdrawal of 0.14% EL-107 (p<0.01) and significantly increased mean heart weight after withdrawal of 1.25% EL-107 (p<0.05). Mean weight of uterus (with ovaries) was similar to controls at recovery.

Relative organ weights- At 90 day sacrifice- relative mean liver weight in males and females was significantly elevated at 0.14 and 1.25% EL-107 (p<0.01).

- At 121 day sacrifice- males in the group which had received 0.14% EL-107 showed increased relative weight of liver, kidneys (with adrenals), heart, testes, and spleen as compared to controls. All were significantly different from controls at p<0.05 except relative kidney weight which was significant at p<0.01. Females which had received 0.14% EL-107 showed lower relative kidney weight (p<0.01) at the 121 day sacrifice.

Relative uterine to body weight ratio was not significantly affected by test compound at 90 or at 121 days, according to analysis by EPA statisticians.

Mean Organ Weights and Relative Organ Weights in Mouse Study With EL-107

Males		Absolute Organ Weight (Rel. Organ Wt. per 100 g. B.W.)	
% EL-107	Body Weight	Liver (g.)	
0	31.9	1.121	(3.51)
0.001	31.4	1.083	(3.45)
0.01	31.1	1.111	(3.57)
0.14	31.0	1.246*	(4.02**)
1.25	31.3	1.377**	(4.40**)

Females	Body Weight	Liver (g.)	Uterus (mg.)
0	25.1	0.939 (3.74)	185.3 (736.4)
0.001	24.2	0.899 (3.71)	161.0 (667.8)
0.01	23.6	0.885 (3.74)	160.2 (674.4)
0.14	24.3	0.979 (4.03**)	161.1 (665.5)
1.25	23.3	1.021 (4.39**)	155.3 (668.4)

* Statistically different from control (p<0.05), Dunnett's T, two-tailed.
** Statistically different from control (p<0.01), Dunnett's T, two-tailed.

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b. Gross pathology

At 3 month sacrifice- one male control had a kidney "lesion", one male at 0.01% EL-107 had a calculus in the urinary bladder, one male at 0.14% EL-107 had a pancreatic cyst, and one male at 0.14% EL-107 had a liver "lesion". One female control showed alopecia at this sacrifice.

At sacrifice after one month recovery- one female control showed an ovarian cyst, one male at 0.001% EL-107 had a cyst of the mammary gland, and twelve animals showed alopecia.

c. Microscopic pathology

1) Non-neoplastic - At 90 day sacrifice- Males showed a dose-related increase in minimal liver hypertrophy, as follows:

<u>Dose</u>	<u>Incidence</u>
0	0
0.001%	1/15
0.01%	0/15
0.14%	3/15
1.25%	9/15

One male at 0.01% EL-107 showed edema of the urinary bladder, and one male and one female at 0.001% EL-107 showed inflammation of the urinary bladder, one male at 0.14% showed minimal glycogen in the liver, and one female at this dose showed multiple inflammation in the liver. One female at 0.14% EL-107 showed histiocytosis in the lung and lymphoid hyperplasia.

At 121 day sacrifice- liver histopathology was observed in a total of 8 females and 1 male (of 100 animals examined), as follows: congestion (1F), fat deposition (2F), minimal glycogen (2F), focal inflammation (1M, 1F), multiple inflammation (1F), and vascular ectasia (1F). None of the findings was dose related. Minimal focal acanthosis and minimal focal hyperkeratosis were noted in the examination of the skin of several animals at this sacrifice.

The no observed effect level (NOEL) for liver hypertrophy was 0.01%. Hypertrophy was the only apparently dose-related histopathological finding. This finding was coupled by significantly increased liver weight and increased liver to body weight ratio.

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2) Neoplastic- no neoplastic lesions were noted at the histopathology examination at 90 or at 121 days.

D. DISCUSSION:

1. Food consumption was not reported for this study. Consequently, compound intake as reported can only be considered approximate for this study. Food consumption for in-house control animals cannot be used to calculate food efficiency for this study. Consumption for study controls and for dose groups would be necessary in order to calculate this parameter. The Pesticide Assessment Guidelines, Subpart F specify that food consumption should be measured weekly [§ 82-1 (g)(8)(v) p.70].

2. Hematology: The following Guideline parameters were not reported: hematocrit, platelet count (or another indicator of clotting time). Initial values were apparently not measured before study initiation.

3. Clinical Chemistry: The following Guideline parameters were not reported: calcium, chloride, magnesium, phosphorous, potassium, sodium, aspartate aminotransferase (SGOT), cholesterol, total protein. Initial values were apparently not measured before study initiation.

4. Ophthalmological examination, hematology, and clinical chemistry parameters were apparently not examined prior to study initiation, as specified in the Guidelines.

5. The Pesticide Assessment Guidelines p. 76 specify: "(I) Detailed description and classification of all histopathological findings" should be part of the data report. There is little description of pathological findings in Report M02782. It is advisable to submit the full pathology report.

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Page _____ is not included in this copy.

Pages 32 through 55 are not included.

The material not included contains the following type of information:

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EPA: 68-01-6561
TASK: 115
August 6, 1985

DATA EVALUATION RECORD

EL-107 (Isomers 121607, 135520 and 173490)

Subchronic Oral Toxicity Study in Dogs

STUDY IDENTIFICATION: Lake, S. G. A subchronic toxicity study of EL-107 (compounds 121607 and 135520) administered orally to beagle dogs for three months. (Unpublished study No. D00783 prepared and submitted by the Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, Greenfield, IN; dated April, 1984.) Accession No. 073293.

APPROVED BY:

I. Cecil Feikner, Ph.D.
Program Manager
Dynamac Corporation

Signature: _____

I. Cecil Feikner

Date: _____

8-6-85

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

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- 1. **CHEMICAL:** EL-107 (Isomers 121607, 135520 and 173490) 121607 = N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide [redacted] 135520=N-[3-(1,1-dimethylbutyl)-5-isoxazolyl]-2,6-dimethoxybenzamide [redacted] 173490 = N-[3-(1,1,2-trimethylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide [redacted]. The combined strength is 95.5% (assayed at end of study).
- 2. **TEST MATERIAL:** EL-107, Technical, Lot No. 710025 with a purity of 95.5%. See Appendix C of the DER for analyses and composition of the lot.
- 3. **STUDY/ACTION TYPE:** Subchronic (3 months) oral toxicity study in dogs.
- 4. **STUDY IDENTIFICATION:** Lake, S. G. A subchronic toxicity study of EL-107 (compounds 121607 and 135520) administered orally to beagle dogs for three months. (Unpublished study No. 000783 by the Toxicology Division, Lilly Research Laboratories, Division of Eli Lilly and Company, Greenfield, IN; dated April, 1984.) Accession No. 073293.

5. **REVIEWED BY:**

Alan C. Levy, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: William L. McLellan for
Date: Aug 6, 1985

William L. McLellan, Ph.D.
Independent Reviewer
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Date: Aug 6, 1985

6. **APPROVED BY:**

Norbert P. Page, D.V.M.
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Technical Quality Control
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W. Thomas Edwards
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Signature: W. Thomas Edwards
Date: 5-15-86

~~MARCIA VAN GEMERT, PhD
Clint Skinner, Ph.D.
EPA Section Head~~
EPA Section Head
Secondary Reviewer

Signature: [Signature]
Date: 12-10-86

7. CONCLUSIONS:

- A. When given orally to beagle dogs by gelatin capsules at doses of 0, 25, 100, or 500 mg/kg/day, the only definitive compound-related effects were increases in the mean absolute and relative liver weights in high-dose (500 mg/kg/day) males. In addition, there was indication of an increase in relative liver weights in high-dose females; however, this increase was not statistically significant. The LOEL based on increased relative liver weight was 500 mg/kg/day, and the NOEL was 110 mg/kg/day.
- B. We assess that this study is adequate to provide the information for which it was intended, i.e., the characterization of possible toxicity when the compound was administered for three months.

Items 8 and 10 - see footnote 1.

9. BACKGROUND:

- A. Previous EL-107 dog studies included: 1) a two-week pilot oral (gavage) non-isometric study (D-3241); 2) a three-month sub-chronic oral (capsule) non-isometric study (D33582), and 3) a two-week pilot oral (capsule) isometric study (D06282). No overt physical signs of toxicity were found in study D-3241 with treatment groups of 0, 1250, 2500, or 5000 mg/kg. Relative liver weight increases occurred in the males of all dose groups and in the high-dose females. Based on these results, study D33582 was conducted with treatment groups of 0, 250, 500, or 1000 mg/kg.

No mortalities or overt physical signs of toxicity occurred in study D33582. Some animals occasionally vomited or had identifiable compound in the stool due to the large dose given. Liver weights were not significantly increased at any treatment level, but all dose groups, both male and female, had increased hepatic p-nitroanisole O-demethylase activities.

A two-week pilot oral study (D06282) with assays for enzyme induction was conducted with treatment groups of 0, 25, and 250 mg/kg. No mortalities or overt physical signs of toxicity occurred. A dose-related trend toward increased activities of p-nitroanisole O-demethylase appeared with increasing dose but the observed increases were not statistically significant. No differences in liver weights were present in control versus treated animals.

¹ Only items appropriate to this DER have been included.

11. MATERIALS AND METHODS (PROTOCOLS):A. Materials and Methods:

Groups of eight purebred beagle dogs, four of each sex, approximately seven months of age, were acclimated to cages, feed, and water, and assigned to dosing groups. They were then dosed once per day, seven days per week, using oral gelatin capsules to administer doses of 0, 25, 110, or 500 mg/kg/day of EL-107 (a mixture of three isomers - compounds 121607, 135520, and 173490); dosing was for three months. Changes in appetite were noted, and body weights were recorded weekly. Hematology, clinical chemistry, and urinalysis examinations were performed prior to the start of the study; at 1, 2, and 4 weeks; and monthly thereafter. At autopsy, organ weights were recorded for liver, kidneys, heart, thyroids, adrenals, and testes or ovaries. Histopathologic examination was performed on more than thirty tissues from each animal in the study. (See Appendix A for a more detailed description.)

B. Protocol: See Appendix B.12. REPORTED RESULTS:

A. Test Material Analyses: The test material was analyzed prior to the start of the study and when the study was completed. In the initial assay it was reported as 94.8 percent active ingredient and when reassayed at termination of the study it was 93.3 percent active ingredient, indicating that the EL-107 formulation was stable.

B. Observations and Mortality: There were no mortalities. The animals were observed daily and it was reported that there were no overt physical signs of toxicity.

Pretermination ophthalmologic examinations indicated no compound-related effects; one control and one high dose had mild follicular conjunctivitis which the authors considered incidental.

C. Body Weights: It was reported that there were no compound-related effects on body weight. However, at least one animal in each group, including controls, lost some weight. Although differences in the mean body weight gains were not statistically significant, there was an apparent dose-related trend in females, weight gains being 0.7 kg in controls, 0.2, 0, and -0.3 kg in the 25, 110, and 500 mg/kg/day dose groups, respectively.

D. Food Consumption: There were no compound-related changes in the food consumption between dosed and control groups.

E. Clinical Studies: There were no effects of dosing on hematology, clinical chemistry, or urinalysis parameters.

- F. Biochemical Toxicology: Enzyme induction was assessed by determining the activity of hepatic p-nitroanisole O-demethylase. Statistically significant treatment-related changes were not found, but a trend toward increased activity in treated male dogs, though not statistically significant, was noted.
- G. Organ Weights: Absolute and relative liver and testes weights were increased in males of the high-dose group. Only liver weight increases were considered to be toxicologically significant (Table 1).

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

- A. The study author concluded that the dogs tolerated daily oral doses of 0, 25, 110, or 500 mg/kg/day of EL-107 for three months without mortality or overt signs of toxicity. The only compound-related effect was increased mean liver weight in high-dose males. The author concluded that the no-effect level of EL-107 under the conditions of this study was 110 mg/kg/day.
- B. A quality assurance statement was present, signed, and dated April 12, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

- A. This study fulfilled the requirements necessary to evaluate compound EL-107 in the dog for three months. Our reviewers agree with the conclusions of the study author, that the only effects of toxicological significance were increases in liver weights. Statistical significance was shown for males only; however, our reviewers assess that there is a possible trend toward liver weight increases in females (see Table 2). Histopathology did not reveal any liver changes. None of the following parameters gave indication of toxicological effects: survival, physical signs, ocular, body weight, food consumption, hematology, clinical chemistry, urinalysis, and pathology.

High-dose (500 mg/kg/day) female dog 172853 showed a relatively severe loss of body weight; a decrease in erythrocyte count and hemoglobin; an increase in the total white blood cell count and neutrophils; a decrease in blood glucose and urea nitrogen; and an increase in alkaline phosphatase activity. This dog was diagnosed as having had an intercurrent inflammatory process which we assessed was not a compound-related effect.

B. Recommendations:

1. Appendix C of the study report, pages 202-214, (Test Article Characterization) is missing. This needs to be included for the report to be acceptable.

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TABLE 1. Liver Weight and Liver-to-Body Weight Ratio in Dogs Dosed with EL-107 for Three Months^a

Dose mg/kg/day	MALES		FEMALES	
	Grams	Percent Body Weight	Grams	Percent Body Weight
0	240.3±10.14	2.509±0.1135	230.5±19.07	2.656±0.2754
25	245.3±32.58	2.590±0.2175	262.3±33.95	2.925±0.4052
110	273.0±2.94	2.887±0.2927	225.3±40.61	3.081±0.1984
500	323.0±20.70**	3.247±0.3710**	274.5±62.05	3.663±1.0223

^a Mean values of 4 dogs/sex/group ± standard deviation.

** Statistically different from control value ($P \leq 0.01$), Dunnett's two tailed t-test.

TABLE 2. Individual Relative Liver Weights for Female Dogs Dosed With EL-107 for Three Months

	Control	25 Mg/Kg/Day	110 Mg/Kg/Day	500 Mg/Kg/Day
	2.33	3.20	3.13	3.28
	2.66	3.06	3.25	2.64
	2.64	3.12	3.15	3.68
	<u>3.00</u>	<u>2.32</u>	<u>2.80</u>	<u>5.06</u>
MEAN	2.656	2.925	3.081	3.663

2. Rounding-Off by Computer: Rounding off by the computer for different tables is not consistent.

EXAMPLE: P.162 versus P.27, Test Group 02, Body Weight (kg)
P.162 = 7.3
P.27 = 7.4

EXAMPLE: P.172 versus P.161, Test Group 01, Organ Weights per 100 G Body Weight, Testes
P.172 (individual weights of 0.1, 0.1, 0.1, 0.1)
P.161 (mean of the 4 dogs is 0.12)

In no instance do these relatively slight differences influence the scientific accuracy of any portion of the report. However, for consistency and clarity, either the values should be the same or the reason for these difference should be explained.

3. Change wording for clarity: P.19, Body Weight (Table 1). "At least one animal from each treatment group, including control, lost up to 10% of its initial body weight during the test."

SUGGESTION: "At least one animal from each of the four groups lost weight when compared to the pretreatment value."

Item 15 - see footnote 1.

16. CBI APPENDIX

Appendix A, Materials and Methods, CBI pp. 12-17.

Appendix B, Protocol, CBI pp. 187-200.

Appendix C. Test Material Analysis and Characterization, CBI p. 215 and Appendix C of Accession No. 073293.

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APPENDIX A
Materials and Methods

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Pages 64 through 89 are not included.

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DATA EVALUATION REPORT

STUDY TYPE: Subchronic liver toxicity in mice for 3 months

TOX. CHEM. NO.: 419F

ACCESSION NUMBER: 265732

MRID NO.:

TEST MATERIAL: Isoxaben technical

SYNONYMS: EL-107

STUDY NUMBER(S): MO 1083

SPONSOR: ELANCO Products Company

TESTING FACILITY: Toxicology Division, Lilly Research Laboratories
Eli Lilly and Company, Greenfield, Indiana

TITLE OF REPORT: A Subchronic Liver Toxicity Study of EL-107
Administered to B6C3F₁ Mice for Three Months

AUTHOR(S): S.G. Lake, R.W. Usher, R.A. Smith

REPORT ISSUED: November, 1985

CONCLUSIONS: EL-107 was administered in the diet to B6C3F₁ mice (15/sex/group) at levels of 100, 1000 and 12500 ppm (0, 0.01, 0.1, and 1.25%). The no observed effect level was 100 ppm which corresponds to 15 mg/kg/day. Males and females had significantly elevated liver weights at 1000 ppm and elevated hepatic p-nitroanisole O-demethylase activity (enzyme induction) at 12500 ppm.

Classification: core-Supplementary

Special Review Criteria (40 CFR 154.7)

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A.6 MATERIALS: MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

1. Test compound: EL-107 (mixture of 3 isomers with [redacted] Lilly compound 121607, N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide, [redacted] Lilly compound 135520 N-[3-(1,1-dimethylbutyl)5-isoxazolyl]-2,5-dimethoxybenzamide, and [redacted] Lilly compound 173490, N-[3-(1,1,2-trimethylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide). Description none given. Lot # Z10025. Combined purity 95.5%, contaminants: listed in CBI appendix (Appendix A).

2. Test animals: Species: mouse, Strain: B6C3F1, Age: 5-6 weeks old at study initiation, Weight: males: 19.8 + 0.2 g., females: 16.4 + 0.2 g., Source: Harlan-Sprague Dawley, Inc., Indianapolis, Indiana.

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned randomly to the following test groups:

Test Group	Dose in diet		Main Study	
	ppm	(mg/kg/day)* (males/females)	3 months male	3 months female
1 Cont.	0	(0/0)	15	15
2 Low (LDT)	100	(13.7/17.0)	15	15
3 Mid (MDT)	1000	(138.7/169.0)	15	15
4 High (HDT)	12500	(1719.7/2101.7)	15	15

* Intake based on in-house control values for food consumption and body weight data for mice in the present study.

2. Diet preparation

Diet was prepared every two weeks and stored at room temperature. Samples of all diet preparations were assayed for EL-107 activity. Results of diets prepared on March 14, 1983, April 25, 1983, and May 23, 1983 were reported. Samples of treated food with 1.25% EL-107 were apparently analyzed for stability and homogeneity of concentration in 1981, as follows: 5 samples of the 1.25% preparation were assayed for EL-107 content; and two samples of diet were stored for 4 weeks at 25°C and 37°C.

Results - Concentrations of the three reported assays of diet preparations were close to theoretical concentrations. Five samples of diet containing 1.25% EL-107 were apparently homogeneous, and four samples taken at 0, 1 week, 2 weeks, and 4 weeks were stable at 25°C and 37°C. Assay at the end of the study for EL-107 activity showed 94.1% purity, 1.4% less than the initial 95.5%, but within an acceptable margin. The test article was considered stable for the duration of the present subchronic study (3 months).

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3. Animals received standard mash diet (Purina Certified Rodent Chow No. 5002) and water ad libitum.
4. Statistics - The following procedures were utilized in ~~005733~~ analyzing the numerical data: Dunnett's two-tailed T test was used to analyze differences between controls and treated group means for body weight, weight gain, and organ weight. Dunnett's one-tailed T test and analysis of variance were used to analyse the results of enzyme activity measurements. Statistical significance was considered demonstrated at $p < 0.05$.
5. Quality assurance inspections were performed on three dates. Various aspects of the study were examined including diet preparation, body weight measurement, clinical observations, gross necropsy, and liver weights.

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C. METHODS AND RESULTS

1. Observations

Animals were inspected daily for signs of toxicity and mortality. Detailed examinations were performed weekly.

Toxicity/Mortality (survival)

Alopecia was noted in all dose groups, with the same frequency in both sexes.

No mortality was associated with the study.

2. Body weight

Animals were weighed weekly.

Results- At the high dose there was a statistically significant increase in mean weight gain in males and females, as shown in the following table.

Mean Body Weight and Weight Gain in Mice Fed EL-107 in the Diet

Dose (ppm) Mean Terminal Body Weight (g) Terminal Body Weight Gain (g)

Males

0	34.4	14.2
100	31.7*	12.4
1000	32.5	12.6
12500	31.6*	11.8*

Females

0	26.7	10.2
100	25.6	9.2
1000	24.8*	8.7
12500	24.8*	8.2**

* Significantly different from controls at the $p < 0.05$ level

** Significantly different from controls at the $p < 0.01$ level.

3. Food consumption and compound intake

Compound intake was measured monthly from in-house control food consumption and body weight from the present study.

Results- Appendix B (p. 71, Test Report) shows the results of in-house control measurements and estimated compound intake. Efficiency of food utilization could not be calculated since actual food consumption was not reported.

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4. Ophthalmological examinations

Performed weekly at the time detailed examinations were performed on all animals.

Results- No toxic signs related to treatment were observed during examination of the eyes.

5. Hematology, clinical chemistry and urinalysis

These investigations were not performed. The purpose of the study was to look at the effect of the compound on the liver and enzyme activity.

6. Sacrifice and Pathology - Livers were weighed and samples taken to use in the test for enzyme activity. Liver samples were taken at necropsy from 5 animals at each dose level. Homogenates were measured for p-nitro-anisole O-demethylase activity. Appendix C (pp. 35-36, Test Report) shows the results of enzyme induction measurements.

No other organs were examined at sacrifice and pathology.

Results- Statistically significant increases in enzyme levels were demonstrated at 12500 ppm. There appears to be a dose-related increase in liver enzyme activity for EL-107. Data are tabulated on appended pages C2 and C3.

Mean Liver Weight and Relative Liver Weight in Mice Fed EL107 in the Diet

Dose (ppm)	Mean Liver Weight (g)	Liver Wt/100 g. Body Weight
Males		
0	1.34	3.9
100	1.26	4.0
1000	1.43	4.4**
12500	1.63**	5.1**
Females		
0	1.03	3.9
100	1.02	4.0
1000	1.06	4.3**
12500	1.21**	4.9**

** Significantly different from control values at $p \leq 0.01$.
Dunnett's T, two-tailed.

Appendix A -

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APPENDIX D

TEST ARTICLE CHARACTERIZATION OF TECHNICAL EL-107, LOT NO. Z10025,
FOR USE IN CHRONIC TOXICOLOGY STUDIES AND REPRODUCTION STUDIES

I-8SR-83-5

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September, 1983

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Pages 96 through 100 are not included.

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Secondary reviewer: Marcia Van Gemert, Ph.D. *M. Van Gemert 1/21/87*
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DATA EVALUATION REPORT

STUDY TYPE: Chronic/Oncogenicity in Mice TOX. CHEM. NO.: 419F

ACCESSION NUMBER: 265737, 265738 MRID NO.:

TEST MATERIAL: Isoxaben Technical

SYNONYMS: EL-107

STUDY NUMBER(S): Combined Report MC0809 (includes replicate studies M00883 and M00983)

SPONSOR: ELANCO Products Co., Eli Lilly and Co.

TESTING FACILITY: Toxicology Division, Lilly Research Labs, Greenfield, Indiana

TITLE OF REPORT: A Two Year Chronic Oncogenic Toxicity Study of EL-107 Administered in the Diet to B6C3F₁ Mice

AUTHOR(S): S.G. Lake, C.L. Gries, R.W. Usher

REPORT ISSUED: November 1985

CONCLUSIONS: Isoxaben was administered for 24 months to B6C3F₁ mice (30/sex/dose) in two replicate studies (combined 60/sex/dose) at doses of 0, 100, 1000, and 12500 ppm. Males and females showed an increase in hepatocellular adenomas at the high dose at terminal sacrifice, and an increase in the combined incidence of hepatocellular adenomas and hepatocellular carcinomas at the high dose. Liver hyperplasia was increased in both males and females at the high dose. Increases in liver nodules were reported at the high dose in males and females. Liver toxicity was also demonstrated by the increased absolute and relative liver weight at the high dose, by hepatocytomegaly in high dose males, and by hepatocellular vacuolation in high dose males and females. Elevated levels of alkaline phosphatase (males only) and alanine transaminase at the high dose support the finding of liver toxicity in this study. The no observed effect level for oncogenic effects was 1000 ppm.

Decreased survival was noted in males at the low dose and in females at the mid dose. Throughout most of the study body weight and body weight gain in males at the high dose was lower than controls. The no observed effect level for systemic effects was 100 ppm.

Classification: core-Minimum
Special Review Criteria (40 CFR 154.7)

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED 05732

A. MATERIALS:

1. Test compound: EL-107, a mixture of isomers consisting primarily of Lilly compound 121607, N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide [redacted] Lilly compound 135520, N-[3-(1,1-dimethylbutyl)-5-isoxazolyl]-2,6-dimethoxybenzamide ([redacted], and Lilly compound 173490, N-[3-(1,1,2-trimethylpropyl)-5-isoxazolyl] 2,6-dimethoxybenzamide [redacted] Description none given, Lot # Z10025, Purity 95.5% (combined purity), contaminants: listed in appended pages 15-17.

2. Test animals: Species: mouse, Strain: B6C3F1, Age: 5-6 weeks old at study initiation (after 7 days acclimation), Weight: M00883: Males: 18.9+ 0.2 g., Females: 15.7+ 0.1g. M00983: Males: 20.6+ 0.2 g., Females: 16.8+ 0.1g. Source: Harlan-Sprague Dawley, Indianapolis, Indiana -332 of each sex were obtained before initiation of replicate studies two weeks apart

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned randomly to the following test groups:

Test Group	Dose in diet (ppm)**	Study M00883		Replicate Study M00983	
		24 months*		24 months**	
		male	female	male	female
1 Cont.	0	30	30	30	30
2 Low (LDT)	100	30	30	30	30
3 Mid (MDT)	1000	30	30	30	30
4 High(HDT)	12500	30	30	30	30
		*start 3/14/83 end 3/15/85		**start 3/31/83 end 4/5/85	

2. Diet preparation

Diets were prepared every two weeks and stored at room temperature. Samples of treated food were analyzed for EL-107 activity at the beginning of studies M00883 and M00983 and at 4 month intervals. The highest concentration of test diet 12500 ppm was analyzed for homogeneity and stability of concentration. Homogeneity was also tested in the 100 ppm preparation. Appended pages 18-20 show the results of testing for homogeneity and stability.

Results - The tests for homogeneity and stability were apparently performed in November 1981. The lot number of the EL-107 tested was not reported. The compound tested was reported stable for 4 weeks and homogeneous in 5 samples tested.

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3. Animals received Purina Certified Rodent Chow No. 5002 and water ad libitum.
4. Statistics - The following procedures were utilized in analyzing the numerical data: see appended pages 1-2. Appended pages 22-28 from the test report show the results of the two year tumor analysis.
5. Quality assurance was reported for 12-13 inspections of various procedures as body weight, diet preparation, clinical observations, euthanasia, blood collected, gross necropsy, etc.

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C. METHODS AND RESULTS:

1. Observations

Animals were inspected weekly for signs of toxicity and mortality.

Toxicity/Mortality (survival) There was a decrease in survival in females at 1000 ppm over the entire study. The greatest decrease appeared at 23 months and was 13% lower than controls. There was a decrease in survival in males at 100 ppm over the first 21 months. The greatest decreases appeared at 18 and 21 months and were approximately 12% lower than controls. Survival is shown in appended pages 3-4. The decrease in survival was apparently not dose-related.

2. Body weight

Animals were weighed weekly for days 1-90 then every other week for the remaining 21 months.

Results- Body weight was significantly lower in males at 12500 ppm from week 25-79 (months 6-20). Body weight gain was significantly lower in males at 12500 ppm from week 25-73 (months 6-18). Body weight in females was similar to controls.

3. Food consumption and compound intake

Mean daily food consumption was determined from in-house controls. An estimated mean daily compound intake was calculated using the in-house control values for 1 month, 3 months and at three month intervals thereafter. Appended page 21 shows the data collected on food consumption and compound intake.

Results- Food consumption/Food Efficiency/Compound Intake

Food consumption reported as mean values for in-house controls appears in appended page 21 along with compound intake calculated from the mean food consumption values.

Estimated Compound Intake for Combined Studies (mg/kg/day)

	<u>Males</u>	<u>Females</u>
100 ppm	11.5	12.2
1000 ppm	113.7	123.9
12500 ppm	1476.4	1567.1

The effect of the test compound on food consumption and food efficiency (as weight gain compared to food consumption) cannot be judged based on the given information.

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4. Ophthalmological examinations

Performed weekly on animals when other toxicity parameters were evaluated. Color and appearance of eyes were evaluated.

Results- Effects noted on test were opacity of one or both eyes, swollen eye(s), microphthalmia, and red fluid from the eye. The incidence of swollen eyes and opacity exceeded control levels only at the low dose as shown in the following table. The test compound does not appear to have an effect on the eyes of the treated mice.

Observations at Ophthalmological Examination of B6C3F₁ Mice
(Summary of Antemortem Observations)

<u>Dose (ppm)</u>	<u>0</u>	<u>100</u>	<u>1000</u>	<u>12500</u>
<u>Males</u> (No. exam.)	60	60	60	60
Opacity - eye(s)	3	4	3	1
Eye or eyes swollen	3	5	3	2
<u>Females</u> (No. exam.)	60	60	60	60
Opacity - eye(s)	1	2	1	0
Eye or eyes swollen	1	2	1	0

5. Blood smears were apparently not obtained at 12 months or at 18 months in 10/sex/dose, as specified in the Pesticide Assessment Guidelines, Subpart F (1982). Instead, blood was collected at sacrifice at approximately 24 months for hematology and clinical analysis from 42-52 animals per group. 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 corpuscular HGB conc. (MCHC)
x	Erythrocyte count (RBC)*	x	Mean corpuscular volume (MCV)
	Platelet count*		Reticulocyte count
	Blood Clotting Measurements		
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* Required for subchronic and chronic studies

Results- Since differential blood counts were not taken at 12 or 18 months it is not possible to compare final results to values on test. Terminal values are shown in Tables 13 and 14 from the test report (appended pages 5-8).

Males- Terminal sacrifice- Mean corpuscular hemoglobin was significantly higher as compared to controls at all doses ($p < 0.05$). Mean corpuscular volume was significantly higher at 12500 ppm. Erythrocyte (RBC) count was significantly lower at 100 ppm and 12500 ppm. RBC was also lower at 1000 ppm but the difference was not significant. Leukocyte counts were significantly lower at 100, 1000, and 12500 ppm ($p < 0.05$). Monocyte counts were significantly higher at 12500 ppm and were also higher at 100 and 1000 with great variation.

Females- Terminal sacrifice- Hemoglobin and mean corpuscular hemoglobin concentration were significantly higher at 12500 ppm ($p < 0.05$) as compared to controls. Leukocyte counts were slightly higher at all doses.

The test compound appears to lower red blood cell count and leukocyte count in males, and to elevate hemoglobin in high dose females, but to raise female leukocyte counts slightly. The data indicate a possible hematopoietic or hemolytic effect of the test compound. There were no additional data to support these findings.

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Hematology Effects in Mice Fed EL-107 for Two Years

<u>Males</u>	RBC (10 ⁶)	MCV (FL)	MCH (PG)	Leuk (10 ³)	Monos (%)
0	9.9	46.0	15.3	5.9	0.9
100	9.4*	46.7	15.6*	4.3*	1.6
1000	9.6	46.7	15.6*	4.0*	1.5
12500	9.1*	47.3*	15.9*	4.2*	1.7*

<u>Females</u>	MCHC (%)	HGB (g/dl)
0	33.6	14.5
100	33.6	14.6
1000	33.5	14.8
12500	34.1*	15.1*

* Significantly different from control values at $p \leq 0.05$; Dunnett's T, two-tailed.

b. Clinical Chemistry

<u>X</u>	Electrolytes:	<u>X</u>	Other:
	Calcium*		Albumin*
	Chloride*	x	Blood creatinine*
	Magnesium*	x	Blood urea nitrogen*
	Phosphorous*		Cholesterol*
	Potassium*		Globulins
	Sodium*	x	Glucose*
	Enzymes	x	Total Bilirubin*
x	Alkaline phosphatase (ALP)		Total Serum Protein*
	Cholinesterase#		Triglycerides
	Creatinine phosphokinase*°		Serum protein electrophoresis
	Lactic acid dehydrogenase		
x	Serum alanine aminotransferase (ALT and also SGPT)*		
	Serum aspartate aminotransferase (AST and also SGOT)*		
	gamma glutamyl transferase		
	glutamate dehydrogenase		

* Required for subchronic and chronic studies

Should be required for OP

° Not required for subchronic studies

Terminal values are shown in attached Table 15 (appended pages 9-10 from the test report).

Results-

Terminal sacrifice- Males- At 1000 and 12500 ppm glucose was significantly higher than controls ($p < 0.05$). Total bilirubin was significantly lower than controls at 100 and 1000 ppm and slightly lower at 12500 (N.S.). Alkaline phosphatase (ALP) and alanine transaminase (ALT) were significantly higher at 12500 ppm ($p < 0.05$). Creatinine was significantly higher at 1000 ppm ($p < 0.05$).

Females- At 12500 ppm blood urea nitrogen and ALT were significantly higher than controls ($p < 0.05$).

Liver involvement was demonstrated by elevated levels of alkaline phosphatase in males at the high dose and in elevated levels of alanine transaminase in males and females at the high dose. There was a dose-related effect on glucose levels in males in which levels were higher than controls. Liver effects are discussed in Section 7 (Sacrifice and Pathology) of this report.

Clinical Pathology Effects in Mice Fed EL-107 for Two Years

Dose (ppm)	Glucose (mg/dl)	Creatinine (mg/dl)	T.Bili (mg/dl)	ALP (IU/L)	ALT (IU/L)
<u>Males</u>					
0	145.5	0.32	0.23	80.0	139
100	151.7	0.35	0.17*	69.1	75
1000	172.2*	0.37*	0.17*	90.1	126
12500	170.0*	0.35	0.20	157.8*	332*
<u>Females</u>	BUN (mg/dl)	ALT (IU/L)			
0	17.5	65.1			
100	15.4	52.3			
1000	18.4	68.6			
12500	18.2*	91.4*			

* Significantly different from controls at $p \leq 0.05$, Dunnett's T, two tailed.

6. Urinalysis

Urinalysis was apparently not performed.

7. Sacrifice and Pathology -

All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

<u>X</u>	<u>X</u>	<u>X</u>
Digestive system	Cardiovasc./Hemat.	Neurologic
Tongue	.Aorta*	x .Brain*†
x .Salivary glands*	xx .Heart*	Periph. nerve*#
.Esophagus*	x .Bone marrow*	Spinal cord (3 levels)*#
x .Stomach*	x .Lymph nodes*	x .Pituitary*
x .Duodenum*	xx .Spleen*	x .Eyes (optic n.)*#
x .Jejunum*	x .Thymus*	Glandular
x .Ileum*	Urogenital	.Adrenals*
.Cecum*	xx .Kidneys*†	Lacrimal gland#
x .Colon*	x .Urinary bladder*	x Mammary gland*#
.Rectum*	xx .Testes*†	.Parathyroids*††
xx .Liver*†	Epididymides	x .Thyroids*††
Gall bladder*#	x Prostate	Other
x .Pancreas*	Seminal vesicle	x Bone*#
Respiratory	xx Ovaries*†	x Skeletal muscle*#
.Trachea*	xx .Uterus*	x Skin*#
x .Lung*	(ovaries and uterus	x All gross lesions
Nose°	were attached)	and masses*
Pharynx°		
Larynx°		

- * Required for subchronic and chronic studies
- ° Required for chronic inhalation
- # In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
- † Organ weights required in subchronic and chronic studies
- †† Organ weight required for non-rodent studies

Results-

a. Organ weight and Relative organ weight- Tables 22 and 25 from the test report (appended pages 11-14) show the values of interest.

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Males- Terminal sacrifice- Kidney weight with adrenals was higher than controls at 100 ppm and 1000 ppm ($p < 0.05$ at 1000 ppm). However, kidney weights were lower than controls at 12500 ppm. Liver weight and relative liver weight was significantly higher than controls at 12500 ppm ($p < 0.05$).

Females- Terminal sacrifice- Liver weight and relative liver weight were significantly higher than controls at 12500 ppm ($p < 0.05$). Relative kidney weight was significantly lower than controls at 12500 ppm as was relative spleen weight at this dose.

b. Gross pathology

At gross examination increases in nodules and lesions were observed in the livers of animals sacrificed at 24 months and dying on test, as shown in the following table.

Gross Pathology of the Liver of B6C3F₁ Mice^a fed EL-107 for Two Years

Dose (ppm)	0	100	1000	12500
<u>Males</u>				
No. examined	60	59	60	60
nodules	26	15	21	35
lesions	6	4	7	9
<u>Females</u>				
No. examined	60	60	59	60
nodules	4	7	7	16
lesions	5	3	4	6

a. Combined Report No. MC0809 (Replicate Report Nos. M00883 and M00983).

c. Microscopic pathology

1) Non-neoplastic - There was a significant increase in hepatocellular cytomegaly, hepatocellular hyperplasia and hepatocellular vacuolation as shown in the following table.

Incidence of Non-neoplastic Lesions in the Liver in B6C3F₁ Mice (MC0809)^a

Dose (ppm)	0	100	1000	12500
<u>Males</u>				
No. examined	60	59	60	59
Hepatocellular-				
Cytomegaly	9	2	7	33
Hyperplasia	19	11	16	27
Vacuolation	5	5	10	22
<u>Females</u>				
No. examined	60	60	59	60
Hepatocellular-				
Cytomegaly	0	0	0	4
Hyperplasia	3	2	6	20
Vacuolation	4	4	11	40

a. From Combined Report No. MC0809 (Replicate Report Nos. M00883 and M00983).

Increases in hepatocellular cytomegaly occurred in males at the high dose. Increases in hepatocellular nodular hyperplasia occurred in males and females at the high dose. Increases in hepatocellular vacuolation occurred in males and females at the mid and high doses. The severity of vacuolation increased with dose as shown in the following table. For numbers examined refer to the above table.

Incidence and Severity of Vacuolation in the Liver of B6C3F₁ Mice

Dose (ppm)	0	100	1000	12500
<u>Males</u>				
minimal vacuolation	3	0	1	1
slight vacuolation	2	3	4	13
moderate vacuolation	0	2	5	8
Total/males	5	5	10	22
<u>Females</u>				
minimal vacuolation	3	1	1	2
slight vacuolation	1	3	10	16
moderate vacuolation	0	0	0	22
Total/females	4	4	11	40

The findings demonstrate that Isoxaben is capable of inducing detectable abnormal cell proliferation and degeneration in the liver of B6C3F₁ mice. These findings support the following neoplastic observations.

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2) Neoplastic- Terminal sacrifice- There were increases in the combined incidence of hepatocellular adenomas and hepatocellular carcinomas as demonstrated in the following table. The increases were related primarily to increases in the numbers of adenomas. There was no apparent decrease in time of onset of the observed hepatocellular histopathology, also shown in the table.

Incidence of Neoplastic Lesions in the Liver of B6C3F₁ Mice^a

Dose (ppm)	0	100	1000	12500
<u>Males</u>				
No. examined	60	59	60	59
Hepatocellular adenomas	3(24) ^b	2(24)	4(23)	12(24)
Hepatocellular carcinomas	9(19)	5 (4)	5(22)	5(24)
Combined adenomas + carcinomas	12	7	9	17
<u>Females</u>				
No. examined	60	60	59	60
Hepatocellular adenomas	0	3(24)	2(24)	7(21)
Hepatocellular carcinomas	0	1(24)	0	2(24)
Combined adenomas + carcinomas	0	4	2	9

a. From combined Report No. MC0809 (Replicate Report No. M00883 and M00983).

b. The number in parentheses shows the month when the first observation was made.

The microscopic pathology findings indicate Isoxaben is capable of inducing detectable abnormal cell proliferation in the liver. There was an apparent increase in hepatic proliferative lesions with the following observations:

1. Males and females at the high dose show an increase in hepatocellular hyperplasia and hepatocellular adenomas.
2. Females at the high dose show a mild increase in hepatocellular carcinomas.
3. Males and females show increases in hepatocellular vacuolation at the mid and high doses.
4. The test compound did not demonstrate decreased latency.
5. The no observed effect level is 100 ppm.

Statistical analysis of the results appears in Appendix I from Test Report MC0809 (appended pages 22-28).

D. DISCUSSION:

1. Comments on report statements which claim effects seen in only one replicate are not significant.

The combined results of both replicate studies show EL-107 produced a decrease in survival at the low dose in males and a decrease in survival at the mid dose in females. The report stated (p.7) "The findings in the replicate studies were similar." Justification for using replicate studies appeared on pp. 13-14 of the test report: This method allows "...comparisons to be made between separate but identical control and treatment groups." The report later states that decreased survival which was apparently seen in one replicate but not the other was not significant. Survival was lower in one replicate than the other at these doses, however, survival was lower in both replicates than in the control groups for most of the two-year period in males and for the last ten months in females.

This review will consider only the combination of the two replicate studies as the basis for any conclusions on the toxicity of the test compound.

2. Food and water consumption were not reported for this study. Food and water consumption should be measured for the first 13 months and then at monthly intervals according to the Pesticide Assessment Guidelines, Subpart F. Compound intake as reported must be considered approximate for this study. Food efficiency cannot be calculated from this information.
3. Hematology: Blood smears in 10/sex/dose were not taken at 12 and 18 months, as specified in the Guidelines. The effect of the test compound on blood cell morphology during the study is therefore unknown. Body weight measurements and clinical observations are the only measures of toxicity during the course of the test.

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Reviewed by: Marcia van Gemert, Ph.D. *M. van Gemert 1.9.87*
Section III, Tox. Branch (TS-769C)
Secondary reviewer: Theodore M. Farber, Ph.D.
Chief, Tox. Branch (TS-769C)

1/15/87
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DATA EVALUATION REPORT

STUDY TYPE: Carcinogenicity/ Oncogenicity
Study in Rats

TOX. CHEM. NO.: 419F

ACCESSION NUMBER: 265735, 265736

MRID NO.: ?

TEST MATERIAL: Isoxaben technical

SYNONYMS: EL 107

STUDY NUMBER(S): KO 1583, KO 1683

SPONSOR: Elianco Products Co.

TESTING FACILITY: Toxicology Division, Lilly Research Laboratories
Greenfield, Indiana 46140

TITLE OF REPORT: A Two-year toxicity/oncogenicity study of EL-107
administration to Fischer 344 rats

AUTHOR(S): S.G. Lake

REPORT ISSUED: Nov. 1985

CONCLUSIONS: EL-107 toxicity seen in both liver and kidney which was manifested in clinical chemistry parameters, histopathology and organ weight parameters. Other organ weight parameters such as brain and heart/body weight, ovaries/body weight and prostate/brain weight were affected in the high dose. Body weights and body weight gains were decreased in the high dose males and females and clinical chemistry parameters mostly associated with liver and kidney were affected in the mid and high dose.
Therefore the NOEL = 0.0125% in the diet or 5 mg/kg for males
and 6.2 mg/kg for females

LEL = 0.125% in diet or 50.7 mg/kg for males and
51.8 mg/kg for females

Classification: core-Minimum, although it would have been a more thorough study if they had investigated ophthalmological parameters, and they should have histopathologically investigated parathyroids more vigorously. Only a few glands were sectioned, and all but one of those investigated showed signs of hyperplasia.

Special Review Criteria (40 CFR 154.7)

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A. MATERIALS: MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

1. Test compound: EL-107, a mixture of isomers consisting of Lilly compound 121607 [redacted] Lilly compound 135520 [redacted] and Lilly compound 173490 [redacted], Description- not given, Batch # 210025, Purity 94.8%,
2. Test animals: Species: Rat, Strain: Fischer 344, Age: 5-6 weeks
 Wt. 1583 males-108.4 ± 1.2 gms Source: Harlan Sprague Dawley Inc.
 females- 90.4 ± 0.9 gms Indianapolis, Ind.
 1683 males- 88.5 ± 0.9 gms
 females- 851 ± 1.1 gms

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned randomly to replicate experiments in the following test groups.

Test Group	Dose in diet %	KO 1583		KO 1683	
		24 months male	24 months female	24 months male	24 months female
1 Cont.	0.0	30	30	30	30
2 Low (LDT)	0.0125	30	30	30	30
3 Mid (MDT)	0.125	30	30	30	30
4 High(HDT)	1.25	30	30	30	30

2. Diet preparation

Diet was prepared every two weeks and stored at room temperature. Samples of all levels of fresh diet mixtures were collected from batches at the beginning and end of the two studies as well as from batches at approximately 4 month intervals after study initiation and assayed for EL-107 activity. Homogeneity and stability were evaluated on 5 samples of feed collected at random. One-half of the samples were stored at 25°C and the other half at 37°C for 0, 1, 2, and 4 weeks for stability measurements.

Results - Total activity of test article determined at approximately 3, 6, 12, and 24 months after study initiation was 95%, 94.8%, 92.2%, 94.2% and 95% respectively. Test article was judged stable for at least 4 weeks in mash diet at both 25°C and 37°C and was shown to be homogeneously distributed throughout the diet.

3. Animals received food, mash feed of Purina Certified Rodent Chow No 5002, and water ad libitum.

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4. Statistics - The procedures on appended page 1 were utilized in analyzing the numerical data.
5. Quality assurance was certified, signed and dated Nov. 27, 1985.

C. METHODS AND RESULTS:

1. Observations

Animals were inspected daily for signs of toxicity and mortality. A detailed exam was performed each week for muscle tone, condition of pelage, color and appearance of eyes, respiration, posture, excreta, locomotion and presence of external lesions or growths.

Mortality (survival): In both replicates there was a decreased survival in high dose males during treatment in the last month.

Survival for 2 years in the combined replicates was 66, 57, 60 and 48% for groups 1,2,3 and 4 respectively.

Since most of the deaths occurred after 700 days, early deaths are not an important consideration in the adjustment of the tumor incidence.

Toxicity: No treatment-related signs of toxicity were evident except for a general thinning condition seen especially in the males toward the last 2-3 weeks of treatment.

2. Body weight

Animals were weighed weekly for the duration of the experiment.

Results: Appended pages 2 and 3 give a summary of each replicate experiment's terminal body weight and body weight gain. In both replicates there was a significant drop in body weight and body weight gain in both males and females at the high dose ($p > 0.05$) throughout the course of the study there was intermittently significant decreases in female body weight at the mid dose. However, the low dose females body weights and body weight gains were similar to controls.

3. Food consumption and compound intake

Consumption was determined and mean daily diet consumption was calculated. Efficiency and compound intake were calculated from the consumption and body weight gain data.

Results: No compound-related decreases in food consumption were noted. As can be seen on appended pages 2 and 3

food efficiency was decreased in both replicates in both males and females at the high dose. Throughout the study mid dose females exhibited some statistically significant decreases in food efficiency.

Compound intake: Time weighted averages of daily doses for combined replicates were 5.0, 50.7, and 526.5 mg/kg for males and 6.2, 61.8, and 646.6 mg/kg for females in groups 2, 3, and 4 respectively.

4. Ophthalmological examinations

These did not appear to be performed on any animals.

b. Blood was collected before treatment and at 6, 12, and 24 months on the first 10 rats of each sex/dose level from study RO 1683 after an overnight fast for hematology and clinical analysis The CHECKED (X) parameters were examined.

a. Hematology

X		X	
X	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*	X	Mean corpuscular HGB (MCH)
X	Leukocyte count (WBC)*	X	Mean corpuscular HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)
X	Platelet count*		Reticulocyte count
	Blood Clotting Measurements	X	Erythrocyte morphology
	(Thromboplastin time)		
	(Clotting time)		
	(Prothrombin time)		

* required for subchronic and chronic studies

Results: Data appear in the hematology section for an unrelated study (RC 1516). It isn't clear what these summary tables were meant to convey. However, after reviewing the data for both replicate experiments, it appears that there are no treatment-related effects.

b. Clinical Chemistry

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<u>X</u>	<u>X</u>
Electrolytes:	Other:
X Calcium*	X Albumin*
X Chloride*	XX Blood creatinine*
Magnesium*	XX Blood urea nitrogen*
X Phosphorous*	X Cholesterol*
X Potassium*	Globulins
X Sodium*	XX Glucose*
Enzymes	X Total Bilirubin*
XX Alkaline phosphatase	XX Total Serum Protein*
Cholinesterase#	X Triglycerides
X Creatinine phosphokinase*°	Serum protein electrophoresis
Lactic acid dehydrogenase	
XX Serum alanine aminotransferase (also SGPT)*	
X Serum aspartate aminotransferase (also SGOT)*	
gamma glutamyl transferase	
glutamate dehydrogenase	

- * Required for subchronic and chronic studies
- # Should be required for OP
- ° Not required for subchronic studies
- XX- all surviving animals were also bled and assayed

Results: Various clinical chemistry parameters were significantly changed from controls in the mid and high dose groups and appeared to be compound related. Blood samples were taken from 10/sex/dose at 6, 12, 18 and 24 months from the orbital sinus from study RO 1683 animals. Blood urea nitrogen levels in these animals were significantly increased ($p > 0.05$) over control levels in the mid and high dose males at 18 months and the high dose males at 24 months. Terminal cardiac puncture blood samples of study RO 1583 and RO 1683 confirm this increase in both mid and high dose males. High dose female BUN was significantly increased in the 12 month interim orbital blood samples only, however, again at the terminal blood samples both high dose replicates had significantly increased BUN levels, and the mid dose RO 1683 females also had increased BUN levels. Refer to table I for details. Appended pages 4 and 5 summarize the statistical significance of the orbital bleeding clinical chemistry.

At 24 months both the orbital sinus samples and the cardiac puncture replicate samples in males showed an increased creatinine level. Both increased BUN and creatinine levels reflect the renal changes that were seen histologically, according to the study text.

Orbital samples at 6, 12 and 18 months revealed a statistically significant decrease in alkaline phosphatase in both males and females at the high dose, and mid dose males at 18 months actually showed an increase in alkaline phosphatase. At terminal sacrifice cardiac puncture samples, only mid dose females in replicate experiment RO 1683 appeared to have decreased alkaline phosphatase levels.

orbital samples at 18 and 24 months both in males and females showed an increase in cholesterol levels at the high dose and the high dose males showed an increased phosphorous level at 24 months as well. The study text regards these changes as secondary to progressive glomerular disease observed histologically.

No other treatment related changes were observed in clinical chemistry parameters.

TABLE I

Cardiac Puncture Terminal Blood Samples
Clinical Chemistry Parameters

	Study RO 1583		Study RO 1683	
Sex:	BUN mg/Dl	Creatinine mg/dl	BUN mg/Dl	Creatinine mg/Dl
Group				
D	17.26	0.6	15.37	0.642
D	2.87	0.17	2.24	0.124
D	22.54	0.732	17.5	0.767
D	10.61	0.197	0.7	0.186
D	20.35*	0.712	16.9	0.8
D	4.16	0.206	3.68	0.194
D	28.81*	0.015*	20.36*	1.043*
D	7.86	0.426	4.19	0.294
Females				
D	22.8	0.535	13.26	0.569
D	37.6	0.443	2.09	0.170
D	14.7	0.437	14.32	0.518
D	4.4	0.154	2.73	0.117
D	14.4	0.433	15.51*	0.553
D	1.7	0.102	2.19	0.106
D	19.8*	0.479	17.82*	0.602
D	15.1	0.204	5.88	0.126

p > 0.05

Urinalysis

Urine was collected from fasted animals at 6, 12, 18 and 24 months. The CHECKED (X) parameters were examined. The same 10/sex/dose of RO 1683 as the clinical chemistry parameters were examined.

(X) Appearance*	(X) Glucose*
(X) Volume*	(X) Ketones*
(X) Specific gravity*	(X) Bilirubin*
(X) pH	(X) Blood*
(X) Sediment (microscopic)*	Nitrate
(X) Protein*	Urobilinogen
(X) Clarity	

* Required for chronic studies

° Not required for subchronic studies

Results: no treatment-related changes in urinalysis were evident.

7. Sacrifice and Pathology -

All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

	X	Cardiovasc./Hemat.	X	Neurologic
Digestive system				
Tongue	X	.Aorta*	XX	.Brain*†
X .Salivary glands*	XX	.Heart*		Periph. nerve*‡
X .Esophagus*	X	.Bone marrow*		Spinal cord (3 levels)*‡
X .Stomach*	X	.Lymph nodes*	X	.Pituitary*
X .Duodenum*	XX	.Spleen*	X	.Eyes (optic n.)*‡
X .Jejunum*	X	.Thymus*		Glandular
X .Ileum*		Urogenital	XX	.Adrenals*
X .Cecum*	XX	.Kidneys*†		Lacrimal gland‡
X .Colon*	X	.Urinary bladder*	X	.Mammary gland*‡
X .Rectum*	XX	.Testes*†		.Parathyroids*††
X .Liver*†		Epididymides	XX	.Thyroids*††
Gall bladder*‡	XX	Prostate		Other
X .Pancreas*		Seminal vesicle	X	.Bone*‡
Respiratory	XX	.Ovaries*†	X	.Skeletal muscle*‡
X .Trachea*	XX	.Uterus*	X	.Skin*‡
X .Lung*			X	All gross lesions and masses*
nose°				
Pharynx°				
Larynx°				

* Required for subchronic and chronic studies

° Required for chronic inhalation

‡ In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement

† Organ weights required in subchronic and chronic studies

†† Organ weight required for non-routent studies

a. Organ weight

Absolute organ weights:

High dose males in replicate 1583 had a significant decrease in prostate weights while high dose males in replicate 1683 showed a significant increase in liver weights. These data are presented on table III.

Organ/body weight ratios:

High dose males in both replicates had increased organ/body weight ratios for liver, kidney, heart and brain. Replicate 1683 also showed an increase in high dose male adrenal/body weight ratios.

High dose females of both replicates also had increased liver, kidney and brain/body weight ratios and in addition high dose females also had increased parathyroid, thyroid/body weight ratios, and ovary/body weight ratios. These data are presented on table IV.

Organ/brain weight ratios:

The picture is not as clear for organ/brain weight ratios, perhaps because brain/body weight ratios were changed so significantly. However, replicate experiment 1583 high dose males had decreased prostate/brain weight and replicate 1683 high dose males had increased kidney and liver/brain weight ratios. These data are presented on table V.

Table III

TERMINAL ORGAN WEIGHTS

Study RO 1583 Males		Study RO 1683 Males	
Group	Prostate g/100g	Group	Liver g/100g
1	0.396	1	12.469
SD	0.137	SD	2.025
2	0.440	2	13.229
SD	0.183	SD	3.983
3	0.375	3	13.135
SD	0.190	SD	2.133
4	0.297*	4	13.882*
SD	0.098	SD	1.792

Table IV

TERMINAL ORGAN WEIGHTS RELATIVE TO BODY WEIGHTS

Study RO 1583

Males:

Group	Liver g/100g	Kidneys g/100g	Heart g/100g	Brain g/100g
1	2.923	0.5569	0.3152	0.463
SD	0.357	0.0970	0.0744	0.069
2	2.387	0.5688	0.3026	0.444
SD	0.503	0.0914	0.0707	0.052
3	3.031	0.5608	0.3082	0.462
SD	0.511	0.1269	0.0739	0.095
4	3.592*	0.3181*	0.3608*	0.526*
SD	0.538	0.1410	0.0160	0.040

Table IV - cont.

Study RO 1683

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Males					
Group	Liver g/100g	Kidneys g/100g	Heart g/100g	Brain g/100g	Adrenals g/100g
1	2.772	0.6525	0.3047	0.454	15.4
SD	0.518	0.0987	0.0691	0.044	4.5
2	2.992	0.6904	0.2971	0.463	29.7
SD	0.817	0.0870	0.0481	0.041	40.1
3	3.040	0.6976	0.3273*	0.470	16.5
SD	0.546	0.0710	0.0420	0.036	6.3
4	3.689*	0.9052*	0.3648*	0.536*	31.8*
SD	0.731	0.2341	0.0617	0.080	35.3

* = p > 0.05

Study RO 1583

Females					
Group	Liver g/100g	Kidneys g/100g	para thy/ Thyroids g/100g	Ovaries g/100g	Brain g/100g
1	2.796	0.6442	7.47	57.98	0.587
SD	0.591	0.1491	1.28	143.92	0.160
2	2.727	0.6480	10.17	42.34	0.559
SD	0.542	0.1536	10.53	33.78	0.089
3	2.755	0.6320	8.13	28.32	0.544
SD	0.432	0.0734	2.73	7.96	0.050
4	3.402*	0.7895*	9.46*	69.79*	0.671*
SD	0.749	0.1781	2.44	120.34	0.156

Study RO 1683

Females					
Group	Liver g/100g	Kidneys g/100gm	Thyroids g/100g	Ovaries g/100g	Brain g/100g
1	2.717	0.6672	3.82	31.23	0.570
SD	0.400	0.0909	3.44	7.97	0.063
2	2.636	0.6376	3.51	32.93	0.540
SD	0.315	0.0480	3.11	7.57	0.043
3	2.833	0.6603	9.35	33.34	0.542
SD	0.393	0.0770	2.57	5.88	0.079
4	3.258*	0.7670*	10.72*	40.40*	0.642*
SD	0.520	0.1497	3.95	8.84	0.074

Table V

TERMINAL ORGAN WEIGHTS RELATIVE TO BRAIN WEIGHTS

Study RO 1583, Males		Study RO 1683 Males		
Group	Prostate g/g	Group	Liver g/g	Kidneys g/g
1	0.199	1	6.100	1.4363
SD	0.073	SD	0.955	0.1543
2	0.222	2	6.527	1.4939
SD	0.089	SD	2.004	0.1605
3	0.188	3	6.461	1.4868
SD	0.096	SD	0.989	0.1637
4	0.148*	4	6.892*	1.6874*
SD	0.049	SD	0.957	0.0890

* = p > 0.05

b. Gross pathology

Results: There appeared to be some treatment-related whole tissue alterations in the kidney. The males showed an incidence of 16/59, 30/60, 27/60 and 39/60 in groups 1, 2, 3, and 4 respectively. Females showed an incidence of 21/61, 15/60, 23/60 and 38/60 in groups 1, 2, 3, and 4 respectively.

c. Non-neoplastic

There was an increased incidence in progressive glomerulonephrosis which appeared to be exacerbated by EL-107b in males and females in the high dose, and females of the mid dose group. There was also an increase in the severity of the glomerulonephrosis in both males and females of the high dose. There was also a slight increase in mineralization of the aorta, stomach mucosa kidney and heart. These numbers can be seen on table II. These mineralization changes are usually secondary to renal failure, and can be explained by the increased glomerulonephritis.

There was an increase in parathyroid hyperplasia at the high dose. Very few animals were examined, but except for one control animal, every parathyroid examined showed hyperplasia. (see table II) The protocol did not specify examination of the parathyroids as routine histopathological tissue treatment, and enlarged parathyroids were not noted on gross examination. So it is unclear how these parathyroids were found. The study text attributes the increased hyperplasia to a consequence of nephrocalcinosis.

d. Neoplastic:

There was a very slight increase in the mid and high dose animals of hepatocellular adenoma. (see table III and appended page 6 for details). The subchronic rat studies had indicated that liver was a target organ, so the study directors had all the liver slides re-examined by a second veterinary pathologist. This is a very low incidence of adenoma. However, they did not submit any historical controls from their laboratory for our consideration.

Data from the mouse oncogenicity study indicates an increased incidence of hepatocellular adenomas. The peer review committee should examine these tumors and decide if there is a compound-related effect.

There was also an increase in adrenal pheochromocytomas in the males at the high dose. These numbers can be seen on table VII and on appended page 7. This increased tumor incidence appears slight however, the peer review committee may also want to examine the incidence before reaching a conclusion. Complete tumor tables have been appended starting on appended page 8 for peer review reference.

Table VI

Non-neoplastic findings

Combined studies K01583 and K01683

Males		Females							
Groups	N	1	2	3	4	1	2	3	4
Kidney	N	59	60	60	60	61	60	60	60
minimal progressive glomerulonephrosis		10	15	12	6	23	17	22	14
slight progressive glomerulonephrosis		11	13	19	15	8	5	11	19
moderate progressive glomerulonephrosis		5	5	8	26	2	5	1	12
severe progressive glomerulonephrosis		0	0	1	4	1	0	1	2
Total		26	33	40	51	34	27	35	47
Heart	N	59	61	60	60	60	60	60	60
Multiple Mineralization		0	0	1	3	0	0	1	1
Aorta	N	59	61	60	60	60	60	60	60
Mineralization		2	0	1	5	0	1	3	0
Stomach	N	59	61	60	60	60	60	60	60
Mineralization of Mucosa		2	1	1	7	1	2	1	0
Parathyroid	N	2	1	1	5	0	1	0	0
Unilateral Hyperplasia		1	1	1	5	0	1	0	0

Table VII

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Neoplastic Findings

Groups		males				females			
		1	2	3	4	1	2	3	4
Liver:	N	59	61	60	60	60	60	60	60
Hepatocellular Adenoma		0	0	3	2	0	0	0	0
Adrenal	N	59	59	60	60	60	60	60	60
Pheochromo- cytomas		10	9	9	18	3	2	4	1

Discussion: EL-107 toxicity was seen in both liver and kidney and this toxicity was manifested in mid and high dose clinical chemistry parameters such as BUN creatinine and cholesterol, alkaline phosphatase and phosphorous. Other organ weight parameters were affected in the high dose, such as heart and brain/body weight, ovaries/body weight and prostate/brain weight.

Therefore the NOEL = 0.0125% in diet or 5 mg/kg for males and 6.2 mg/kg for females

LEL = 0.125% in diet or 50.7 mg/kg in males and 61.8 mg/kg for females.

The core classification was Minimum, however, several troubling points were raised in this study. No ophthalmological examinations were performed. Parathyroids should be investigated as part of the histopathological regimen. However, only a few tissues were examined, and of those few all but one control exhibited hyperplasia. The study text states that this is a common phenomenon found in animals with progressive glomerulonephrosis, however, this should have been more vigorously investigated.

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Pages 155 through 180 are not included.

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EPA: 68-02-4225
DYNAMAC No. 261-E1-2
February 2, 1987

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DATA EVALUATION RECORD

EL-107

Three-Generation Reproduction Study in Rats

STUDY IDENTIFICATION: Lake, S. G., and Hoyt, J. A. A three-generation reproduction study with EL-107 in the Wistar rat. (Unpublished study Nos. R15382, R03783, and R14183 conducted by the Toxicology Division, Lilly Research Laboratories, Greenfield, IN; dated August 1984.) Accession Nos. 073297 and 073298.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 2-2-87

1. CHEMICAL: EL-107 is a mixture of N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide (Lilly compound 121607), N-[3-(1,1-dimethylbutyl)-5-isoxazolyl]-2,6-dimethoxybenzamide (Lilly compound 135520), and N-[3-(1,1,2-trimethylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide (Lilly compound 173490).
2. TEST MATERIAL: Technical EL-107 from lot No. H02-266-118 was 93.7% pure and from lot No. Z10025 was 94.5% pure.
3. STUDY/ACTION TYPE: Three-generation reproduction study in rats.
4. STUDY IDENTIFICATION: Lake, S. G., and Hoyt, J. A. A three-generation reproduction study with EL-107 in the Wistar rat. (Unpublished study Nos. R15382, R03783, and R14183 conducted by the Toxicology Division, Lilly Research Laboratories, Greenfield, IN; dated August 1984.) Accession Nos. 073297 and 073298.

5. REVIEWED BY:

Michael J. Norvell, Ph.D. and
Michael A. Gallo, Ph.D.
Principal Reviewers
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Signature: G. Hillebray for
Date: 2-2-87

Patricia A. Turck, M.S.
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Date: 2/2/87

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Margaret L. Jones
EPA Reviewer

Signature: Margaret Jones
Date: 2-3-87

Maria Van Gemert, Ph.D., D.A.B.T.
EPA Section Head

Signature: Maria Van Gemert
Date: 2/5/87

7. CONCLUSIONS:

- A. The parental NOEL and LOEL values for rats fed EL-107 continuously in the diet are 0.05 and 0.25% or 500 and 2500 ppm EL-107, respectively. These values are based on statistically significant ($p \leq 0.05$) depressions in mean body weight, mean body weight gain, and mean efficiency of food utilization during growth, gestational, and lactational periods of parental females at the 0.25% (2500 ppm) and 1.25% (12,500 ppm) dietary level and on decreased body weights during growth phases of parental males at the 1.25% (12,500 ppm) level. In addition, these effect levels are based on increased mean relative liver weights of parental males and females at the 1.25% (12,500 ppm) dietary level and of parental females at the 0.25% (2500 ppm) level.

The reproductive toxicological NOEL and LOEL values for rats are 0.25 and 1.25% or 2500 and 12,500 ppm EL-107, respectively, based on decreased numbers ($p \leq 0.05$) of viable pups born during the F_{2a} and F_{2b} generations, statistically significant ($p \leq 0.05$) depressions in the mean body weights of progeny on postpartum day 21, and increased incidences of microphthalmia at the 1.25% (12,500 ppm) dietary level.

The NOEL and LOEL for embryo/fetal toxicity in rats are 0.25 and 1.25% or 2000 and 12,500 ppm EL-107, respectively, based on significant ($p \leq 0.05$) decreases in the numbers of viable fetuses per litter, increases in resorptions and postimplantation losses, and increased incidences of hydroureter at the 1.25% (12,500 ppm) dietary level when compared to controls for both generations. The biological meaning of increased incidences of microphthalmia in the 1.25% (12,500 ppm) groups is unclear; however, a possible teratogenic effect cannot be completely ruled out.

- B. This study was adequately designed and conducted. The study is classified Core Minimum.

Items 8-10--see footnote 1.

11. MATERIALS AND METHODS (PRGTOCOLS):

A. Materials and Methods:

1. Test Material: The test material consisted of two lots of a mixture of three N substituted 5-isoxazoly-2,6-dimethoxybenzamides, which was incorporated into the diet at concentrations of 0, 0.05, 0.25, and 1.25% or 0, 500, 2500, and 12,500 ppm. Initially, lot No. H02-2G6-118 was used; however, lot No. Z10025 was used from approximately 14 weeks after study initiation. Time-weighted estimates of the intake of the test material during the growth phases (based on average

¹ Only items appropriate to this DER have been included.

daily food consumption, individual body weights, and theoretical dietary concentrations) were approximately 0, 40, 200, or 1000 mg/kg/day, respectively.

Based on an earlier fertility study with this same strain of rat, it was estimated that the test material intake may have been as high as 60, 300, or 1600 mg/kg/day during gestation and lactation.

The test material was administered continuously in the diet over three generations. Each generation was maintained on the test diets for a growth period of 70 days, and rats from the F₀, F₁, and F₂ generations were continued on the test diets for two, three, and one breeding trial, respectively (see Appendix B). All initial batches of the diets used for the F₀ and F₁ generations and all final batches of the diets that were used during the F₁ and F₂ generations were analyzed for EL-107 content. Homogeneity was determined on a single batch of the 0.05% EL-107 test diet. In a previous study, the test material was found to be stable for up to 4 weeks at 25 or 37°C.

2. Reproduction Phase:

- a. Animals and Test System: Weanling Wistar rats were obtained from Harlan Sprague-Dawley, Inc., Indianapolis, IN, and randomly assigned to one of four groups consisting of 25 males and 25 females each.

Females were mated on a one-to-one basis with males after approximately 10 weeks on test diets. Siblings were not mated. The day on which a copulatory plug was found was designated as gestation day (GD) 0; all animals were separated after 14 days and allowed to deliver. For the F₀, F₁, and F₂ parental generations, two, three, and one breeding trial, respectively, were conducted; litters from the last breeding trial of the F₁ generation (F_{2c}) and the F₂ generation breeding trial (F_{3a}) were subjected to complete teratologic evaluation upon sacrifice of dams on GD 20. For the reproduction phases, litters were randomly culled on postpartum day 4 to five males and five females (when possible) and weaned on postpartum day 21.

Twenty-five F_{1a} weanlings/sex/groups were randomly selected to be the F₁ parental animals. In addition, five weanlings/sex/group were grossly examined, and their tissues were collected for histopathologic evaluation. After weaning, F_{1b} offspring were sacrificed and examined for external abnormalities. Rats from the F_{2a} breeding trial were weaned and five/sex/group were randomly chosen for gross necropsy and collection of tissues for histopathological evaluation. Weanlings from the F_{2b} breeding trial were given ophthalmic examinations

prior to the random selection of weanlings for the F₂ parental generation. All remaining F_{2b} weanlings were examined for external and internal abnormalities.

After weaning, parental animals were sacrificed and grossly examined. Livers and reproductive organs were collected for histopathological examination. In addition, eyes from F₂ parental animals were saved and examined histologically. The pregnancy status of uteri with no macroscopic evidence of implantations was confirmed using 10% ammonium sulfide.

- b. Parameters Measured: Parameters determined during the growth phases included daily mortality, weekly body weight and food consumption, average test material intake, and daily clinical observations. Food consumption was not measured during gestation or lactation in this study. Body weights for mated females were measured on GD 0 and 20 and postpartum day 21. Adult and progeny mortality, daily clinical observations, uterine weights, gestation length, the number of corpora lutea and implantations, preimplantation losses, resorptions, numbers of live and dead fetuses, litter size, progeny survival (postpartum days 1-24), adult liver weight, and adult and progeny necropsy findings were evaluated. Litter weights of surviving progeny were measured on postpartum days 1, 7, and 14, and the individual body weights of pups were measured on postpartum days 4 and 21. Pup sex was recorded at the final weighing (day 21). In addition, the eyes of the F₂ adults and the F_{2b} progeny were subjected to histopathologic evaluation.

3. Teratogenicity Phase:

- a. Animals and Test System: Teratologic evaluations were conducted on two generations of rats. From the F_{2b} litters, 25 weanlings/sex/group were randomly selected as F₂ parental animals. In addition, high-dose progeny with microphthalmia (eight males and eight females), 10 control weanlings with linear retinal dysplasia (diagnosed during ophthalmic examinations), and 10 normal control weanlings from each sex were retained as F₂ parental animals. After approximately 70 days on test diets, all animals were mated in the manner described in Section 11.A.2a (Materials and Methods). To obtain F_{2c} litters, F₁ parental animals were again paired. Dams were sacrificed on GD 20, and litters were delivered by caesarean section. Females were grossly examined and livers and reproductive organs were collected for histopathological evaluation. Uteri with no macroscopically visible implantations were immersed in a 10% ammonium sulfide solution to confirm pregnancy status (Salewski, 1964).

- b. Parameters Measured: Clinical observations were conducted daily, and maternal body weights were measured on GD 0, 7, 14, and 20. Food consumption was not measured during gestation. Weights of gravid uteri and maternal livers were recorded at necropsy. The number of corpora lutea and the number, distribution, and type of implantations were recorded. Live fetuses were then weighed, sexed, and examined for external abnormalities. Visceral examinations were conducted on approximately one-half of the fetuses by the methods described by Wilson, and skeletal examinations were conducted on the remaining fetuses by the methods described by Staples and Schell.

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

A. Dietary Analysis: The concentrations of all diet batches analyzed were near the nominal values throughout the study. The test material was found to be homogeneously distributed within the feed and remained stable in diets at 25 and 37°C for up to 4 weeks.

B. Test Material Intake: During the growth periods, rats administered diets containing 0, 0.05, 0.25, or 1.25% EL-107 ingested approximately 0, 40, 200, or 1000 mg/kg/day of the test material, respectively. Male animals consumed slightly less EL-107 than females on a per kilogram of body weight basis.

C. Reproduction Phase:

1. Parental Mortality: One F₀ and two F₁ parental males (in the 0.25% and 1.25% groups, respectively) died. Necropsies of each of these animals indicated that none of the deaths were related to ingestion of the test material.

2. Physical Signs of Toxicity: There were no compound-related physical signs of toxicity in the parental animals. Clinical observations included malocclusion of incisors, chromadocryorrhea, chromorhinorrhea, alopecia, ocular opacity, mechanical or cage injury, urogenital soiling, nodules, and a transient subcutaneous mass.

There was a suggestion of insufficient milk production in at least four females in the 1.25% dose group. Milk was not being produced from the mammary glands of these females, and pup weights indicated that the progeny were not thriving. This problem was observed in the F_{1b} litters and in F_{2a} and F_{2b} litters.

3. Food Consumption and Efficiency of Food Utilization: During the growth periods, there were no significant compound-related differences in cumulative mean daily food consumption for F₀, F₁, and F₂ males and F₀ females (Table 1). During the F₁ and F₂ growth period, food consumption was significantly depressed in females from the high-dose group. There were no significant compound-related differences in feed efficiency in the F₀ and F₁ generation animals. However, during the F₂ growth period, feed efficiency was significantly depressed in females fed 0.25 and 1.25% EL-107. There were no differences in feed efficiency in the F₂ males.
4. Body Weights and Growth: During the growth period, body weight gain for all male rats in the dose groups did not differ significantly from the control values (Table 1). Mean body weights of weanling F₁ males in the high-dose group (1.25%) were depressed at initiation of the growth period. Body weights of the F₂ males of the high-dose group were depressed at the initiation of the growth period and remained depressed throughout the breeding trial. However, there were no significant differences from controls in body weights of either F₀ or F₁ parental males exposed to the test material during the breeding trials. Body weights and body weight gains of F₁ parental females in the high-dose group and of F₂ parental females in the mid- and high-dose groups were depressed during the growth periods. Body weight depression was also observed in pregnant and lactating females in the mid- and high-dose groups (Table 2). During the F₀ generation, significant body weight decreases were observed in females in the high-dose group during the second breeding trial. During the F₁ generation, body weights of females in the mid- and high-dose groups were depressed during the first and second breeding trials. A significant depression in weight gain during gestation was observed in the high-dose group females during all breeding trials. Body weights and weight gain in females from the low-dose group were not adversely affected.
5. Mating Performance and Fertility: A summary of the effects of EL-107 on reproductive parameters during the reproduction phases is presented in Table 3. The test material had no adverse effects on mating performance or fertility during the reproduction phase of this study. The mating of one F₀ control female, one control male, and two females and one male in the low-dose group did not result in pregnancies in either of the two breeding trials. At necropsy, pyometra was found in the control female and one female from the low-dose group. The control male had complete aspermatogenesis of the left testis, focal aspermatogenesis of the right testis, and scant sperm in the tubules of the epididymis. In the F₀ generation, all males of the mid- and high-dose groups sired

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TABLE 1. Summary of Body Weight, Body Weight Gain, and Food Consumption During the Growth Phases of Rats Fed EL-107^a

Sex and Generation	Dietary Concentration (%) ^b	Mean Body Weight (g) on Study Day:		Mean Body Weight Gain (g)	Mean Daily Food Consumption (g)
		0	70		
F ₀ Males	0	77	404	327	21.2
	0.05	75	407	333	21.3
	0.25	76	401	325	21.9
	1.25	75	397	322	21.6
F ₀ Females	0	71	261	190	16.7
	0.05	70	266	196	16.6
	0.25	70	257	188	16.6
	1.25	69	259	189	16.8
F ₁ Males	0	134	474	340	24.6
	0.05	138	482	344	25.0
	0.25	130	460	330	24.5
	1.25	121*	447	326	24.2
F ₁ Females	0	116	287	171	18.4
	0.05	125	291	167	18.7
	0.25	116	271	155	17.8
	1.25	108	252*	145*	17.0*
F ₂ Males	0	172	477	306	24.9
	0.05	167	474	307	24.8
	0.25	161	458	297	24.4
	1.25	141*	427*	286	23.8
F ₂ Females	0	144	307	163	18.1
	0.05	148	305	158	18.3
	0.25	141	278*	137*	17.2
	1.25	121*	251*	131*	16.5*

*Significantly different from control value ($p \leq 0.05$).^aTaken from Table 6 of Test Report for Studies R15382, R03783, R14183.^bCorresponds to 0, 500, 2500, and 12,500 ppm, respectively.

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TABLE 2. Summary of Maternal Body Weights During Gestation and at the End of Lactation of Rats Fed EL-107^a

Progeny Generation	Dietary Concentration (%)	Mean Body Weight (g) on GD:		Mean Body Weight Gain During Gestation (g)	Mean Body Weight (g) on Postpartum Day 21
		0	20		
F1a	0	260	382	122	317
	0.05	264	374	110	314
	0.25	252	366	114	307
	1.25	257	363	107*	300
F1b	0	312	429	117	346
	0.05	316	432	116	345
	0.25	304	420	116	338
	1.25	289*	381*	92*	318*
F2a	0	291	396	106	319
	0.05	292	399	107	317
	0.25	267*	366*	99	300
	1.25	251*	336*	86*	273*
F2b	0	310	428	119	345
	0.05	314	426	112	350
	0.25	286*	391*	105	324*
	1.25	268*	352*	84*	308*

*Significantly different from control value ($p \leq 0.05$).^aTaken from Table 8 of Test Report.

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TABLE 3. Summary of Effects on Reproductive Parameters in Rats Fed EL-107^c

Generation	Dietary Concentration (%)	No. Females		Fertility Index (%)	No. Live Litters Born	Mean No. Live Pups/Litter	Gestation Survival (%) ^a
		Mated	Pregnant				
F _{1a}	0	25	23	92	23	12.8	99.7
	0.05	25	21	84	21	11.5	99.2
	0.25	25	24	96	24	11.9	99.0
	1.25	25	25	100	25	11.5	93.5
F _{1b}	0	25	23	92	23	12.8	97.9
	0.05	25	22	88	22	12.6	93.5
	0.25	25	23	92	23	12.9	99.3
	1.25	25	24	96	24	11.0	98.3
F _{2a}	0	25	21 ^b	84	21	11.0	98.0
	0.05	25	24	96	24	11.2	94.3
	0.25	25	24	96	24	10.0	93.8
	1.25	25	21	84	21	9.6	93.8
F _{2b}	0	25	18	72	18	12.2	98.3
	0.05	25	24	96	23	11.2	94.3
	0.25	25	23	92	23	11.6	97.2
	1.25	25	20	80	18	8.1*	88.6

^aPercentage of newborn progeny alive on postpartum day 0.^bReported value was 20.^cTaken from Tables 10 and 11 of Test Report.*Significantly different from control value ($p < 0.05$).

at least one litter and the majority sired two litters. At least one pregnancy, and in most cases two pregnancies, were recorded for each mid- and high-dose female. In the F₁ generation, only one control male failed to sire a litter during the three breeding trials. This rat had chronic prostatitis, which may have contributed to its failure to sire a litter. All treated F₁ males sired at least one litter and at least one pregnancy occurred for every F₁ female.

6. Gestation Length, Gestation Survival, and Live Born Litter Size: There were no significant compound-related differences in gestation length or in the percentage of F₁ and F₂ pups alive on postpartum day 0. There were no significant differences in the number of liveborn pups per litter in the F_{1a}, F_{1b}, and F_{2a} generations; however, live litter size in the F_{2b} generation was significantly depressed in the high-dose group when compared with controls (Table 3).
7. Progeny Survival: There were no significant variations in survival of liveborn progeny through postpartum day 21, although slight depression in F_{1b} and F_{2b} pup survival was apparent in groups exposed to 1.25% (Table 4). The mean number of pups per litter surviving through postpartum day 21 was similar in the control and low- and mid-dose groups.
8. Progeny Body Weight and Sex Distribution: There were no significant differences in progeny body weights between control and dose groups at postpartum day 1. However, in all four delivery trials, significant depressions in the body weights of high-dose pups were noted at postpartum day 21 (Table 4); mean body weights in the high-dose group were 13-25% less than that of control progeny. Body weights of progeny in the low- and mid-dose groups were not significantly different from the controls at postpartum day 21. The sex distribution of progeny ranged between 46 and 54% males and was reportedly not affected by the test material.
9. Progeny Observations and Examinations: Of the pups that were born dead or died prior to weaning and that were examined externally, one high-dose pup (F_{1a} litter) had exencephaly and a second high-dose pup (F_{2a} litter) had multiple anomalies (exencephaly, edema, protruding tongue, cleft palate, and bilateral microphthalmia). Due to cannibalization not all the pups were examined. Microphthalmia was also observed in two weanlings, one control male and one F_{1a} female in the high-dose group.

Specific abnormalities associated with disruption of fetal development were observed with increased frequency in the F_{2b} pups of high-dose rats. Two litters containing four and five pups, respectively, with exencephaly were born to females of this dose group. Bilateral microphthalmia was

TABLE 4. Summary of Effects on Progeny Survival and Body Weights in Rats Fed EL-107^a

Generation	Dietary Concentration (%)	Pup Survival (%) on Postpartum Days		Pup Body Weight (g) on Postpartum Day		
		0-4	4-21 ^b	4	14	21
F1a	0	92.7	93.7	8.0	23.6	36.1
	0.05	87.1	90.9	8.2	24.1	37.5
	0.25	96.9	92.5	8.0	23.5	35.8
	1.25	87.4	94.5	7.8	21.5	31.4*
F1b	0	93.4	91.7	8.9	26.1	40.6
	0.05	93.0	93.2	8.6	25.5	39.1
	0.25	94.1	97.2	8.4	23.9	36.5
	1.25	91.5	84.3	7.8*	22.5*	32.5*
F2a	0	87.6	99.5	9.2	24.7	36.5
	0.05	92.0	98.3	9.3	24.5	36.7
	0.25	93.7	98.6	9.1	22.6*	33.6
	1.25	95.5	99.3	8.4	20.1*	27.2*
F2b	0	92.2	95.7	8.7	24.4	37.0
	0.05	94.1	96.4	8.8	24.3	37.0
	0.25	98.3	98.0	8.7	23.3	35.3
	1.25	90.7	90.1	8.3	20.6*	30.7*

^aTaken from Tables 12 and 14 of Test Report.

^bLitters were culled on day 4 to a maximum of 10 pups (5 males and 5 females, when possible).

*Significantly different from control value ($p < 0.05$).

associated with the exencephaly, and two of the nine affected pups had additional abnormalities. On examination of the 21-day-old pups, microphthalmia was observed in 10 additional pups from six different litters; one of these pups was from the litter that contained five pups with exencephaly. Microphthalmia was observed in one pup each in the control (F_{1a}) and low-dose (F_{2b}) groups.

Findings in the progeny of the low-dose group included one pup with cleft palate and one pup from a different litter with edema and microstomia. No developmental abnormalities were noted in progeny from the mid-dose group.

Other clinical observations noted in the surviving progeny included ring tail and stub tail, alopecia, progeny cool to the touch, dehydration, injuries, distended abdomen and labored respiration (one low-dose pup), and weakness with uncoordinated movement (one pup from the high-dose group). Although most of these conditions occurred in both control and dose groups, more litters were affected in the high-dose group. Apparent hydronephrosis and hydroureter were noted in both culled and weanling progeny of the control and dose groups, with no indication of a dose response. Additional necropsy findings in the weanling progeny, consisting of incidental changes in the spleen, testes, and uterus, were not considered to be related to test material administration.

10. Ophthalmic Examination: Of the 695 F_{2b} weanling rats subjected to ophthalmic examinations, 79 had ocular abnormalities. Significant compound-related ocular abnormalities were limited to progeny of the high-dose group (Table 5). Ten of these progeny had some degree of microphthalmia and four had a coloboma of the optic disc. The fundus of most of the microphthalmic globes could not be visualized to determine the presence or absence of colobomas.

The degree of microphthalmia noted ranged from mild cases to severe malformation of the globe where only remnants of ocular tissue were discernible behind the eyelids. Varying degrees of iris coloboma and cataract and optic disc coloboma were often associated with microphthalmia. No significant compound-related ocular lesions of postnatal origin were found in this study.

11. Liver Weights: Mean relative liver weights (liver-to-body weight ratio) were significantly increased in F₀ and F₁ parental male rats in the high-dose group (Table 6). The mean relative liver weights were significantly increased among F₀ females from the mid-dose group and in F₁ females from the mid- and high-dose groups.

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TABLE 5. Summary of Eye Defects Noted in F_{2b} Progeny
After Ophthalmic Examination of Rats Fed EL-107^a

Eye Defect	Number (and Percent) of Animals Affected at Dietary Concentration			
	0	0.05	0.25	1.25
Total no. examined	163	204	212	116
Microphthalmia (uni- or bilateral)	0(0)	0(0)	0(0)	10(8.6)
Coloboma optic disc	1(0.6)	1(0.5)	0(0)	4(3.4)
Linear retinal dysplasia (uni- or bilateral)	10(6.1)	5(2.5)	3(1.4)	10(8.6)

^aTaken from individual animal data in Appendix F of Test Report.

TABLE 6. Relative Liver Weights for Three Generations of Parental Rats Fed EL-107^a

Dietary Concentration (%) ^b	Mean Relative Liver Weight (g) for Parental Generation:					
	F ₀		F ₁		F ₂	
	M	F	M	F	M	F
0	4.1	4.8	4.0	4.8	4.1	5.1
0.05	4.2	4.6	4.0	5.0	4.2	5.2
0.25	4.2	5.3*	4.2	5.4*	4.2	5.5*
1.25	4.5*	5.0	4.4*	5.4*	4.2	5.7*

*Significantly different from control value ($p \leq 0.05$).

^aTaken from Table 23 of Test Report.

^bCorrespond to 0, 500, 2500, and 12,500 ppm, respectively.

12. Gross and Microscopic Findings: For the F_{1a} weanlings, only ring tail and hydronephrosis were reported at necropsy. Both findings occurred at low incidences and were unrelated to test material administration.

Minimal vacuolar changes in the liver were observed sporadically in F_{2a} weanlings of both sexes from the control and dose groups. This change appeared to result from increased glycogen accumulation within hepatocytes. The vacuolization was minimal, not dose related, and not considered to be compound related. All other lesions occurred sporadically and were unrelated to test material administration.

Since no compound-related lesions were found in F₀ and F₁ adults, only tissues from the control and high-dose groups were examined microscopically.

In the F₂ adults, retinal folds and/or retinal dysplasia were present in control and dose groups. Retinal folds were more prominent in the control and low- and mid-dose rats whereas retinal dysplasia, anophthalmia, and microphthalmia were more frequent in high-dose animals. Except for the retinal folds, these changes appeared to be compound related. Retinal dysplasia suggested developmental pathogenesis, and the diagnosis of microphthalmia was based on both histologic and clinical examinations. All other lesions occurred in low and sporadic incidences and were not considered compound related.

D. Teratogenicity Phase:

1. Maternal Effects: One high-dose female (F₂ parental generation) died prior to pairing, but this death was not considered to be compound related. No other clinical observations were noted. During the teratogenicity phase, maternal body weights for the F₁ generation were significantly decreased in the high-dose group (Table 7). During the F₂ generation, maternal body weights were significantly lower in the mid- and high-dose groups. Body weights for low-dose animals were similar to controls.

No adverse compound-related effects were noted in the pregnancy rate for either generation (Table 8).

2. Reproductive Parameters: The number of corpora lutea, implantations, and live fetuses were significantly decreased in both F₁ and F₂ high-dose females (Table 8). There were no compound-related differences in preimplantation loss in either group of animals when compared to controls. There were also no significant differences in the proportions of live fetuses, dead fetuses, and resorptions. However, two dead fetuses and one late resorption were observed only in the high-dose group during the third breeding of the F₁ animals.

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TABLE 7. Summary of Maternal Body Weights and Body Weight Gains During Gestation in Rats Fed EL-107^a

Generation	Concentration (%) ^b	Mean Body Weight on GD:				Mean Total Body Weight Gain (g)
		0	7	14	20	
F ₁	0	323	344	370	436	113
	0.05	343	358	386	465	123
	0.25	322	336	364	432	110
	1.25	297	311*	336*	391*	94*
F ₂	0	305	327	355	420	115
	0.05	306	331	355	425	120
	0.25	275*	299*	322*	392	117
	1.25	253*	268*	287*	336	82*

*Significantly different from control value ($p \leq 0.05$).

^aTaken from Table 9 of Test Report.

^bCorresponds to 0, 500, 2500, and 12,500 ppm.

TABLE 8. Summary of Reproductive and Fetal Parameters in Rats Fed EL-107^a

Genera- tion	Dietary Concen- tration (\$) ^g	No. Females		Pregnant Rate(%) ^b	Mean No. Corpora Lutea	Mean No. Implan- tations	Preimplan- tation Loss (%) ^c	Mean No. Live Fetuses	Post- implantation Loss (\$) ^d	Mean No. Resorp- tions	Mean Fetal Body Weight (g) Ratio		Male Sex Ratio (%)
		Mated	Pregnant								Male	Female	
F _{2c}	0	25	21	84	15.2	13.5	13.1	12.0	14.5	1.5	3.7	3.6	51
	0.05	25	19	76	15.4	14.8	5.3	12.9	13.3	1.9	3.7	3.5	49
	0.25	25	24	96	15.0	13.2	12.2	11.0	16.0	2.1	4.1	3.7	50
	1.25	25	23	92	13.2*	12.1*	9.6	9.6*	19.6	2.4	3.8	3.7	48
F _{3a}	0	25	18	72	15.9	14.4	9.4	13.1	10.3	1.3	3.6	3.3	48
	0.05	25	21	84	15.3	12.1	10.9	10.7	20.0	1.4	3.9	3.7	48
	0.25	25	22	88	14.6	12.4	15.1	11.8	5.7	0.6	3.8	3.5	50
	1.25	25	23	92	12.8*	11.3*	12.2	9.1*	21.6	2.1	3.7	3.4	52
of	1.25 ^e	8	7	88	12.6*	11.1*	13.0	7.7*	30.8	3.4	3.9	3.6	44
	10	10	10	100	14.0	13.6	5.8	12.6	6.9	1.0	3.8	3.7	49

^aTaken from Tables 20 and 22 of Test Report.

^bNo. females pregnant/No. females mated x 100.

^c(No. corpora lutea - No. implantations)/No. corpora lutea x 100.

^dRecalculated by the reviewers from individual animal data using (No. implantations - No. live fetuses)/No. implantations x 100.

^eAdditional animals with microphthalmia were mated to produce offspring.

^fAdditional animals with linear retinal dysplasia were mated to produce offspring.

^gCorresponds to 0, 500, 2500, and 12,500 ppm, respectively.

*Significantly different from controls (p ≤ 0.05).

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3. Fetal Parameters: There were no compound-related differences in fetal body weights and sex ratios (Table 8). There were no statistically significant differences in the proportions of normal, variant, and abnormal or in runted fetuses (Table 9). With the exception of one fetus in the low-dose group with multiple anomalies (including exencephaly and cleft palate), the occurrence of frank malformations was limited to six fetuses from three litters in the control group and 12 live and one dead fetus from seven litters of the high-dose group (Table 10). Four of the six control fetuses had cleft palate, and one of the four fetuses with cleft palate also had brachygnathia, microstomia, and microphthalmia. Two control fetuses had omphaloceles. The 12 live fetuses in the high-dose group had unilateral or bilateral microphthalmia. Four of the 12 high-dose fetuses had additional craniofacial malformations, which included hydrocephaly, craniorachischisis, microcephaly, brachygnathia, and cleft palate. The dead fetus from the high-dose group had multiple abnormalities, which included microphthalmia, exencephaly, edema, and missing diaphragm with abdominal viscera displaced into the thoracic cavity. No fetuses with frank malformations were observed in the mid-dose group. Other anomalies were considered developmental variations and were randomly distributed across all groups with no indication of a compound-related effect.
- E. Genealogy: The genealogies of litters that contained abnormal progeny showed interrelationships suggesting that genetic factors may have placed this population of rats at risk with regard to craniofacial defects. In the F_0 generation, the control dam and a dam in the high-dose group with affected progeny were siblings. Of the 17 litters in the high-dose group with abnormal progeny, five resulted from matings of related parents. Eight of the 17 affected litters had rats from supplier litter numbers 13 and 29 in their lineages. One F_1 female in this group had three affected litters with a total of six abnormal pups. Three of the four affected F_{3a} litters also belonged to this lineage. Two of the dams and one of the sires of these three litters were siblings. Two F_1 animals (which were siblings) were parents of the two most severely affected litters; observations for these litters included both exencephaly and bilateral microphthalmia.
13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:
- A. The authors concluded that the NOELs for parental and developmental toxicity were 0.05% (approximately 40 mg/kg/day) and 0.25% (approximately 200 mg/kg/day) EL-107, respectively. The authors identified an "effect level" value of 1.25% EL-107 for both parental and developmental toxicity. This "effect level" value was based on depressed body weights and body weight gains and increased relative liver weights for the parental animals and a

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TABLE 9. Summary of Effect of on Fetal Development
in Rats Fed EL-107^a

	Incidence of Fetuses Affected (%) at Dietary Concentration (%) ^b			
	0	0.05	0.25	1.25
F_{2c} Generation				
Normal fetuses				
Male	63	62	59	46
Female	60	53	51	49
Variant fetuses				
Male	36	35	39	46
Female	35	46	49	42
Abnormal fetuses				
Male	2	2	2	9
Female	5	1	0	9
F_{3a} Generation				
Normal fetuses				
Male	65	74	56	64
Female	56	64	65	50
Variant fetuses				
Male	34	25	44	34
Female	41	35	33	40
Abnormal fetuses				
Male	1	1	0	2
Female	3	1	2	10

^aTaken from Table 22 of Test Report.

^bCorresponds to 0, 500, 2500, and 12,500 ppm, respectively.

TABLE 10. Summary of Fetal Findings in Rats Fed EL-107
Continuously for Three Generations^a

Findings	No. Fetuses (Litters) Affected at Dietary Concentration (%): ^g							
	0		0.05		0.25		1.25	
	F ₁	F ₂	F ₁	F ₂	F ₁	F ₂	F ₁	F ₂
Total No. examined	202(16)	345(27) ^b	245(19)	225(19)	265(24)	259(22)	208(22)	264(27) ^b
Cleft palate	4(2)	0(0)	1(1) ^d	0(0)	0(0)	0(0)	0(0)	1(1) ^e
Brachygnathia	1(1) ^c	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	2(2) ^{e, f}
Exencephaly	0(0)	0(0)	1(1) ^d	0(0)	0(0)	0(0)	0(0)	0(0)
Omphalocele	2(1)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Microphthalmia	1(1) ^c	0(0)	0(0)	0(0)	0(0)	0(0)	5(3)	7(3) ^{e, f}
Hydrocephaly	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	1(1)	1(1)
Hydroureter	4(3)	7(6)	11(8)	6(5)	14(8)	7(5)	28(13)	26(16)

^aTaken from Table 21 of Test Report.

^bIncluded are additional groups with eye defects.

^{c, d, e, f} Same superscript indicates same fetus.

^gCorresponds to 0, 500, 2500, and 12,500 ppm, respectively.

decrease in live litter size due to depressed ovulation. The "effect level" for developmental toxicity was based on retarded growth of progeny and increased incidences of microphthalmia and associated craniofacial anomalies.

B. A quality assurance statement was signed and dated August 8, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

- A. 1. Reproduction Phase: We assess the NOEL and LOEL for parental and developmental toxicology for this study to be 0.05 and 0.25% EL-107, respectively. These values are based on reduced body weight, weight gain, feed efficiency, and increased relative liver weight of parental animals.

The reproductive toxicological NOEL and LOEL values are 0.25 and 1.25% EL-107, respectively. The data indicate a significant depression in body weight on postpartum day 21 and increased incidences of eye defects (such as microphthalmia) in pups from animals in the 1.25% group.

2. Teratogenicity Phase: We agree with the authors that there were significant decreases in the mean numbers of corpora lutea and implantations in the high-dose groups from both generations, with preimplantation losses being comparable between control and dose groups. This suggested that there was a depression in ovulation. Significant decreases in mean numbers of live fetuses/litter and increases in the mean numbers of resorptions and postimplantation losses in the high-dose groups from both generations compared to control indicate a embryo/fetotoxic effect at 1.25% EL-107 in the diet. Furthermore, we assess that the dose-related increases in the incidence of hydroureter in the dose groups when compared to controls from both generations were an indication of embryo/fetotoxicity at the high-dose level.

Increased incidences of microphthalmia in high-dose fetuses from two generations suggest that EL-107 may be teratogenic at 1.25% in the diet. However, because the increases were not dose related and small numbers of fetuses and litters were affected, the results are inconclusive and we were unable to assess the teratogenic potential of EL-107.

- B. The differences between the study authors' and the reviewers' conclusions are summarized as follows:

<u>Item</u>	<u>Study Authors</u>	<u>Reviewers</u>
Parental LOEL	Not stated; an "effect level" is suggested at 1.25% EL-107.	0.25% EL-107
Developmental toxicology LOEL	An "effect level" is suggested at 1.25% EL-107.	0.25% EL-107
Teratogenicity LOEL	Not clearly stated.	1.25% EL-107

- C. The following deficiencies and discrepancies were noted in the study report:

1. The frequency of diet analyses was not adequate for a three-generation reproduction study. Homogeneity was determined on a single batch of test diet at one mixing and according to the authors, diets were prepared biweekly. Test material concentrations were analyzed only on initial batches at the start of the F₀ and F₁ parental generations and final batches at the termination of the F₁ and F₂ parental generations. Therefore, analyses were conducted on only four batches of the test diet during the 16-month study. Furthermore, data were not presented for the concentration analysis and we could not verify data presented in the summary table. In addition, data presented in the text (Table 2) for the homogeneity analyses indicated that the 0.05% (500 ppm) dietary level was analyzed; in Appendix D of the test report, the data presented indicated that the 1.25% (12,500 ppm) dietary level was analyzed.
2. Feed consumption was not measured during the gestation/lactation periods. Had this parameter been measured during these periods, the ingestion of the test material would have been more accurately calculated, and its relationship to body weight depressions during those phases of the study could have been assessed.
3. Two different lots of the test material were used (the lots were changed on March 14, 1983) during the course of the study; reporting of the composition of the test material was not clear. Two chemical compositional profiles were reported for lot No. H02-2G6-118 and a third chemical profile was given for lot No. Z10025. Although not specifically stated, the first lot (H02-2G6-118) was apparently reanalyzed, and both analyses were reported. The major difference between the lots was in the percent 121607 (the major constituent) present. However, we do not consider this difference (3.2%) to be significant.

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4. The actual numbers of animals per group subjected to ophthalmic examination was not reported.
 5. Several clerical errors were also noted in the final report.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Protocol, CBI pp. 846-885; Appendix B, Breeding scheme for three-generation reproductive study, CBI pp. 57-58.

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APPENDIX A
Protocol

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Page is not included in this copy.

Pages 206 through 248 are not included.

The material not included contains the following type of information:

- Identity of product inert ingredients.
- Identity of product impurities.
- Description of the product manufacturing process.
- Description of quality control procedures.
- Identity of the source of product ingredients.
- Sales or other commercial/financial information.
- A draft product label.
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January 21, 1987

Addendum to Dynamac Review
Teratogenicity Study in Rats
EL-107

EPA: 68-02-4225
DYNAMAC No. 261-F2

The following correction should be inserted under Conclusions,
Section 7, page 3 of the Data Evaluation Record:

Delete: "Although male fetal weights were significantly less than controls ($p < 0.05$) at all dose levels tested, this finding does not appear to be toxicologically meaningful since females were unaffected."

Insert: "In addition, mean male fetal body weights were significantly lower than controls ($p < 0.05$) at all doses and differences appeared to be dose-related. None of the differences between dose groups and controls was greater than 5%."

Approved by:

Margaret L. Jones
EPA reviewer

Signature: Margaret L. Jones

Date: 1-21-87

Marcia Van Gemert, Ph.D.
EPA Section Head

Signature: M Van Gemert

Date: 1-21-87

BEST AVAILABLE COPY

EPA: 68-02-4225
DYNAMAC No. 261-F2
January 20, 1987

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DATA EVALUATION RECORD

EL-107

Teratogenicity Study in Rats

STUDY IDENTIFICATION: Lake, S. G., and Byrd, R. A. A teratology study of EL-107 administered orally to Wistar rats. (Unpublished study No. R09483 prepared by Lilly Research Laboratories, Greenfield, IN, for Elanco Products Co., Indianapolis, IN; dated July 1984.) Accession No. 073229.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: _____

I. Cecil Felkner

Date: _____

1-20-87

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

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1. CHEMICAL: EL-107: Lilly compound 121607, N-[3-(1-ethyl-1-methyl-propyl)-5-isoxazolyl]-2,6-dimethoxybenzamide [redacted]; Lilly compound 135520, N-[3-(1,1-dimethylbutyl)-5-isoxazolyl]-2,6-dimethoxybenzamide [redacted] and Lilly compound 173490, N-[3-(1,1,2-trimethylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide [redacted]
2. TEST MATERIAL: EL-107 from lot No. Z10025 was described as containing a mixture of isomers consisting primarily of Lilly compounds 121607, 135520, and 173490. The combined purity was 95.5%.
3. STUDY/ACTION TYPE: Teratogenicity study in rats.
4. STUDY IDENTIFICATION: Lake, S. G., and Byrd, R. A. A teratology study of EL-107 administered orally to Wistar rats. (Unpublished study No. R09483 prepared by Lilly Research Laboratories, Greenfield, IN, for Elanco Products Co., Indianapolis, IN; dated July 1984.) Accession No. 073229.

5. REVIEWED BY:

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Principal Reviewer
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Date: 1/20/87

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Marcia Van Gemert, Ph.D.
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Date: 1-21-87

7. CONCLUSIONS:

- A. We assess the LOEL and NOEL for maternal toxicity of EL-107 in rats to be 1000 and 320 mg/kg/day, respectively, based on decreased body weight gain at the highest dosage during the dosing period. We assess the LOEL and NOEL for embryo/fetotoxicity to be 1000 and 320 mg/kg/day, respectively, based on the increased preimplantation loss, increased incidence of resorptions, smaller litter size, and the increased incidence of runt fetuses at the highest dosage tested when compared to the control group. [Although male fetal weights were significantly less than controls ($p < 0.05$) at all dose levels tested, this finding does not appear to be toxicologically meaningful since females were unaffected.] *See Attachments*
- B. Core Classification: This study is classified Core Minimum.

Item 8--see footnote 1.

9. BACKGROUND: The study authors referred to a preliminary study involving pregnant Wistar rats in which "gavage doses as high as 1000 mg/kg/day did not produce maternal or prenatal toxicity." They stated that "additional teratology studies with EL-107 have been conducted in the rat as well as in the rabbit," but provided no additional information about those studies.

Item 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):A. Materials and Methods:

1. Test Material: EL-107 was described as a mixture of isomers with a combined activity of 95.5%. No physical properties of the test material were reported. Dose suspensions were prepared daily on a weight per volume basis in 10% aqueous acacia. Animals were dosed by gavage on gestation days 6-15 at 0, 100, 320, or 1000 mg/kg/day. Dose volumes were 10 mL/kg, based on body weights measured on gestation day 6.
2. Test Animals: Virgin adult female Wistar rats (Hsd:(WI)BR) were supplied by Harlan Sprague Dawley, Inc. (Indianapolis, IN). The females were acclimated for 3 days before being mated 1:1 with adult males from the same stock colony. Observation of an expelled copulatory plug was considered to be day 0 of gestation. A total of 100 mated females with a mean weight of 217 g were randomized into four study groups and individually identified. Food and water were available ad libitum.

¹ Only items appropriate to this DER have been included.

3. Study Design and Parameters Evaluated: During the study, all females were observed daily for survival and clinical signs of toxicity. Body weights and food consumption were recorded on gestation days 0, 6, 11, 16, and 20. On gestation day 20, females were sacrificed and the litters were delivered by caesarean section. The dams were necropsied and gravid uterine weights were recorded. Corpora lutea were counted, as well as numbers and distribution of implantations, resorptions, and live and dead fetuses. Uteri that appeared non-pregnant were immersed in ammonium sulfide to confirm the absence of implantation scars. Live fetuses were examined externally, sexed, and weighed. Fetuses weighing 33.3% less than the mean weight of controls were classified as runts. Approximately one-half of the fetuses from each litter were fixed in Bouin's solution and examined viscerally (Wilson, 1965). The remaining half were eviscerated and processed for skeletal examination (Staples and Schnell, 1964). Fetuses were classified as either normal, variant, or abnormal (runts and malformed fetuses). All findings were recorded.

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

- A. Test Material: The study authors did not indicate if chemical analyses were performed on dosing solutions; no analytical results were reported. Data on test material characterization indicated that the EL-107 used for this study contained 95.5% active ingredient.
- B. Maternal Effects: No females died during the study. In-life clinical observations and necropsy findings were considered incidental. Net weight gain (total gain minus gravid uterine weight) and food consumption were comparable among all groups (Tables 1 and 2). Reproduction parameters, including pregnancy rate, numbers of corpora lutea and implantations, and proportions of resorptions and live fetuses, were reported to be similar for all groups (Table 3).
- C. Fetal Effects: No dead fetuses were observed in this study. Fetal sex ratios were not affected by compound administration. Male fetal body weights in all dosage groups were significantly lower than the control, but female fetal body weights were similar for the control and dosage groups (Table 3). The authors did not attribute the lower male body weights to compound administration because the control fetuses were "atypically high in weight," the weights were comparable to historical control data, and the depression of male fetal body weights did not occur in two subsequent teratology studies with EL-107. The authors reported one, one, two, and four small (runt) fetuses in the 0-, 100-, 320-, and 1000-mg/kg/day groups, respectively. Visceral and skeletal variations and/or malformations were randomly distributed among the study groups with no evidence of compound

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TABLE 1. Mean Maternal Body Weight and Weight Gain (g)
During Gestation in Rats Dosed with EL-107

Dosage (mg/kg/day)	Mean Body Weight on Gestation Day				
	0	6	11	16	20
0	215	249	271	308	366
100	218	249	274	309	368
320	216	248	270	304	362
1000	218	251	271	301	358

Dosage (mg/kg/day)	Mean Body Weight Gain on Gestation Days				
	0-6	6-16	16-20	Total 0-20	Corrected ^a 0-20
0	34	59	58	151	79
100	31	60	59	150	80
320	32	56	58	146	76
1000	33	50	57	140*	76

^aCorrected weight gain = total weight gain - gravid uterine weight.

*Significantly different from control value (p <0.05).

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 TABLE 2. Mean Maternal Food Consumption (g/day \pm S.E.) During Gestation in Rats Dosed with EL-107

Dosage (mg/kg/day)	Gestation Days			
	0-5	6-10	11-15	16-19
0	19.9 \pm 0.3	20.8 \pm 0.6	25.7 \pm 0.7	24.3 \pm 0.8
100	19.6 \pm 0.4	22.4 \pm 0.5*	25.4 \pm 0.8	24.5 \pm 0.7
320	19.7 \pm 0.4	21.3 \pm 0.4	25.5 \pm 0.7	24.6 \pm 0.8
1000	19.8 \pm 0.5	21.3 \pm 0.5	24.8 \pm 0.7	24.4 \pm 0.8

*Significantly different from control value (p <0.05).

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TABLE 3. Summary of Mean Reproduction and Litter Data for Rats Dosed with EL-107

	Dosage (mg/kg/day)			
	0	100	320	1000
No. of females mated	25	25	25	25
No. pregnant (%)	23 (92)	23 (92)	22 (88)	24 (96)
Mean per litter:				
No. corpora lutea	13.4	13.7	13.8	14.0
No. implantations	12.2	11.9	12.6	11.6
Preimplantation loss (%) ^a	8.4	12.6	8.4	16.5
No. resorptions	0.7	0.5	1.1	1.6
No. live fetuses	11.5	11.4	11.5	10.0
Male fetal weight	4.31	4.11*	4.08*	4.08*
Female fetal weight	4.03	3.96	3.91	4.00

^aCalculated by the reviewers from the individual animal data.

*Significantly different from control value ($p < 0.05$).

effects (Tables 4 and 5). Malformations were limited to two control fetuses, one with inguinal hernia and one with an absent thoracic rib, and one 1000-mg/kg/day fetus with cryptorchism and renal agenesis.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

- A. The authors concluded that the NOEL for maternal and fetal toxicity and teratogenicity of EL-107 in the rat is 1000 mg/kg/day, the highest dosage tested.
- B. A signed quality assurance statement, dated July 31, 1984, was presented in the final report.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

- A. Maternal/Fetal Effects: Based on lower body weight gain during the dosing period, we assess that mild maternal toxicity was evident at the highest tested dosage, 1000 mg/kg/day. Furthermore, we assess that embryo/fetotoxicity was also evident at the highest dosage, based on increased preimplantation loss, the increased incidence of resorptions, smaller litter size, and the increased incidence of runt fetuses when compared to the control group. These assessments differ from the study authors' conclusion that no maternal or fetal effects were evident at 1000 mg/kg/day. We cannot assess the biological significance of the significantly decreased male fetal body weights in all dosage groups. Although the authors stated that similar effects did not occur in other teratology studies, those data were not presented for our review. The incidence, distribution, and type of fetal variations and/or abnormalities observed in this study did not indicate compound-related teratogenic effects.
- B. Deficiencies: Although data for the characterization of the test material were presented in the final report, it appears that no chemical analyses were performed on the dosing solutions administered to the test animals.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Protocol, CBI pp. 144-151.

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TABLE 4. Incidence Summary of Selected Fetal Variations and Malformations in Rats Dosed with EL-107

	<u>Dosage (mg/kg/day)</u>			
	0	100	320	1000
	<u>Fetuses (Litters) Examined</u>			
<u>Examination Type</u>				
External	265 (23)	262 (23)	253 (22)	241 (24)
Visceral	136 (23)	137 (23)	131 (22)	128 (24)
Skeletal	129 (23)	125 (23)	122 (22)	113 (24)
	<u>Fetuses (Litters) Affected</u>			
<u>External</u>				
Runt	1 (1)	1 (1)	2 (2)	4 (4)
<u>Visceral</u>				
Cryptorchism	0	0	0	1 (1) ^a
Inguinal hernia	1 (1)	0	0	0
Hydronephrosis	64 (20)	81 (22)	71 (21)	59 (21)
Renal agenesis	0	0	0	1 (1) ^a
<u>Skeletal</u>				
Incomplete develop- ment of sternbrae	0	0	2 (2)	2 (2)
Incomplete develop- ment of/or absent vertebral centra	23 (13)	33 (16)	18 (10)	21 (13)

^aSame fetus (also a runt).

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TABLE 5. Incidence of Normal, Variant,^a and Malformed^b Fetuses
in Rats Dosed with EL-107

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	Dosage (mg/kg/day)			
	0	100	320	1000
Normal fetuses (%)				
Male	61.5	51.8	57.3	57.9
Female	61.0	51.3	59.4	63.9
Variant fetuses (%)				
Male	37.1	47.7	40.6	36.8
Female	34.6	48.7	39.4	35.4
Abnormal fetuses (%)				
Male	1.4	0.5	2.0	5.1
Female	4.4	0.0	1.1	0.7

^aIncludes fetuses with developmental variations.

^bIncludes runts and malformed fetuses.

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APPENDIX A
Protocol

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Pages 261 through 268 are not included.

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- Identity of product inert ingredients.
- Identity of product impurities.
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EPA: 68-02-4225
DYNAMAC No. 261-F1
January 28, 1987

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DATA EVALUATION RECORD

EL-107

Teratogenicity Study in Rabbits

STUDY IDENTIFICATION: Lake, S. G. and Byrd, R. A. A teratology study of EL-107 administered orally to Dutch Belted rabbits. (Unpublished study No. 803383 prepared and submitted by Eli Lilly and Co., Greenfield, IN; dated May 1984.) Accession No. 073299.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: _____

Date: _____

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1. CHEMICAL: EL-107 is a mixture of isomers consisting primarily of N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide [redacted], N-[3-(1,1-dimethylbutyl)-5-isoxazolyl]-2,6-dimethoxybenzamide [redacted] and N-[3-(1,1,2-trimethylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide [redacted]

2. TEST MATERIAL: EL-107, lot No. Z10025, is a mixture of isomers listed above consisting of 95.5% active ingredient (combined).

3. STUDY/ACTION TYPE: Teratogenicity study in rabbits.

4. STUDY IDENTIFICATION: Lake, S. G. and Byrd, R. A. A teratology study of EL-107 administered orally to Dutch Belted rabbits. (Unpublished study No. B0338j prepared and submitted by Eli Lilly and Co., Greenfield, IN; dated May 1984.) Accession No. 073299.

5. REVIEWED BY:

Patricia A. Turck, M.S.
Principal Reviewer
Dynamac Corporation

Signature: Patricia A. Turck
Date: 1-28-87

Guillermo Millicovsky, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: Guillermo Millicovsky
Date: 1-23-87

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Teratogenic and Reproductive
Effects
Technical Quality Control
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 1-28-87

Margaret L. Jones
EPA Reviewer

Signature: Margaret L. Jones
Date: 1/29/87

Marcia Van Gemert, Ph.D.
EPA Section Head

Signature: Marcia Van Gemert
Date: 1/29/87

7. CONCLUSIONS:

- A. No compound-related maternal, embryonic, fetal, or teratogenic effects were noted at any dose level of EL-107 tested; 0, 100, 320 or 1000 mg/kg/day. Therefore, the NOEL for maternal and embryo/fetal toxicity in rabbits is 1000 mg/kg, the highest dose level tested.
- B. Although no maternal toxicity was noted, the administration of 1000 mg/kg satisfied the limit test; testing at higher doses was therefore not required. No analyses of the dose formulation were conducted and the method of fetal sacrifice was not specified. This study is classified Core Minimum.

Item 8—see footnote 1.

9. BACKGROUND: A preliminary study was conducted on nonpregnant rabbits to assess the toxicity of EL-107. An unspecified number of rabbits were given daily oral doses (gavage) of 0, 500, 1000, 2000, and 3000 mg/kg of EL-107 for 13 days. The authors reported no signs of toxicity, mortality, or weight loss at any dose level tested.

Item 10—see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):Materials and Methods:

1. Test Material: The test material, EL-107 (lot No. Z10025), was a mixture of isomers (combined purity of 95.5%) consisting primarily of Lilly compound 121607 [REDACTED], N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide; Lilly compound 135520 [REDACTED], N-[3-(1,1-dimethyl-butyl)-5-isoxazolyl]-2,6-dimethoxybenzamide; and Lilly compound 173490 [REDACTED], N-[3-(1,1,2-trimethylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide. The dose formulations were prepared daily by suspending 0, 1.06, 3.38, and 10.6 percent (w/v) of EL-107 in 10% aqueous acacia oil to give concentrations of 0, 100, 320, and 1000 mg/kg. The dose volume was adjusted to 10 mL/kg.
2. Animals and Test System: Eighty virgin female Dutch Belted rabbits were received from Langshaw Farms, Augusta, MI, and were acclimated for at least 24 days. On consecutive days, sets of 20 females were injected intravenously with 20 IU/kg of chorionic gonadotrophin (A.P.L., Ayerst Laboratories) and artificially inseminated approximately 3 hours later with semen from male rabbits of the same strain and from the same source as the females. The day of insemination was designated gestation day (GD) 0. Two rabbits each were used as semen donors for the first two insemination blocks, and one rabbit

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

each was used for the remaining two insemination blocks; a total of six males were used for the study. Mated females from each set were then randomly assigned to one of four groups. Rabbits received daily oral (by gavage) doses of 0, 100, 320, or 1000 mg/kg of EL-107 on GD 6 through 18. Food consumption was measured daily and body weights were measured and recorded on GD 0, 6, 13, 19, 24, and 28. The mated females were observed daily for signs of toxicity.

On GD 28, dams were sacrificed by intravenous injection of 0.5 mL of T-61 (National Laboratories Corp., Somerville, NJ). A laparohystero-oophorectomy and subsequent necropsy were performed. The uteri of animals without fetuses were stained with 10% aqueous ammonium sulfide to confirm pregnancy status. Gravid uteri were weighed and examined for number, type, and position of implantations. Corpora lutea were counted. Fetuses were then weighed and examined externally for abnormalities. Fetuses weighing 33.3% less than the mean fetal body weights of controls were designated as runts. Visceral and skeletal examinations were performed using the methods of Staples (1974) and Staples and Schnell (1964), respectively. See Appendix B for statistical methods.

B. Protocol: See Appendix A.

12. REPORTED RESULTS:

- A. Test Material Analysis: No analyses of the dose formulations to determine homogeneity, stability, or concentration of the test material were conducted.
- B. Maternal Effects: Upon arrival at the facility, the rabbits were treated for ear mites by treatment with Otomite (Carson Chemical, Inc.) and given an injection (route not specified) of chorionic gonadotrophin. During the first 14 days of acclimation, the rabbits were given sulfaquinoxaline (Salbury Sulquin 6:50, Salbury Laboratories) in the drinking water as a prophylactic treatment for coccidiosis. This treatment was discontinued at least 10 days prior to study initiation.

One mortality occurred during the study (GD 28) in the 1000-mg/kg dose group. Prior to death this animal was reportedly anorectic (food consumption and body weight were decreased) and cachectic; necropsy findings included trichobezoar (hair in the stomach) and an empty gastrointestinal tract; this female was reportedly pregnant. Another animal in the 1000-mg/kg dose group aborted four live fetuses on GD 22 and was subsequently sacrificed. The authors attributed the finding in both of these animals to partial or complete blockage of the digestive system by hair in the stomach and attributed the abortion and death to compound-related nutritional deprivation. No other clinical observations attributable to test material administration were noted. No adverse

effects were noted in body weight gains (Table 1) or food consumption (Table 2) between control and dose groups. There were significant increases ($p < 0.05$) in food consumption for the test groups when compared to controls during GD 0 through 5; however, the animals were not dosed until GD 6. Therefore, the authors did not consider these increases biologically significant. No compound-related findings were noted at necropsy. Lung abscesses noted in one animal in the 100-mg/kg dose group and one in the 320-mg/kg dose group were not considered to be related to compound administration.

- C. Embryonic/Fetal Effects: A summary of the effects of EL-107 on reproductive parameters is presented in Table 3. There were no compound-related differences noted in pregnancy rates, the number of corpora lutea, or type and number of implantations. Sex ratios, the number of runts, and fetal body weights were comparable between control and dose groups. A summary of the incidences of fetal malformations and variations is presented in Table 4. Multiple anomalies including protruding tongue, incomplete development of the sternbrae, vertebral centra (cervical and thoracic), ilium, ischium, and pubis, and short, spaded ribs were noted in one fetus from the 1000-mg/kg dose group. Hydrocephaly was observed in one fetus each from the control and 100- and 1000-mg/kg dose groups, and incomplete development of calvaria was observed in one fetus from the 1000-mg/kg dose group. The authors considered these findings to be random and not compound-related occurrences. No differences in the incidences of minor skeletal variants were noted between control and test groups.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

- A. The authors concluded that based on the death and abortion that occurred at the 1000-mg/kg dose level, the NOEL for maternal toxicity in rabbits is 320 mg/kg of EL-107. The NOEL for embryonic/fetal toxicity and teratogenicity is at least 1000 mg/kg of EL-107 and exceeds the maternal NOEL in the rabbit.
- B. A quality assurance statement was signed and dated May 7, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

- A. Maternal Effects: One mortality occurred during the study in the 1000-mg/kg dose group. This female began losing weight steadily after GD 13, and food consumption was also dramatically decreased after this point; the clinical observations noted were anorexia and cachexia. This animal died on GD 28; necropsy findings included hair in the stomach and an empty intestinal tract. A second female in the 1000-mg/kg dose group aborted her litter on GD 22. Clinical observations and necropsy findings were similar to those of the female dying prior to study termination. No other abnormal observations were reported for animals in the 1000-mg/kg dose group or in the other groups during the study. Body weight gain and food consumption during gestation were comparable between the control and test groups.

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TABLE 1. Effects of EL-107 on Mean Maternal Body Weights in Pregnant Rabbits

Dosage (mg/kg/day)	Mean Body Weight (kg) at GD			
	0	6	19	28
0	2.79 ^a ±0.24	2.81 ±0.24	2.87 ±0.28	2.97 ±0.28
100	2.98 ±0.36	2.90 ±0.32	2.96 ±0.28	3.08 ±0.28
320	2.94 ±0.28	2.97 ±0.31	2.96 ±0.38	3.07 ±0.38
1000	2.86 ±0.16	2.91 ±0.20	2.95 ±0.20	3.07 ±0.20

^aMean ± SD.

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TABLE 2. Effects of EL-107 on Mean Food Consumption in Pregnant Rabbits

Dosage (mg/kg/day)	Mean Food Consumption (g/day) at GD Interval			
	0-5	6-12	13-18	19-27
0	135 ^a ±28	132 ±32	128 ±43	129 ±27
100	161* ±23	138 ±27	130 ±36	135 ±15
320	163* ±30	129 ±41	99 ±54	133 ±34
1000	158* ±24	132 ±32	127 ±46	135 ±24

^aMean ± SD.*Significantly different from controls at $p < 0.05$.

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TABLE 3. Effects of EL-107 on Reproductive Parameters in Rabbits

Parameter	Dosage (mg/kg/day)			
	0	100	320	1000
No. females inseminated	20	20	20	20
No. females pregnant	16	16	12	18
Pregnancy rate (%)	80.0	80.0	60.0	90.0
Mean No. corpora lutea ^a	9.8 ±2.8	9.6 ±2.8	11.2 ±2.8	9.6 ±2.4
Mean No. implantations ^a	6.6 ±2.8	6.6 ±2.8	7.2 ±3.1	7.2 ±2.1
Preimplantation losses (%) ^b	30.2	31.9	29.2	24.8
Mean No. viable fetuses ^a	6.3 ±2.8	6.3 ±2.8	6.3 ±3.8	6.9 ±2.4
Postimplantation losses (%)	8.2	11.3	20.3	3.4
Mean No. resorptions	0.3	0.3	0.9	0.3
Mean fetal body wt. (g) ^{a, b}	36.5 ±5.1	36.7 ±4.1	36.0 ±3.9	35.4 ±4.6
Sex ratio ^c	1.9	1.6	1.9	1.5

^aMean ± SD.^bCalculated by the reviewers from individual animal data.^cNo. males/No. females.

TABLE 4. A Summary of Effects of EL-107 on Litter Incidences of Malformations and Variations in Rabbits

	Dosage (mg/kg/day)			
	0	100	320	1000
No. litters examined	15	15	10	16
<u>Visceral Malformations:</u>				
Hydrocephaly	1 (6.7) ^a	1 (6.7)	0 (0)	1 (6.3)
<u>Skeletal Variants:</u>				
13 ribs (uni- or bilaterally)	9(60.0)	10(66.7)	7(70.0)	9(60.0)
Missing rib	0 (0)	0 (0)	0 (0)	1 (6.3)
Incomplete development of sternbrae	1 (6.7)	2(13.3)	1(10.0)	2(12.5)
Incomplete development of vertebral centra	0 (0)	0 (0)	0 (0)	1 (6.3)

^aNumber of litters affected (% incidence).

- B. Embryonic/Fetal Effects: A decrease in the pregnancy rate and increases in the number of resorptions and postimplantation loss occurred at the 320-mg/kg dose level. However, no dose-related response was noted; these parameters were comparable among the control and 100- and 1000-mg/kg dose groups. Therefore, these differences were not considered to be compound related or toxicologically significant. The incidences of variations and malformations were comparable between control and test groups.
- C. The only difference between the study authors' interpretation and our assessment of the study results was in the presence of maternal toxicity. One animal died and another animal aborted and was subsequently sacrificed in the 1000-mg/kg dose group. The author attributed both the death and the abortion to nutritional deprivation caused by EL-107 administration because of observations of hair in stomach and empty gastrointestinal tract of both animals at necropsy. These findings were not reported for the remaining animals of this group, and clinical signs of maternal toxicity were not noted during the study. Body weight gains and food consumption for this group were comparable to controls. The presence of hair or hair balls in the stomach is not uncommon and sometimes causes gastrointestinal obstruction. Pregnant rabbits frequently pull hair out for nesting purposes and often retain hair in their mouths. In addition, rabbits groom themselves by licking their fur and, in the process, swallow hair. Spontaneous abortion frequently occurs in rabbits. Therefore, we do not agree that the death and abortion were due to EL-107 administration; no maternal toxicity was demonstrated. However, the lack of maternal toxicity does not compromise the acceptability of the study because a dose level of 1000 mg/kg produced no developmental toxicity or teratogenicity, thus satisfying the limit test. Therefore, testing at higher dose levels is not required.
- D. The following deficiencies in the conduct or reporting of this study were noted:
1. Analyses were not conducted to determine the homogeneity, stability, and concentrations of EL-107 in the dose formulations. Therefore, we were unable to verify that the animals received the intended dosages.
 2. The animals were sacrificed and delivered by caesarian section on GD 28. This is generally considered too early because it may introduce abnormally high incidences of incomplete fetal development (i.e., delayed ossification) in the data. Sacrifice on GD 29 is more acceptable.
 3. The method of fetal sacrifice was not specified; we could therefore not assess its acceptability for use in a teratogenicity study.

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These deficiencies did not, however, compromise the validity of the study results.

Item 15--see footnote 1.

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16. CBI APPENDIX: Appendix A, Protocol, CBI Appendix C, pp. 102-108.
Appendix B, Statistical Methods, CBI p. 14.

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APPENDIX A

Protocol

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Pages 281 through 289 are not included.

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- Identity of product inert ingredients.
- Identity of product impurities.
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- Sales or other commercial/financial information.
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TB 7-0037
(7-0139)

TOXICOLOGY BRANCH: DATA REVIEW

Caswell: 419F
EPA Chem: 125851

Chemical: Isoxaben (EL-107)

Study Type: Mutagenicity - Chromosome
aberrations in vivo (mouse micronucleus)

Citation: Test for Genotoxicity of EL-107 Using a Micronucleus
Technique in the Mouse

Accession No: 265739

MRID: N/A

Sponsor: Eli Lilly France SA

Testing Lab: C.E.R.T.I. (G. Siou, L. Lerond-Conan, M. el Haitem,
and M. Lacrampe)

Study No: 871

Date: September 6, 1984

Test Material:

EL-107 (Lot No. HO2-266-118), wettable powder (% ai not
stated), suspended in peanut oil for oral administration.

TB Conclusion/Evaluation:

Inconclusive. Presumptive positive results should be
confirmed in a repeat assay, employing additional procedures as
outlined below (TB Evaluation).

Procedures:

Following toxicity testing, three groups of ten adult male
Swiss mice (25-30 g) were intubated twice 24 hours apart with
test material at a dose of 5000 mg/kg/day and sacrificed 24, 48,
and 72 hours following the second dose. Negative controls (a
fourth group given peanut oil vehicle only), and a fifth group of
ten males administered benzene as reference clastogen (positive
control) were killed 24 hours after dose. Bone marrow poly-
chromatic erythrocytes (PCE, 2000 from each animal) were examined
for the presence of micronuclei, and group means of micronucleated
PCE compared statistically by Student's t-test and the Mann-Whitney
(U) test.

Study Results:

In a preliminary dose-selection test, two of five animals died following two doses of 10,000 mg/kg 24 hours apart, and all treated mice showed signs of prostration. Therefore, a schedule of 2X 5000 mg/kg was chosen as the MTD.

No animals died in the main test (no evidence of clinical toxicity was reported). Group mean percent of PCE with micronuclei of benzene-treated mice was significantly elevated (5.54, $p < 0.01$) over peanut oil controls (0.10) by both statistical analyses. In EL-107 treated groups, slight but significant ($p < 0.01$) increases were found in both the 24-hour (0.22) and 48-hour (0.17) groups, but not at 72 hours (0.16).

Study Conclusions:

The author concluded there may be a "slight clastogenic effect of EL-107" in the mouse, and suggest further study (at 24 hours) to assess the validity of this initial assay.

TB Assessment:

The inclusion of individual animal data support the summary results presented, indicating a consistently slight but statistically significant increase in micronucleated PCE over controls at the 24-hour sacrifice following a dose of 5000 mg/kg. We note that no justification for not testing females was offered. Further, although the single level used was not shown to be a MTD (no clinical toxicity was discussed, nor evidence that a sufficient concentration of test material was absorbed to cause cytotoxicity at the target), we concur with the study author's conclusion that EL-107 induces a cytogenetic effect. Thus, the study can be considered inconclusive evidence for a presumptively positive result in males, deserving of a repeat study to confirm this effect.

We recommend the following procedural details for the repeat study:

1. An equal number of females be tested with EL-107.
2. Evidence of cytotoxicity (e.g., altered ratios of PCE to NCE) be demonstrated at an MTD (higher than 5000 mg/kg, but less than 10,000 mg/kg).

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3. Sampling bone marrow cells be started as early as 12 hours after dosing, and other groups at 24, 36 and 48 hours postdose.

Reviewed by: Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division

J. W. Hausman, 61-67-17

*Judith W. Hausman
1/9/87*

Primary Reviewer: Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division

Irving Mauer
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Secondary Reviewer: *Quarsh W. Sansworth 1/9/87*

TB #7-0037
(7-0139)

TOXICOLOGY BRANCH: DATA REVIEW

Caswell: 419F
EPA Chem: 1256J1

Chemical: Isoxaben (EL-107)

Study Type: Mutagenicity - Chromosome aberrations in vivo
(dominant lethal test in rats)

Citation: A Male Effect and Dominant Lethal Study With EL-107 in
the Wistar Rat.

Accession No.: 265739

MRID: N/A

Sponsor: Elanco

Testing Lab.: Toxicology Division, Eli Lilly Research Lab.

Study No.: R01984

Date: October 1985

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

Test Material: EL-107 technical (Lot #Z10025), 95.5% ai, a mixture
of isomers (Lilly Compounds: 121607, [REDACTED] 135520, [REDACTED] and
173490, [REDACTED], incorporated into feed for dietary administration.

TB Conclusions/Evaluation:

Provisionally acceptable in demonstrating no dominant lethality
at dietary concentrations up to 1.25% EL-107 (12,500 ppm, providing
an intake of 932 mg/kg/day). Data on a positive control study
with triethylenemelamine (TEM) are required.

Procedures:

Proven F2 Wistar males (25/treatment group) from a
three-generation reproduction study with EL-107 (R14183) were
maintained on test diets containing 0, 0.05, 0.25, and 1.25% EL-107
(equivalent to 0, 500, 2500, and 12,500 ppm) until they were about
19 weeks old, then mated to untreated virgin Wistar females (1:1)
for two 7-day mating periods. Females were sacrificed on day 20
of gestation, ovaries and intrauterine contents removed, and fetuses
examined for external and intracranial anomalies. The numbers
and distribution of corpora lutea, implantations, live and dead

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fetuses, and resorptions were recorded. Live fetuses were sexed, weighed, and classified as normal, variant (i.e., with anomalies frequently seen among historical control fetuses and/or not considered to affect postnatal function or survival), or abnormal (fetuses with anatomical malformations or changes that would affect postnatal function or survival).

Means and standard errors for the following reproduction and developmental indices were calculated: fertility (% pregnant), preimplantation loss (% corpora lutea minus implantation sites) and postimplantation loss (% dead implants per total implant sites). Dominant lethal mutations (increase in preimplantation loss/postimplantation death) were statistically analyzed by Dunnett's t-test on rank-transformed data; fertility rates were compared by chi-square analysis.

No positive control group was included in this study. The sensitivity of Wistar rats to dominant lethals was stated to have been demonstrated in an earlier (unpublished) study* with TEM, but no data were presented.

The following were included in the Final Report: complete characterization, stability and homogeneity data on the test substance; a GLP-QA Statement; full protocol and (minor) amendments (none of which affected the conduct of the study or the integrity of the derived data); both summary tabulations of reproduction, developmental and fetal parameters and individual animal data; and cumulative anomaly data in control (vehicle) fetuses from 17 previous studies (spanning the last 4 years).

Study Results:

Assays for potency of the test article in the feed throughout the study indicated no substantive changes from theoretical limits (within 1.15 to 3.50%). Determinations of mean daily food consumption, individual body weights, and theoretical dietary concentrations of test substance provided the following time-weighted estimates of test article intake for male rats fed 0, 0.05, 0.25 and 1.25% EL-107: 0, 34, 173, and 932 mg/kg/day, respectively.

No animals died during the study, and no clinical toxicity attributable to EL-107 treatment was observed in the males; low incidences of incisor malocclusion, chromodacryorrhea, alopecia, chromorhinorrhea, and labored respiration were evenly distributed

* Markham, J.K. and Hoyt, J.A. (1983). A dominant lethal study with triethylenemelamine (TEM) in the Wistar rat. Toxicology Division, Lilly Research Lab.

among all groups. Body weights of high-dose males were significantly lower than controls at the beginning of the study, and remained so throughout the mating trials.

As indicated in the summary tabulation from the Final Report (attached), there was no apparent effect of treatment on mating performance and fertility of treated males; of the 25 males fed 0, 0.05, 0.25 and 1.25% EL-107, respectively, 20, 23, 24, and 24 sired at least one litter (a total 32 of 50 control females, and 35, 45, and 36 of the respective treatment groups were pregnant). The numbers of live or dead implantations were comparable in all groups, and no differences were found in either preimplantation or postimplantation loss.

Fetal parameters appear to have been unaffected by EL-107 treatment. As indicated by the summary group values and individual data tabulations, examination of 453, 465, 585, and 507 live fetuses from the four groups (respectively, control, 0.05, 0.25, and 1.25% EL-107) revealed no differences from control values for incidences of runting (defined as fetal weight less than one-third control mean), gross abnormalities, body weights, or sex ratios.

Study Conclusions:

The authors concluded that there was no evidence in this study of a dominant lethal effect in the progeny of male rats fed diets containing 0, 0.05, 0.25, and 1.25% EL-107 (equivalent to 0, 34, 173, and 932 mg/kg/day) throughout the spermatogenic cycle (9 to 10 weeks).

TB Evaluation:

This study appears to have been conducted according to adequate procedures to generate valid results. The treated males were derived from EL-107-treated parents (constituting the third generation [F₂] of a three-generation study with this test article), and a clinical effect was evident at least in the high-dose group (reduced body weight), although no reproductive parameters were affected at this level (intake of ca 932 mg/kg/day). Repeated administration of a test substance over the entire period of germ cell maturation is an acceptable alternative treatment protocol for this type of assay, but supported by published data on only a few reference mutagens (notably TEM), and the data base comparing the effects with the conventional protocol (acute or subacute treatment followed by 8- to 10-week matings) is sparse. Further, the authors are faulted for not including the results of the study stated to demonstrate the sensitivity of this strain of rat to TEM-induced dominant lethals (Markham and Hoyt, 1983).

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Although the EDT (1.25% of the diet) provided a fairly high intake, the clinical toxicity at this level was a carryover of parental toxicity, with no apparent reproductive effects (such as reduced fertility, as recommended by EPA Guidelines*). Thus, it could be argued that this top dose was insufficient to satisfy the Guidelines criterion that, in the absence of frank toxicity of the test article by the dietary route, sufficient concentration of the substance to affect reproductive performance shall be demonstrated.

This could have been obviated by treating this satellite group of males (derived from the reproductive segments) by oral intubation, or parenterally (e.g., i.p.), at a sufficiently high dosage to guarantee transport of effective concentrations to the target.

Attachment s

* Gene-Tox Health Effects Test Guidelines, FEDERAL REGISTER Volume 50, No. 188, Friday, September 27, 1985.

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Pages 297 through 301 are not included.

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TB 7-0037
(0337)

TOXICOLOGY BRANCH: DATA REVIEW

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Caswell: 419F
EPA Chem: 125851

Chemical: Isoxaben (EL-107)

Study Type: Mutagenicity - Gene mutation in
bacteria (S. typhimurium, Ames Test)

Citation: The Effect of EL-107 on the Induction of Reverse
Mutation in Salmonella typhimurium Using the Ames
Test

Accession No: 75-47

MRID: N/A

Sponsor: Elanco (Division of Eli Lilly & Company)

Testing Lab: Toxicology Division, Lilly Research Labs

Study No: 841001AMS1378

Date: October 1984

Test Material:

Technical EL-107 (Lilly Compound No. 121607, (Lot Z10025,
a mixture of three active isomers), 95.5% ai.

TB Conclusions/Evaluation:

Acceptable in demonstrating negative results for induction
of revertents in five histidine auxotrophic tester strains of
S. typhimurium LT-2 (i.e., not mutagenic in Ames tests) for
technical El-107.

Procedures:

Following cytotoxicity and precipitation tests with strain
TA 100, cultures of five Salmonella typhimurium LT-2 histidine
(his⁻) auxotrophs (TA 1535, TA 1537, TA 1538, TA 98, TA 100)
were exposed for 48 hours to test material at concentrations of
0 (DMSO solvent control), 31, 62.5, 125, 250, and 500 ug/plate
(in triplicate), in the absence and presence of a mammalian
metabolic activation (MA) system consisting of microsomal enzymes
(S9) from the livers of Arochlor 1254-treated male Fischer 344

rats plus cofactors, according to standardized (referenced) procedures. After the 2-day incubation period, revertent (his⁺) colonies were enumerated using an electronic colony counter. Appropriate controls were run concurrently, namely: the solvent (dimethylsulfoxide, DMSO); the mutagens N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), 2-nitrofluorene (2NF), and 9-aminoacridine (9AmAc) for nonactivated tests; and 2-aminoanthracene (2AA) for activated tests.

A GLP-QA statement, abbreviated protocol, references to appropriate procedures, and criteria for positive response were included in the Final Report.

Study Results:

EL-107 with/without MA was not toxic to TA 100 cells exposed to concentrations up to 5000 $\mu\text{g}/\text{plate}$ (% survival = 109% and 113%, respectively, of solvent control), but dose-related increased precipitation was evident at doses of 500 $\mu\text{g}/\text{plate}$ and above. Hence, 500 $\mu\text{g}/\text{plate}$ was considered the limit of solubility of the test material, and the highest dose assayed.

Neither in the presence or absence of MA were increased counts of revertent colonies observed in EL-107-treated cultures (as noted in Table 3 from the Final Report, attached), in contrast to positive controls which responded with greatly increased colony counts.

Study Conclusions:

The authors concluded that technical EL-107 was not mutagenic for his reversion in Salmonella typhimurium Ames Assays.

TB Evaluation:

The study was well conducted under procedures providing valid results, and demonstrating EL-107 is not mutagenic in standard Ames Assays when tested to the limit of solubility.

Attachment

Reviewed by: Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division

Irving Mauer
01-07-87

J.W. Hauswirth
1/8/87

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TABLE 3. AN EVALUATION OF EL-107 FOR THE INDUCTION OF BACTERIAL MUTATION USING THE AMES TEST. STUDY 841001AMS1378.

Treatment	$\mu\text{g}/\text{plate}$	Revertant Colony Counts (Mean \pm S.D.) ^a				
		TA1535	TA1537	TA1538	TA98	TA100
<u>TEST WITHOUT METABOLIC ACTIVATION</u>						
EL-107	500	21 \pm 1	8 \pm 1	16 \pm 3	28 \pm 4	109 \pm 5
	250	20 \pm 3	9 \pm 3	21 \pm 1	28 \pm 2	126 \pm 4
	125	25 \pm 5	10 \pm 4	12 \pm 4	24 \pm 6	110 \pm 4
	62.5	20 \pm 1	6 \pm 1	13 \pm 7	23 \pm 3	118 \pm 2
	31	21 \pm 2	8 \pm 1	16 \pm 1	25 \pm 5	124 \pm 15
DMSO ^b	0.05 ml	22 \pm 1	7 \pm 0 ^e	15 \pm 1	23 \pm 4	113 \pm 3
DMSO ^c	0.05 ml	19 \pm 5	8 \pm 2	21 \pm 3	23 \pm 5	122 \pm 12
MNNG ^d	5	3494 \pm 64				3476 \pm 76
MNNG	2.5	500 \pm 21				1409 \pm 215
9AmAc ^d	100	951 \pm 97				
9AmAc	50	94 \pm 43				
2NF ^d	5			528 \pm 43	680 \pm 26	
2NF	0.5			78 \pm 8	83 \pm 10	
<u>TEST WITH METABOLIC ACTIVATION</u>						
EL-107	500	25 \pm 9 ^f	11 \pm 4 ^f	31 \pm 4	48 \pm 6	118 \pm 3
	250	21 \pm 5	10 \pm 3	30 \pm 3	40 \pm 1	117 \pm 13
	125	20 \pm 1	8 \pm 4	33 \pm 3	37 \pm 4	120 \pm 12
	62.5	16 \pm 1	9 \pm 3	31 \pm 3	42 \pm 6	123 \pm 16
	31	21 \pm 3	9 \pm 3	30 \pm 4	37 \pm 6	108 \pm 17
DMSO ^b	0.05 ml	23 \pm 4	8 \pm 2	23 \pm 3	32 \pm 8	108 \pm 9
DMSO ^c	0.05 ml	21 \pm 1	9 \pm 2	22 \pm 1	36 \pm 4	101 \pm 11
2AA ^d	2.5	229 \pm 19	215 \pm 11	886 \pm 76	1657 \pm 72	1549 \pm 60
2AA	1.25	118 \pm 15	70 \pm 16	409 \pm 17	702 \pm 37	768 \pm 27

^a Mean \pm standard deviation of counts from triplicate plates. Values represent corrected counts for 100 percent of the plate area.

^b DMSO control value for the tester strain plated at the initiation of plating.

^c DMSO control value for the tester strain plated at the termination of plating.

^d In the non-activated test MNNG served as the positive control for strains TA1535 and TA100; 9AmAc was the positive control for strain TA1537; and 2NF served as the positive control for strains TA1538 and TA98. In the activated test, 2AA served as the positive control for all tester strains.

^e One of the plates was believed to have been inoculated twice; therefore, this value is the mean of two plates.

^f Plates were not counted due to presence of a chemical precipitate.

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EPA: 68-02-4225
DYNAMAC NO. 261-82
January 20, 1987

DATA EVALUATION RECORD

EL-107

Percutaneous Absorption Study in Monkeys

STUDY IDENTIFICATION: J.F. White and R.B.L. van Lier. Percutaneous absorption of ^{14}C -EL-107 in Rhesus monkeys. (Unpublished study Nos. PO 3983 and PO 4083 conducted by Eli Lilly and Company, Greenfield, IN; dated November 1983.) Accession No. 073293

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner
Date: 1-26-87

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

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1. CHEMICAL: EL-107; N-[3-(1-ethyl-1-methylpropyl)-5-isoxazdyl]-2, 6-dimethoxybenzamide.
2. TEST MATERIAL: EL-107, compound 121607, lot No. 553-02V-094/553-1X7-018, contained █████ percent EL-107, █████ percent 135520, and █████ percent 173490.
3. STUDY/ACTION TYPE: Percutaneous absorption in monkeys.
4. STUDY IDENTIFICATION: J.F. White and R.B.L. van Lier. Percutaneous absorption of ¹⁴C-EL-107 in Rhesus monkeys. (Unpublished study Nos. PO 3983 and PO 4083 conducted by Eli Lilly and Company, Greenfield, IN; dated November 1983.) Accession No. 073293

5. REVIEWED BY:

Finis Cavender, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: *Finis Cavender*

Date: 1/20/87

William L. McLellan, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: *William L. McLellan*

Date: 1/20/87

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Acute Toxicology
Technical Quality Control
Dynamac Corporation

Signature: *I. Cecil Felkner*

Date: 1-20-87

Marcia Van Gemert, Ph.D.
EPA Section Head

Signature: *Marcia Van Gemert*

Date: 1/21/87

7. SUMMARY:

One group of two male and two female adult Rhesus monkeys received an intravenous dose of 2 mg/kg [¹⁴C]EL-107, and radioactivity was monitored in their plasma and excreta for 168 hours. Approximately 1 month later, the same monkeys were given a topical dose of 2 mg/kg [¹⁴C]EL-107, which was covered by a dressing. After 24 hours, the dressing was removed and the plasma and excreta of the monkeys were monitored for radioactivity for 168 hours. Based on the area-under-the-curve (AUC) data at 7 days for each administration, approximately 11 percent of the topical dose was absorbed through the skin (Table 1). Based on the urinary excretion data for the two routes of administration, the topical dose absorption through the skin was approximately 7.5 percent of the intravenous dose (see Table 2). Percutaneous absorption studies are not Core studies; however, the study is acceptable.

8. REVIEWERS' COMMENTS AND QUALITY ASSURANCE MEASURES:

The study was conducted in an acceptable manner, and a quality assurance statement was signed and dated. Based on the plasma AUC data, approximately 11 percent of the topically applied EL-107 was absorbed through the skin.

9. CBI APPENDIX: Appendix A, CBI Protocol, pp.29-42.

Table 1. Mean (\pm S.E.M.) Plasma or Serum Levels of Radiolabel in Rhesus Monkeys Receiving 2 mg/kg [14 C]-EL-107 Intravenously or Topically (studies P03983 and P04083)

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Time (h)	iv (μ g-eq/ml)	Topical (ng-eq/ml)
0.25	1.21 \pm 0.08	0.875 \pm 0.502
0.50	1.17 \pm 0.05	0.700 \pm 0.406
1	0.969 \pm 0.035	3.35 \pm 0.773
2	0.624 \pm 0.056	1.125 \pm 0.718
4	0.251 \pm 0.012	3.25 \pm 1.44
6	0.173 \pm 0.009	2.08 \pm 1.35
24	0.064 \pm 0.004	2.50 \pm 1.20
48	0.031 \pm 0.002	7.25 \pm 2.91
72	0.021 \pm 0.004	6.70 \pm 2.66
96	0.012 \pm 0.003	6.23 \pm 2.77
120	0.007 \pm 0.002	5.95 \pm 2.85
144	0.003 \pm 0.002	4.83 \pm 1.90
168	0.013 \pm 0.004	3.85 \pm 2.05
Total AUC	7.94 μ g-eq/ml	873 ng-eg/ml

$$\text{Relative Plasma Concentration} = \frac{\text{AUC (Topical)}}{\text{AUC (iv)}} \times 100 = \frac{0.873^a}{7.94} \times 100 = 11.0\%$$

^aMean AUC values at 168 hours.

Source: CBI, p. 24.

Table 2. Urinary Excretion of Radiolabel From Rhesus Monkeys Receiving 2 mg/kg [¹⁴C]EL-107 Intravenously or Topically

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Time (h)	iv (% of Dose)	Topical (% of Dose)
24	48.08	0.80
48	8.24	2.19
72	2.00	0.68
96	0.68	0.36
120	0.87	0.47
144	0.07	0.10
168	<u>0.08</u>	<u>0.10</u>
Total	60.02 ± 2.55	4.53 ± 2.22

Mean Urinary Excretion: iv - 60.02 ± 2.55
 Topical - 4.53 ± 2.22

Relative Urinary Excretion = $\frac{\text{Mean Urinary Excretion (Topical)}}{\text{Mean Urinary Excretion (iv)}} \times 100 = \frac{4.53}{60.02} \times 100 = 7.55 \text{ percent.}$

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Appendix A
CBI Protocol

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Page _____ is not included in this copy.

Pages 311 through 324 are not included.

The material not included contains the following type of information:

- Identity of product inert ingredients.
- Identity of product impurities.
- Description of the product manufacturing process.
- Description of quality control procedures.
- Identity of the source of product ingredients.
- Sales or other commercial/financial information.
- A draft product label.
- The product confidential statement of formula.
- Information about a pending registration action.
- FIFRA registration data.
- The document is a duplicate of page(s) _____.
- The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

005732

1. CHEMICAL: EL-107; N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide.

2. TEST MATERIAL: [¹⁴C]-EL-107 (lot No. 553-3N1-056), labeled at position 5 of the isoxazole ring (specific activity = 11.21 μCi/mg). had a radiochemical purity of 98.6 percent as determined by thin-layer chromatography.

3. STUDY/ACTION TYPE: Metabolic study in rats.

4. STUDY IDENTIFICATION: Magnussen, J. D. and Rainey, D. P. Metabolism of ¹⁴C EL-107 in male and female Wistar rats. (Unpublished study No. ABC-0153 prepared by Lilly Research Laboratories, Greenfield, IN. for Elanco Products Co., Indianapolis, IN; dated August 1984.) Accession No. 073293.

5. REVIEWED BY:

Charles E. Rothwell, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: Charles Rothwell
Date: 1-20-87

William L. McLellan, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: 1-20-87

6. APPROVED BY:

Nicolas P. Hajjar, Ph.D.
Metabolism
Technical Quality Control
Dynamac Corporation

Signature: Nicolas P. Hajjar
Date: Jan 20, 1987

Marcia Van Gemert, Ph.D.
EPA Section Head

Signature: M. van Gemert
Date: 1/21/87

7. CONCLUSIONS:

A. When adult rats were given single oral doses of 250 mg/kg of [¹⁴C]-EL-107, virtually the entire dose was excreted by 48 hours. After 72 hours, 90 percent of the administered dose was recovered in the feces. The majority of this fecal [¹⁴C] (90 percent) was identified as unmetabolized EL-107, indicating that the 250-mg/kg oral dose was poorly absorbed. Of the estimated 20 percent of the dose that was absorbed, about half was excreted in the urine and half into the feces as metabolites. The urine contained an estimated 15 to 20 metabolites; fecal metabolites were not characterized. For the generation of urinary metabolites, the major metabolic transformations involved oxidation of the EL-107 molecule at position 2 (see Appendix A, Metabolite Structures and Names, CBI pp. 22-23) of the alkyl side chain to produce either an alcohol or a ketone, hydroxylation of the aromatic ring adjacent to one of the methoxy substituents, and O-demethylation of one of the methoxy substituents. Generally, there were no major differences between male and female rats with regard to their excretion or metabolism of EL-107, although minor differences in the urinary metabolic profiles were described. No studies were conducted on the tissue distribution of EL-107.

B. This metabolic study is acceptable.

Items 8-10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS): (See Appendix B for details.)A. Materials and Methods:

Dosing: Five male and five female Wistar rats (Harlan Industries) having body weight ranges of 250-275 g and 160-180 g, respectively, were fasted overnight. These animals were then administered by gavage a single 250-mg/kg oral dose of [¹⁴C]-EL-107 (specific activity = 0.5 μ Ci/mg) suspended in 10 percent acacia. After dosing, the animals were housed in metabolism cages, and their urine and feces were collected separately every 24 hours for a period of 72 hours. Food was withheld for 4 hours after dosing, after which time food and water were provided ad libitum. Aliquots of urine and feces were radioassayed at the time of collection, and the remainder was stored frozen.

¹ Only items appropriate to this DER have been included.

Radioactivity was quantified in urine by direct liquid scintillation counting (LSC) and in the feces using combustion followed by LSC.

Extraction of Urine: Raw urine was diluted with water and extracted three times with ethyl acetate. The organic phase was dried over sodium sulfate and concentrated, and aliquots were radioassayed by LSC. Aliquots of the spent urine were also radioassayed by LSC and the remainder treated with β -glucuronidase/aryl sulfatase by incubating overnight at 37°C. Following the incubation period, the urine was acidified (pH 1.5) and extracted three times with ethyl acetate. Aliquots of the ethyl acetate extracts and the aqueous phases were radioassayed by LSC.

Extraction of Feces: Fecal samples were extracted by refluxing with methanol for 1 hour. Aliquots of the spent feces were dried, combusted, and radioassayed by LSC. The methanol extracts were concentrated, and aliquots were radioassayed by LSC.

Isolation and Cleanup of Metabolites: Urine extracts were initially cleaned up by silica-gel chromatography. Fractions constituting the same radioactive peak were pooled, concentrated under vacuum, and subjected to additional separation and cleanup by thin-layer chromatography (TLC) using the solvent systems listed in Appendix C, TLC Solvent Systems, CBI p. 24. In addition, TLC was used for the initial isolation and cleanup of fecal radioactivity. Radioautographs or spark chamber radiograms were made from these plates, and the radioactive zones were removed and eluted with methanol. Each zone removed from the TLC plates was purified by high pressure liquid chromatography (HPLC) and the isolated metabolites were analyzed by mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy.

12. REPORTED RESULTS:

- A. The entire radioactive dose was essentially eliminated by both males and female rats within 48 hours (Table 1). However, an additional 1 to 2 percent of the dose was excreted between 48 and 72 hours. The major route of excretion in both males and females was via the feces; they excreted an average of 81.2 (males) and 90.0 percent (females) of the dose within 48 hours. Both males and females excreted an average of approximately 8.5 percent of the dose in the urine. The cumulative percent of the dose recovered in the urine and feces after 72 hours was 90.4 and 100.6 percent for the males and females, respectively. The low recovery (as well as the large standard deviation) of [^{14}C] in the male rats was largely a result of low fecal excretion in rat No. QCS. If this rat was excluded as an outlier, the cumulative percent recovery after 72 hours would be 94.8 percent.

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TABLE 1. Cumulative Excretion of Radioactivity in Male and Female Wistar Rats Receiving a Single 250-mg/kg Oral Dose of [^{14}C]-EL-107

	Cumulative Percent of Administered Dose ^a					
	Male			Female		
	Urine	Feces	Total	Urine	Feces	Total
24 hours	7.0 ± 1.4 ^b	74.2 ± 8.9 ^c	80.9 ± 9.0 ^c	5.6 ± 1.5	78.2 ± 9.4	83.8 ± 8.7
48 hours	8.4 ± 1.8 ^b	81.2 ± 12.1	89.6 ± 12.6	8.3 ± 2.8	90.0 ± 4.0	98.3 ± 5.4
72 hours	8.6 ± 1.9 ^b	81.8 ± 11.8	90.4 ± 12.1	8.5 ± 3.0	91.8 ± 4.3	100.6 ± 6.4

^a Mean ± SD of five animals/group. The standard deviations were calculated by our reviewers.

^b These values were incorrect in the final report.

^c Only four animals were used to calculate these values (24-hour feces from rat No. 001 contained inordinately low levels (3.3 percent) of [^{14}C]).

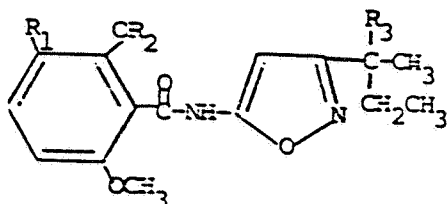
- B. Extraction of feces with methanol was essentially quantitative with 97.1 and 97.5 percent of the fecal [^{14}C] extracted from the male and female fecal samples, respectively. The major component in fecal extracts was EL-107, which represented 87.5 and 90.8 percent of the extractable fecal radioactivity in males and females, respectively. The remainder of the extractable fecal radioactivity (10-12 percent) consisted of highly polar [^{14}C] material (2-4 percent) that remained at the origin of the TLC plates, a band of radioactivity representing 1 to 2 percent of the radioactivity that chromatographed as a broad, diffuse band starting just above the origin, and two to three minor metabolite zones that as a group accounted for 2 to 4 percent of the extractable fecal radioactivity. No further characterization of this radioactivity was performed.
- C. The 0- to 24-hour and the 24- to 48-hour urine collections for each sex were pooled. Extraction of urine with ethyl acetate removed 14.2 and 23.2 percent of the urinary [^{14}C] from male and female urine, respectively (this fraction of radioactivity was designated the neutral fraction or NF). The aqueous fractions remaining after the ethyl acetate extraction were treated with aryl sulfatase/ β -glucuronidase to hydrolyze conjugated metabolites of EL-107. Following this hydrolysis procedure, 53.6 and 42.9 percent of the [^{14}C] in male and female urine, respectively, were extractable into ethyl acetate (this fraction of radioactivity was designated the aglycone fraction or AF). Of the remaining radioactivity, 10.3 and 14.9 percent was nonextractable from the male and female urine, respectively, following enzyme hydrolysis and approximately 15 to 20 percent could not be accounted for following enzyme hydrolysis. The cause for this lost radioactivity was not known, but could have resulted from incomplete phase separation due to emulsion formation.
- D. The neutral and aglycone fractions from both male and female urine were each initially characterized by silica-gel column chromatography (Appendix D, Silica-gel Elution Profiles, CBI pp. 27-30, Figures 2-5). The authors stated that except for some minor differences, the elution profiles for the neutral fraction from male and female urine (Figures 2 and 4) were basically identical as were the profiles for the aglycone fraction from males and females (Figures 3 and 5).
- E. The peak fractions eluted from the silica-gel columns were pooled as shown in Figures 2 to 5 and further analyzed by TLC. The distribution of metabolites in the neutral and aglycone fractions from female urine were essentially identical to the distribution found in the comparable fractions from male urine. With the exception of peaks MAF-2, FAF-1, and FNF-5, each of which contained a single radioactive component, all other peaks contained multiple components. In several cases, up to five metabolite zones were observed in a single column peak. Comparison of the R_f values of the major metabolite zones in the various column peaks indicated that for the most part, the same metabolites were present in both

the neutral and aglycone fractions. The major exception to this was the two very polar zones of radioactivity observed in fractions MNF-6 and FNF-6. When these two column fractions were treated by enzyme hydrolysis as described previously for unextractable urinary radioactivity, TLC analysis of radioactivity in the subsequent ethyl acetate extracts showed them to be virtually free of the previously observed polar radioactivity. This analysis further showed that the extractable [¹⁴C] now contained the same metabolite spectrum present in the comparable aglycone fraction. 005732

- F. As a result of the characterization work, it was estimated that 15 to 20 metabolites of EL-107 were present in urine. Quantitative TLC analysis indicated that six to seven of the urinary metabolites were present at concentrations equal to approximately 2 to 10 percent of the urinary radioactivity. The remainder of the metabolites was considered to be minor since they were present in concentrations ranging from 0.1 to about 1.0 percent of the urinary [¹⁴C].
- G. Only the major EL-107 metabolites were isolated from the TLC plates for structure determinations. Table 2 summarizes the structures and percent distribution of the major urinary metabolites of EL-107. Structural determinations were made based on analysis by TLC, HPLC, MS, and NMR. Quantitation of [¹⁴C] was accomplished by scraping the corresponding radioactive zone from TLC plates and subjecting the material to radioassay by LSC.

Metabolite A represented about 80 percent of the radioactivity in fractions MAF-2 and FAF-2. This compound was also detected in fraction MNF-1. Metabolite B₁ accounted for 40 percent of the radioactivity in fractions MAF-3 and FAF-3. It was also present in FNF-1 and MNF-1 where it represented about 50 and 15 percent of the radioactivity, respectively. Metabolite B₂ represented approximately 16 percent of the [¹⁴C] in fraction MAF-3 and 25 percent in FAF-3. It was also a minor component in MNF-1 and FNF-1. Metabolite B₃ represented 20 percent of the [¹⁴C] in MAF-3 and was a minor component in MNF-1 and FNF-1. It did not appear to be present in the aglycone fraction of female urine. Metabolite C was the major metabolite in fractions MAF-5 and FAF-5. In addition, it was the major metabolite released by enzyme hydrolysis of fractions MNF-6 and FNF-6. Metabolite D₁ + D₂ constituted the major [¹⁴C] zone in fractions MAF-6 and FAF-6. This major zone represented 70 percent of the [¹⁴C] in these two fractions. Metabolite E was isolated from fraction MNF-3. Metabolite F was isolated from fractions MNF-4 and MAF-4. Metabolite G was the sole component in fractions MAF-1 (radioautograph data not included) and FAF-1. Metabolite H was the major radioactive component in fraction FAF-4 and comprised 40 percent of the radioactivity in that fraction. It also appeared to be present in MAF-4 and FNF-2 based on TLC comparisons.

TABLE 2. Structure and Percent Distribution of Radiolabeled EL-107 Urinary Metabolites from Male and Female Wistar Rats Administered a Single 250-mg/kg Oral Dose of [¹⁴C]-EL-107



Compound	Urinary [¹⁴ C] (percent)		Structure		
	Male	Female	R ₁	R ₂	R ₃
EL-107 ^a	1.0-1.5	0.3-0.5	-H	-CH ₃	-CH ₂ CH ₃
Metabolite A	5-6	1-1.5	-H	-H	$\begin{array}{c} \text{O} \\ \parallel \\ \text{-C-CH}_3 \end{array}$
Metabolite B ₁ + B ₂ ^b (diastereomers)	7-9	3-4	-H	-H	$\begin{array}{c} \text{OH} \\ \\ \text{-CHCH}_3 \end{array}$
Metabolite B ₃ ^b	2.5-3.5	< 0.2	-H	-H	-CH ₂ CH ₂ OH
Metabolite C	12-14	10-12	-OH	-CH ₃	$\begin{array}{c} \text{O} \\ \parallel \\ \text{-CCH}_3 \end{array}$
Metabolite D ₁ + D ₂ (diastereomers)	8-9	11-12	-OH	-CH ₃	$\begin{array}{c} \text{OH} \\ \\ \text{-CHCH}_3 \end{array}$
Metabolite E ^a	0.5-1.0	ND ^c	-H	-CH ₃	$\begin{array}{c} \text{O} \\ \parallel \\ \text{-CHCH}_3 \end{array}$
Metabolite F ^a	0.5-1.0	ND	-H	-CH ₃	-CH ₂ CH ₂ OH
Metabolite G	ND	0.5-1.0	-H	-H	-CH ₂ CH ₃
Metabolite H	ND	2.3	-OH	-CH ₃	-CH ₂ CH ₃

^aStructures supported by comparison to authentic standards.

^bStructures consistent with available data but not definitive.

^cNot determined.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

When administered to rats in single oral doses, [¹⁴C]-EL-107 was poorly absorbed. Virtually all of the administered dose was excreted within 48 hours of its administration, and 90 percent was found in the feces as unmetabolized EL-107. The small amount of EL-107 (approximately 8 to 10 percent of the dose) that was absorbed from a single oral dose was extensively metabolized into 15 to 20 metabolic products, which were subsequently excreted primarily via the urine. The major metabolic transformations involved oxidation of the EL-107 molecule at position 2 of the alkyl side chain to produce either an alcohol or ketone, hydroxylation of the aromatic ring adjacent to one of the methoxy substituents, and O-demethylation of one of the methoxy substituents.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

This study was well conducted. The test material, animals, and methodology were adequately described, and the results supported the authors' conclusions. The authors concluded that virtually all of the EL-107 equivalents were excreted by the end of 3 days. Although this statement is generally correct, there are some points that should have been more thoroughly clarified. The sum of urinary and fecal [¹⁴C] was approximately 100 percent after 72 hours; however, it would have been more convincing had the authors measured the [¹⁴C] remaining in the carcasses at 72 hours. This would have answered most questions about the extent of the excretion of EL-107 equivalents if the total recovery was still approximately 100 percent. In addition, it would help in explaining why the fecal excretion of male rat No. 005 was so low; i.e., if the [¹⁴C] retained in the carcass was comparable to the other male rats then maybe rat No. 005 was not administered the proper dose. The authors also made a misstatement in their conclusions. They stated that virtually the entire dose was excreted within 48 hours of administration, 90 percent of which was found in the feces as unmetabolized EL-107. In fact, 90 percent of the administered dose was found in the feces at 48 hours, but only 90 percent of the fecal [¹⁴C] (81 percent of the administered dose) was identified as unmetabolized EL-107. It is not clear why the authors made no attempt to identify the fecal metabolites. There was clearly enough fecal radioactivity present as metabolites (9-11 percent of the administered dose versus 8.5 percent of the administered dose for the urinary metabolites). The identification of the fecal metabolites would have added important information to the overall metabolic fate of EL-107.

With regards to the identification of the urinary metabolites, the combination of silica-gel chromatography, TLC, HPLC, MS, NMR, and comparisons to authentic standards when possible gave strong evidence for the authors' conclusions on metabolite profile and structures. The three positions of attack on the EL-107 molecule are well demonstrated as well as the conclusion that, generally, the male and

female rat metabolize EL-107 at the same rate and at the same positions. However, the unaccountable loss of approximately 20 percent of the urinary [¹⁴C] during extraction could mean that minor differences may exist between the reported results and the true metabolic profile of EL-107.

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Item 15--see footnote 1.

16. CBI APPENDIX:

Appendix A, Metabolite Structures and Names, CBI pp. 22-23;
Appendix B, Materials and Methods, CBI pp. 3-8; Appendix C, TLC
Solvent Systems, CBI p. 24; Appendix D, Silica-gel Elution Profiles,
CBI pp. 27-30.

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APPENDIX A
Metabolite Structures and Names

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Page ___ is not included in this copy.

Pages 336 through 356 are not included.

The material not included contains the following type of information:

- Identity of product inert ingredients.
- Identity of product impurities.
- Description of the product manufacturing process.
- Description of quality control procedures.
- Identity of the source of product ingredients.
- Sales or other commercial/financial information.
- A draft product label.
- The product confidential statement of formula.
- Information about a pending registration action.
- FIFRA registration data.
- The document is a duplicate of page(s) _____.
- The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

Reviewed by: Marcia van Gemert, Ph.D. *Marcia van Gemert 1/14/87*
Head, Section III, Tox. Branch (TS-769C)
Secondary reviewer: Theodore M. Farber, Ph.D.
Chief, Tox. Branch (TS-759C)

DATA EVALUATION REPORT

005732

STUDY TYPE: Excretion of ^{14}C -EL-107 in expired air
TOX. CHEM. NO.: 419F
ACCESSION NUMBER: 265710
MRID NO.: ?

TEST MATERIAL: EL-107

SYNONYMS: Isoxaben

STUDY NUMBER(S): R02185

SPONSOR: Elanco

TESTING FACILITY: Toxicology Division, Lilly Research Laboratories
Greenfield, Indiana 46140

TITLE OF REPORT: Radiocarbon excretion of ^{14}C in the expired air from Fischer 344 rats given single oral doses of EL-107 (121607)

AUTHOR(S): E.G. Gries

REPORT ISSUED: June 1985

CONCLUSIONS: Total radioactivity recovered from all sources, eg. urine and feces, carcass and expired air at 48 hours was 85% for males and 92.6% for females of the dose administered. Expired air accounts for a very small amount of the total radioactivity. Males expired 2.4% and females 2.8% of the dose administered in 48 hours. There is not complete recovery at least in males at 48 hours of total radioactivity. There may be some interference with sample recovery, or incomplete recovery from the carcass. This phenomenon was disturbing enough to Elanco to run this study, looking for residual radioactivity in expired CO_2 . If the residual radioactivity is residing in the carcass, there may be some concern for bioaccumulation.

Classification: core-acceptable

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~~005733~~

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

A. MATERIALS

1. Test compound: EL-107, Batch # Z 10025.

EL-107 is a mixture of two predominant isomers designated 121607 and 135520. These are known chemically as N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide and N-[3-(1,1-dimethylbutyl)-5-isoxazole]-2,6-dimethoxybenzamide. Initial lot analysis indicated [redacted] 121607 and [redacted] 135520 with a combined purity of 94.8%. The final analysis indicated [redacted] 121607 and [redacted] 135520 by weight with a combined purity of 93.3%.

Lot number 553-301-055 of compound 121607 was used to radiolabel. ¹⁴C labeled in the isoxazole ring with a specific activity of 10.6uc/mg with a 99.9% purity was used.

Test Article preparation:

Unlabeled and labeled EL-107 were combined in acetone to yield 250 mg/ml solution at 0.08 uCi/mg specific activity. The solution was suspended in 10% aqueous acacia to yield a 25 mg/ml suspension and then the solvent was evaporated off.

2. Test animals: Species: rat, Strain: Fischer 344, Age: 8-9 weeks
Source: Harlan Sprague Dawley, Indianapolis Indiana.
Weight: males: 212.2 ± 4.3, females: 154.2 ± 2.6

B. STUDY DESIGN:

1. Animal assignment

The study consisted of one treatment group of 5/sex. 3 males and 2 females received a single oral dose of approximately 250 mg/kg. Another group of 2 males and 3 females received a similar dose about one week later using a second dose solution. 48 hours after dosing animals were sacrificed.

2. Test Dose Administration

Each rat received 1 ml/100 gms body weight a gavage dose after an overnight fast. Each dose was approximately 250 mg/kg EL-107.

3. Sample collection and Preparation:

Tissue preparation is on appended page 1.2. Appended page 2 details radioactivity quantifications.

4. Quality assurance statement was given and signed June 30, 1986.

5. Procedures:

After animals were administered the dose they were placed in glass metabolism cages. Air flow was 500-1000 cc/min. Water was available ad libitum but food was withheld until 6 hours post-

dosing.

RESULTS:

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Expired $^{14}\text{CO}_2$

Expired $^{14}\text{CO}_2$ levels are presented in Table I on appended page 3. Combining the replicate experiments, the mean percent of dose administered that was recovered in the expired air as $^{14}\text{CO}_2$ for males was 0.4, 1.7 and 0.3% for 6, 24 and 48 hours respectively. The total collected for the 48 hour period for males was 2.4%. Female $^{14}\text{CO}_2$ expired as the percent of dose administered was 0.5, 1.7, and 0.6% for 6, 24, and 48 hours respectively with a total of 2.8% in the expired air.

Urinary and Fecal Excretion:

The data in the study were presented as combined urinary and fecal excretion levels of radioactivity because the cages inadequately separated urine from feces, according to the study text. These data are summarized on table I on appended page 3. Male 24 and 48 hour excretion are 60.2 and 17.9% of administered dose. Female 24 and 48 hour excretion data are 55.6 and 18.2% respectively. Total cumulative excretion for males was 78.1% and for females 73.8% of administered dose by 48 hours. As mentioned in the review of metabolism study R11285 there appears to be some concern that the majority of this compound is not excreted by 48 hours. If this is the case, there may be some bioaccumulation occurring.

Residual Radioactivity in Carcass and Cages

Residual radioactivity at 48 hours was minimal in males, being 1.5% of dose administered. The residual left in female carcasses was somewhat more substantial owing to one outlier animal, 1055, with a residual of 46.3% in the carcass remaining at 48 hours. Cage rinsing did not contribute greatly to the total amount of radioactivity recovered, being 3.9% in males and 1.6% in females of the dose administered.

Discussion:

Total radioactivity recovered from all sources was 85% for males and 92.6% for females of the dose administered. Expired CO_2 accounts for a very small amount of the total radioactivity, 2.4 and 2.8% for males and females respectively.

However, there is some concern that at least in males at 48 hours there is not complete recovery of radioactivity. This may be the result of incomplete recovery from the carcass and/or quenching interference from tissue, urine and fecal samples. However, this was a disturbing enough phenomenon found in the other metabolism studies that the firm decided to check expired air for residual $^{14}\text{CO}_2$ levels.

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Pages 360 through 362 are not included.

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- Identity of product impurities.
- Description of the product manufacturing process.
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Reviewed by: Marcia van Gemert, Ph.D. *Manu (cont) 1/12/87*
Head, Section III, Tox. Branch (TS-769C)
Secondary reviewer: Theodore M. Farber, Ph.D.
Chief, Tox. Branch (TS-769C)

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1/12/87

005732

DATA EVALUATION REPORT

STUDY TYPE: Distribution of EL-107 in rats TOX. CHEM. NO.: 419F

ACCESSION NUMBER: 265740 MRID NO.: ?

TEST MATERIAL: EL-107

SYNONYMS: Isoxaben

STUDY NUMBER(S): R11285

SPONSOR: Elanco

TESTING FACILITY: Toxicology Division, Lilly Research Laboratories
Greentfield, Indiana 46140

TITLE OF REPORT: Distribution of Radioactivity into tissues and
organs from Fischer 344 rats given single
oral doses of ¹⁴C EL-107

AUTHOR(S): E.G. Gries

REPORT ISSUED: January 1986

CONCLUSIONS: Radioactivity was measured in tissues at 4 and 24 hours post dosing with ¹⁴C- EL-107. Most of the radioactivity appears to remain in the intestinal tract. However, significant amounts of radioactivity that was present in tissues at 4 hours remains in tissues after 24 hours post dosing. Tissue to plasma ratios suggest that there is some accumulation in several tissues at levels above those found in plasma. This study would have been more informative if one or more time periods beyond 24 hours were used for evaluation, since it appears that significant radioactivity remains in tissues. Section F Guidelines recommend that the study be carried out long enough to eliminate 90% of the radioactivity or that the study be carried out for 7 days, whichever comes first.
Classification: core-supplementary

Special Review Criteria (40 CFR 154.7)

005732

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

A. MATERIALS:

1. Test compound: EL-107, Batch # Z 10025,
EL-107 is a mixture of two predominant isomers designated 121607 and 135520. These are known chemically as N-(3-(1-ethyl-1-methylpropyl)-5-isoxazolyl)-2,6-dimethoxybenzamide and N-(3-(1,1-dimethylbutyl)-5-isoxazole)-2,6-dimethoxybenzamide. Initial lot analysis indicated [redacted] 121607 and [redacted] 135520 with a combined purity of 94.8%. The final analysis indicated [redacted] 121607 and [redacted] 135520 by weight with a combined purity of 93.3%.

Radiolabeled and unlabeled material were mixed to yield a 333 mg/kg solution at 0.666Ci/mg, which was suspended in 10% aqueous acacia to a final concentration of 100 mg/kg, and was then evaporated.

2. Test animals: Species: rat, Strain: Fischer 344, Age: 7-8 weeks, Weight: not given, Source: Charles River Breeding Laboratories, Wilmington Mass. However, replacement animals of the same age were used for the 4-hour dose groups and these were obtained from Harlan Sprague Dawley, Indianapolis Indiana. The strain was not specified.

B. STUDY DESIGN:

1. Animal assignment

Five animals sex were assigned to the either the 4 hour or the 24 hour test group.

2. Procedures:

Rats were fasted overnight prior to dosing. The dose/rat for 4 hour groups was 1.0 ml and for 24 hour groups was 1.1 ml/100 gm. body weight by gavage giving about 1000 mg/kg of EL-107 as a final dose.

3. Sample collection and Preparation:

At 4 and 24 hours after dosing animals were bled from the abdominal aorta with heparinized syringe. After ether anaesthesia, samples of blood were taken and centrifuged for packed cell volume. Animals were exanguinated and organs were removed. Tissue preparation is on appended page 1. Appended page 2 details radioactivity quantification and packed cell volume determinations.

RESULTS:

Appended pages 3-8 contain mean ug-eq/g tissue as well as tissue/plasma levels. All tissue concentrations of radioactivity except colon (appended page 4) were lower at 24 hours than at 4

hours. Many tissue/plasma concentrations were higher than 1.0 by 24 hours. Table I details these ratios.

TABLE I
Tissue/plasma ratios

females Tissue	females		males	
	4 hrs	24 hrs	4 hrs	24 hrs
Carcass	39.9	19.3	65.62	34.94
Kidney	2.64	2.32	3.2	2.8
Liver	11.85	6.54	10.95	7.53
Adrenal	2.26	0.75	1.09	1.28
Fat	3.65	3.70	2.10	1.71
Duodenum	26.94	5.45	30.65	13.44
Jejunum	21.15	9.47	42.98	25.38
Ileum	155.65	13.99	177.31	37.83
Colon	4.60	11.67	10.70	15.03
Pancreas	1.65	1.30		
Prostate			1.69	1.67

Appended pages 9 and 10 present tables of the percent of 4 hour radioactivity remaining in the tissues after 24 hours. This gives some indication of the tissue retention after 24 hours. For the sake of comparison, the previous metabolism study using 250 mg/kg was presented.

Table II gives some indication of the body retention of the administered radioactive dose in the carcass and intestinal contents.

Table II
% of Administered Dose

males Tissue	males		females	
	4 hr	24 hr	4 hr	24 hr
Carcass	77.85	31.01	76.94	19.85
Intestinal contents	14.62	6.07	15.42	3.68

Discussion:

As can be seen on appended pages 9 and 10, significant amounts of radioactivity remain in tissues after 24 hours, contrary to the study text which discounts amounts of radioactivity remaining in the tissues. Most of the radioactivity remains in the intestinal tract, particularly in the colon. However, from examination of the tissue/plasma ratios, it appears that significant amounts of radioactivity remain in the tissues after 24 hours. In addition, Table II shows that there is a significant amount of the administered dose remaining in the carcass after 24 hours, 31.01%

in males and 19.85% in the female carcasses. This study would have been of much greater value if an additional time period, eg. 48 and/or 72 hours was used to determine more clearly the residence time for radioactivity remaining in the tissues. Section F guidelines recommend that the study be carried out long enough to eliminate 90% of the radioactivity or for the study to run 7 days, whichever comes first.

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Head, Section III, Tox. Branch (TS-769C)
Secondary reviewer: Theodore M. Farber, Ph.D.
Chief, Tox. Branch (TS-769C)

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DATA EVALUATION REPORT

STUDY TYPE: Repeated dose distribution study TOX. CHEM. NO.: 419F
in rats

ACCESSION NUMBER: 265740 MRID NO.: ?

TEST MATERIAL: EL-107

SYNONYMS: Isoxaben

STUDY NUMBER(S): R13885

SPONSOR: Elianco

TESTING FACILITY: Toxicology Division, Lilly Research Laboratories
Greenfield, Indiana 46140

TITLE OF REPORT: Distribution of radioactivity into tissues and
organs from Fischer 344 rats given oral doses of unlabeled EL-107
daily for two weeks followed by a single dose of ¹⁴C EL-107

AUTHOR(S): E.G. Gries

REPORT ISSUED: January 1986

CONCLUSIONS: Predosing of the animals with cold EL-107 at 250 mg/kg did not significantly change the pattern of urinary and fecal excretion of EL-107, compared to the results of previously reviewed excretion studies, ie ABC-0153 and R11285. 80.59% of the radioactivity was excreted within 7 days for males and 96.1% excreted for females in combined urinary and fecal excretion. Significant amounts of radioactivity did not remain in the organs and tissues after 7 days post dosing with radiolabeled EL-107. In male carcasses the percent radioactivity remaining was 0.3% and for females, the remaining radioactivity was 0.4%.

Classification: core-acceptable

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED 005732

A. MATERIALS:

1. Test compound: EL-107, Batch # 2 10025,
EL-107 is a mixture of two predominant isomers designated 121607 and 135520. These are known chemically as N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide and N-[3-(1,1-dimethylbutyl)-5-isoxazole]-2,6-dimethoxybenzamide. Initial lot analysis indicated [redacted] 121607 and [redacted] 135520 with a combined purity of 94.8%. The final analysis indicated [redacted] 121607 and [redacted] 135520 by weight with a combined purity of 93.3%.

Lot number 553-3nl-056 of compound 121607 was used to radiolabel. ¹⁴C labeled in the isoxazole ring with a specific activity of 10.6uc/mg with a 99.9% purity was used.

Test Article preparation:

For the two weeks prior to radioactive EL-107 administration, unlabeled EL-107 was given at a dose of 25 mg/kg in 10% aqueous acacia and prepared daily. Each rat was given 1 ml/100 kg body weight by gavage which equals 250 mg/kg/day. The radioactive dose on day 15 consisted of both label and unlabeled EL-107 combined to yield 25 mg/ml solution with 0.13 uCi/mg specific activity. This solution was suspended in 10% acacia to yield 25 mg/ml and then evaporated.

2. Test animals: Species: rat, Strain: Fischer 344, Age: 9-10 weeks
Source: Harlan Sprague Dawley, Indianapolis Indiana.
Weight: males: 175.8 ± 3.5, females: 157.6 ± 2.0

B. STUDY DESIGN:

1. Animal assignment

Five animals/sex were given 250 mg/kg daily of unlabeled EL-107 for 14 days. On day 15 they received a single 250 mg/kg oral gavage dose of labeled ¹⁴C-EL-107. Urine and feces were collected in metabolism cages for 7 days after dosing with the radioactive EL-107. After 7 days tissues and organs were collected and analyzed for radioactivity content.

2. Sample collection and Preparation:

Tissue preparation is on appended page 1, 2 and 3. Appended page 3 details radioactivity quantification and packed cell volume determinations.

3. Quality assurance statement was given and signed April 3, 1986.

RESULTS:

Urinary and Fecal Excretion:

Table I lists urinary and fecal excretion of radioactivity at the percent of administered dose for the 7 days post radioactive

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dosing. Males excreted 5.59% in the urine and 75% in the feces within 7 days compared to females who excreted 7.53% in urine and 88.57% in feces. At 24 hours males had excreted 4.67 and 61.32% of the administered dose in urine and feces respectively and females had excreted 5.94 and 69.57% of the radioactivity in urine and feces respectively.

Table I

	Mean Percent of Administered Dose			
	Urine		Feces	
	Male	Female	Male	Female
Day 16	4.67	5.94	61.32	69.57
17	0.79	1.36	13.12	17.75
18	0.10	0.19	0.42	1.11
19	0.02	0.03	0.06	0.07
20	0.01	0.01	0.01	0.03
21	0.00	0.00	0.01	0.02
22	0.00	0.00	0.02	0.01
Total	5.59	7.53	75.00	88.57

Tissue levels of Radioactivity:

Residual levels of radioactivity remaining in the body after 7 days were very low. Table II details male and female carcass levels which were 0.3 and 0.4% of the initial radioactive dose respectively. Intestinal tract by 7 days was devoid of radioactivity and liver of males and females was extremely low, 0.01 and 0.02% respectively. See table II for details.

Table II
Mean Percent of Dose Remaining after 7 Days

Tissue	Males	Females
Carcass	0.30	0.40
Intestines	0.00	0.00
Liver	0.01	0.02

RBC/Plasma ratios

Plasma levels were so low at termination that the calculation was determined by the study directors not to be "meaningful."

Cage Washing Rinse

Cage washing resulted in a recovery of 0.08% for males and 0.13% for females of initial radioactive dose for females. Less than 0.02% of the total radioactivity can be accounted for by the cage washing procedure. 005732

Discussion:

Pre-dosing of the animals with cold EL-107 at 250 mg/kg did not significantly change the pattern of urinary and fecal excretion of EL-107 when compared to the previous rat metabolism studies ABC-0153 and K11285. 80.59% of the radioactivity was excreted within 7 days for males and 96.1% excreted in females when combining urinary and fecal excretion. Significant amounts of radioactivity did not remain in the organs and tissues after 7 days post dosing with radiolabeled EL-107, with carcass radioactivity under 0.3% for males and 0.4% for females of the administered dose.

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Secondary reviewer: Theodore M. Farber, Ph.D.
Chief, Tox. Branch (TS-769C) *Theodore M. Farber*

005732

DATA EVALUATION REPORT

STUDY TYPE Absorption and disappearance of plasma ^{14}C -EL-107 in mice
TOX. CHEM. NO. 419F
ACCESSION NUMBER: 265729
MRID NO.: ?

TEST MATERIAL: EL-107

SYNONYMS: Isoxaben

STUDY NUMBER(S): M03182

SPONSOR: Elanco

TESTING FACILITY: Toxicology Division, Lilly Research Laboratories
Greenfield, Indiana 46140

TITLE OF REPORT: Overview of [^{14}C]- EL-107 disposition in mice

AUTHOR(S): R.B.L. van Lier, E.G. Gries

REPORT ISSUED: Sept. 1986

CONCLUSIONS:

Plasma half-life was calculated to be 8.3 to 8.9 hours for radioactivity. Most of the plasma radioactivity was eliminated by 72 hours. The data suggest that the amount of EL-107 absorbed is limited to about 100 mg/kg. Plasma elimination is not affected by increasing doses. This was a dose-range finding study to determine doses for the mouse oncogenicity study and made no pretense as a full metabolism study.

Classification: core-supplementary

005732

A. MATERIALS:

1. Test compound: EL-107, Batch # 121607. Isomeric component is $> 85\%$, The label is in the alpha position of the isoxazole ring.

Test Article preparation:

1, 15, 100 or 200 mg/kg EL-107 were prepared in polyethylene glycol -200 (PEG-200) and given at 10 ml/kg body weight. Except for the 1 mg/kg dose each animal received around 100 uCi [^{14}C]-EL-107/kg. The 1 mg/kg dose was 11.21 uCi/kg.

2. Test animals: Species: mouse, Strain: ICR, Age: not given
Source: not given
Weight: females: 17-22 grams

B. STUDY DESIGN:

1. Animal assignment

33 females/group were given a single gavage dose of 1, 15, 100 or 200 mg/kg.

2. Quality assurance statement was not given.

3. Procedures:

Cardiac puncture blood samples were obtained from anaesthetized animals at 0.5, 1, 2, 4, 6, 16, 24, 48, 72, 120, and 168 hours after dose administration. Plasma was centrifuged and 0.075 to 0.25 ml aliquots were mixed with liquid scintillation cocktail and were assayed in liquid scintillation spectrometer for radioactivity.

4. Results:

Figure 1 on appended page 1 details the plasma levels of radioactivity over a 72 hour period. In this figure the plasma levels of both 100 and 200 mg/kg dose were virtually identical. This confirms the finding of the earlier study M03182 that above 100 mg/kg the distribution changes. Plasma elimination does not appear to change with increasing doses, but the amount absorbed from the gut is somewhat limited above 100 mg/kg. These values are presented on appended pages 2 and 3 on table I.

The study text states that the half-life for elimination of ^{14}C from the plasma is 8.3 to 8.9 hours and did not appear to change with dose. The area under the curve values support the idea that

absorption appears to be limited beyond 100 mg/kg doses. AVC values extrapolated to infinity were 1.52, 21.5, 88.7 and 114.4 ug-eq/hr/ml for the 1, 15, 100 and 200 mg/kg doses respectively. These AVC/dose ratios come out to be 1.52, 1.43, 0.89 and 0.57 for 1, 15, 100 and 200 mg/kg doses respectively. There was only a 29% increase in AVC obtained for the two-fold increase in dose from 100 to 200 mg/kg according to the study text.

It appears that the kinetics of EL-107, most likely absorption, are changed above 100 mg/kg doses.

Appended pg 1

005732

TABLE 1. CUMULATIVE EXCRETION OF RADIOACTIVITY IN MALE MICE RECEIVING A SINGLE ORAL DOSE OF RADIOLABELED EL-107. STUDY M03082

CAGE NUMBER	DOSE GROUP (HG/KG)	CUMULATIVE PERCENT OF DOSE															
		URINE					FECEs					TOTAL URINE AND FECEs					
		24 HR.	48 HR.	72 HR.	24 HR.	48 HR.	72 HR.	24 HR.	48 HR.	72 HR.	24 HR.	48 HR.	72 HR.				
1001	1.0	16.1	16.3	16.4	38.4	41.0	41.4	54.5	57.3	57.9							
1002		40.7	42.0	42.2	41.0	44.2	44.4	81.7	86.2	86.6							
1003		50.5	50.9	51.0	35.2	36.1	36.3	85.7	87.0	87.4							
1004		47.8	48.3	48.5	43.5	44.5	44.7	91.3	92.8	93.2							
1005		36.5	37.3	37.3	47.2	49.1	49.5	83.7	86.4	86.9							
MEAN		38.3	39.0	39.1	41.1	43.0	43.3	79.4	82.0	82.4							
S.E.		6.1	6.1	6.2	2.1	2.2	2.2	6.4	6.3	6.2							
2001	15.0	46.2	46.5	46.6	47.8	48.9	49.1	94.0	95.4	95.7							
2002		40.8	41.5	41.6	46.4	47.7	47.8	87.2	89.2	89.4							
2003		41.1	43.0	43.4	42.8	47.2	47.8	93.9	90.2	91.2							
2004		35.7	37.6	37.8	48.1	51.2	51.6	84.7	88.7	89.3							
2005		36.0	36.7	36.7	57.9	58.9	59.4	93.9	95.5	96.1							
MEAN		40.2	41.0	41.2	48.6	50.8	51.1	88.7	91.8	92.3							
S.E.		1.8	1.8	1.8	2.5	2.1	2.2	2.2	1.5	1.5							
3001	100.0	21.5	21.9	21.9	67.4	68.7	68.9	88.9	90.6	90.8							
3002		22.3	22.5	22.6	71.5	72.3	72.4	93.7	94.8	95.0							
3003		18.6	18.5	19.0	71.1	72.1	72.3	89.8	91.1	91.3							
3004		21.8	22.7	22.8	68.1	69.4	69.5	89.9	92.1	92.3							
3005		25.2	25.5	25.6	71.3	73.0	73.3	96.4	98.5	98.9							
MEAN		21.9	22.3	22.4	69.9	71.1	71.3	91.7	93.4	93.7							
S.E.		1.0	1.1	1.1	0.9	0.9	0.9	1.4	1.5	1.5							

Appendix pg 2

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TABLE 1. (CONTINUED)
STUDY M03082

CAGE NUMBER	DOSE GROUP (MG/KG)	CUMULATIVE PERCENT OF DOSE											
		URINE			FECES			TOTAL URINE AND FECES					
		24 HR.	48 HR.	72 HR.	24 HR.	48 HR.	72 HR.	24 HR.	48 HR.	72 HR.	24 HR.	48 HR.	72 HR.
4001	300.0	15.3	16.2	16.3	65.6	69.8	70.2	80.9	86.0	86.5			
4002		17.1	18.5	18.8	53.7	66.7	68.0	70.8	85.2	86.8			
4003		13.7	16.8	17.0	62.8	80.1	80.4	76.5	96.9	97.4			
4004		13.9	14.7	14.8	74.1	75.6	75.9	88.0	90.3	90.7			
4005		14.8	15.5	15.6	73.7	77.5	77.7	88.5	93.0	93.3			
MEAN		15.0	16.3	16.5	66.0	73.9	74.5	81.0	90.3	91.0			
S.E.		0.6	0.6	0.7	3.8	2.5	2.3	3.4	2.2	2.0			

Reviewed by: Marci van Gemert, Ph.D. *M. van Gemert 12.22.86*
Section III, Tox. Branch (TS-769C)
Secondary reviewer: Theodore M. Farber, Ph.D.
Chief, Tox. Branch (TS-769C)

Wolfschlaeger

005732

DATA EVALUATION REPORT

STUDY TYPE: 1-year dog study

TOX. CHEM. NO.: 419F

ACCESSION NUMBER: 265733

MRID NO.: ?

TEST MATERIAL: EL-107 (Isomers 121607, 135520, 173490)

SYNONYMS: Isoxaben technical

STUDY NUMBER(S): DO-4783

SPONSOR: Elanco

TESTING FACILITY: Toxicology Division, Lilly Research Labs
Eli Lilly and Co, Greenfield Ind. 46140

TITLE OF REPORT: A one-year toxicity study of EL-107 administered orally to Beagle dogs

AUTHOR(S): S.G. Lake, J.R. Means

REPORT ISSUED: Dec. 1985

CONCLUSIONS: There was an increased alkaline phosphatase levels at the mid and high dose group animals. Liver/brain weight ratios were elevated in the high dose males and females, and liver/body weight ratio was elevated in females. There appears to be some liver microsomal enzyme induction in the high dose males.

NOEL = 10 mg/kg
LEL = 100 mg/kg

Classification: core-Minimum

Previous Dog studies included:

1. a two-week pilot, oral (gavage) non-isomeric study (D-3241)
2. A 3-month subchronic oral (capsule) non-isomeric study (D-33582)
3. A two-week pilot oral (capsule) isomeric study (DO-6282)
4. A 3-month subchronic oral (capsule) isomeric study (D-00783)

A. MATERIALS: MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

1. Test compound: EL-107 is a mixture of isomers (Lilly compound 121607, [REDACTED] Lilly compound 135520, [REDACTED] Lilly compound 173499, [REDACTED])
Description; not given:
Batch # 210025

005732

2. Test animals: Species: Dog, Strain: Beagle, Age: 6 months,
Weight: males- 7.8 ± 0.14 kg, females- 7.1 ± 0.15 kg
Source: Marshall Research Animals Inc. North Rose, New York

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned randomly to the following test groups:

Test Group	Dose in diet (ppm)	Main Study 12 months	
		male	female
1 Cont.	0	4	4
2 Low (LDT)	10	4	4
3 Mid (MDT)	100	4	4
4 High(HDT)	1000	4	4

2. Diet preparation

Dogs received single oral doses in gelatin capsules. The amounts given each animal were adjusted weekly for body weight variations. Control dogs received empty gelatin capsules.

Results - Test compound was assayed for stability and found to be stable stored under normal laboratory conditions.

3. Animals received Purina Certified Canine Diet No. 5007 and water ad libitum.
4. Statistics - The statistical procedures utilized in analyzing the numerical data are on appended page 1.
5. Quality assurance statement was signed and dated Dec. 5, 1985.

C. METHODS AND RESULTS:

005732

1. Observations

Animals were inspected "frequently" on first day of dosing and several times a day during the week and once daily on weekends and holidays for the duration of the experiment for signs of toxicity and mortality.

Physical exams by a Veterinarian were given to each dog before start of the experiment and at termination.

Mortality:(survival) - No animals died on test

Toxicity: No treatment-related signs of toxicity were noted, except for a dose-related increased frequency of soft stools and diarrhea, test compound was also evident in the high dose animals' stool samples on numerous occasions.

2. Body weight

Animals were weighed before study initiation and at weekly intervals for the test duration except during the Christmas holiday.

Results: no compound-related changes were evident.

3. Food consumption and compound intake

Consumption was determined visually and changes in appetite were noted if they occurred.

Results: no compound-related changes in food consumption were noted.

4. Ophthalmological examinations

Performed on all animals 3 weeks prior to study initiation and at termination of the study.

4B. Electrocardiograms: According to the study text, Lead II EKG's were recorded on each animal prior to study initiation and 1.5 hours after dosing at 6 and 12 months. Lead II EKGs were also recorded one month after dosing. However, due to error this time period was before dosing instead of 1.5 hours after dosing.

Results: No treatment-related effects were noted. One female in the mid-dose group displayed skipped beats on one occasion during the test, and had showed similar problems prior to study initiation. This was not thought to be a compound-related phenomenon.

5. Blood was collected before treatment and at study initiation at 1,2 and 4 weeks and monthly thereafter for hematology and clinical analysis from all animals. 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 corpuscular HGB conc.(MCHC)
X	Erythrocyte count (RBC)*	X	Mean corpuscular volume (MCV)
	Platelet count*		Reticulocyte count
	Blood Clotting Measurements	X	Packed cell volume
	(Thromboplastin time)		
	(Clotting time)		
X	(Prothrombin time)		
X	Thrombocyte count		

* Required for subchronic and chronic studies

Bone marrow smears were taken at study termination and an estimated M:E ratio was performed.

Results: no compound-related changes in hematological parameters occurred.

b. Clinical Chemistry

X	Electrolytes:	X	Other:
X	Calcium*	X	Albumin*
X	Chloride*	X	Blood creatinine*
	Magnesium*	X	Blood urea nitrogen*
X	Phosphorous*	X	Cholesterol*
X	Potassium*	X	Globulins
X	Sodium*	X	Glucose*
	Enzymes	X	Total Bilirubin*
X	Alkaline phosphatase	X	Total Serum Protein*
	Cholinesterase#	X	Triglycerides
X	Creatinine phosphokinase*°		Serum protein electrophoresis
	Lactic acid dehydrogenase		
X	Serum alanine aminotransferase (also SGPT)*		
X	Serum aspartate aminotransferase (also SGOT)*		
	gamma glutamyl transferase		
	glutamate dehydrogenase		

* Required for subchronic and chronic studies

Should be required for OP

° Not required for subchronic studies

B1. A portion of the liver was taken from each animal for measurement of in vitro hepatic p-nitroanisole o-demethylase activity.

Results: High dose males showed a statistically significant increase

in P-nitroanisole O-demethylase activity. See appended page 2 for details. Females had values similar to controls. (see appended page 3) EL-107 can be considered an inducer of hepatic microsomal metabolism in males only.

B2. Antipyrine half life determinations were scheduled to be performed before study initiation, at 6 months. However, the 6 month values were not determined and the study initiation values are not presented in the study text.

Results: Clinical Chemistry-

Alkaline phosphatase was elevated in the mid and high dose groups for various time periods. The appended table on appended page 4 has calculated the percentages at several time periods of control values. Alkaline phosphatase values as seen in the control and low dose groups are expected to fall over the course of the experimental time period ie, 1 year. However, the mid and high dose groups remained high during this entire period. Total bilirubin levels were also decreased in the mid and high dose groups in a somewhat inconsistent manner in both males and females.

Creatinine levels in the mid and high dose males were significantly elevated in the pretest blood samples, and remained sporadically elevated throughout the study. Creatinine levels in the low and high dose females were significantly decreased in pretest blood samples but showed some inconsistent elevations in all three dose groups throughout the study. No dose-response was evident and the overall effects on creatinine in males and females did not appear to be treatment-related.

6. Urinalysis°

Urine was collected from each dog at study initiation, at 1, 2 and 4 weeks and monthly thereafter. The CHECKED (X) parameters were examined.

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

Results: no treatment-related changes were evident.

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7. Sacrifice and Pathology:

All animals that died and that were sacrificed on schedule were subject to gross pathological examination and the CHECKED (X) tissues were collected for histological examination. The (XX) organs in addition were weighed.

<u>X</u>	<u>X</u>	<u>X</u>
Digestive system	Cardiovasc./Hemat.	Neurologic
Tongue	X .Aorta*	XX.Brain*†
X .Salivary glands*	XX.Heart*	Periph. nerve*#
X .Esophagus*	X .Bone marrow*	Spinal cord (3 levels)*#
X .Stomach*	X .Lymph nodes*	X .Pituitary*
λ .Duodenum*	X .Spleen*	X .Eyes (optic n.)*#
X .Jejunum*	X .Thymus*	Glandular
X .Ileum*	Urogenital	XX.Adrenals*
.Cecum*	XX.Kidneys*†	Lacrimal gland#
X .Colon*	X .Urinary bladder*	X .Mammary gland*#
.Rectum*	XX.Testes*†	X .Parathyroids*††
XX.Liver*†	Epididymides	X .Thyroids*††
λ .Gall bladder*#	λ .Prostate	Other
X .Pancreas*	Seminal vesicle	X .Bone*#
Respiratory	XX.Ovaries*†	X .Skeletal muscle*#
X .Trachea*	X .Uterus*	X .Skin*#
X .Lung*		X .All gross lesions and masses*
Nose°		
Pharynx°		
Larynx°		

- * Required for subchronic and chronic studies
- ° Required for chronic inhalation
- # In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
- † Organ weights required in subchronic and chronic studies
- †† Organ weight required for non-rodent studies

a. Organ weight

Results:

There was a statistically significant increase in relative liver weight at the high dose with a trend toward increases in the mid dose male groups. Organ to body or brain weights were significantly elevated in the high dose males and females. See table 1 for details.

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TABLE I

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Males:

Groups	Absol. wt.	liver/body wt g/100g	liver/brain wt g/g
1.	249.75	2.331	3.071
2.	246.25	2.350	3.120
3.	273.25	2.953	3.808
4.	300.50	2.819	4.236*

Females:

1.	233.25	2.320	3.150
2.	235.00	2.455	3.087
3.	233.50	2.544	3.085
4.	281.75	2.991*	3.997*

* p < 0.05, two-tailed Dunnett t

b. Gross pathology : No treatment-related gross pathology was found at necropsy.

c. Microscopic pathology: There was "minimal to slight" hypertrophy of hepatocytes in one high dose male and two high dose females. These effects have to be considered compound-related.

No other compound-related effects were seen.

Discussion:

Treatment-related effects were seen at the mid and high dose males and females in elevated alkaline phosphatase at various time periods. High dose males showed a significant increase in p-nitroanisole o-demethylase active indicative of hepatic microsomal enzyme induction. There appears to be a somewhat inconsistent decrease in bilirubin levels in the mid and high dose males and females. High dose males and females showed an increased liver/brain weight ratio and high dose males showed an increased liver/body weight ratio as well.

NOEL = 10 mg/kg

LEL = 100 mg/kg based on increased alkaline phosphatase levels

Core classification: Minimum

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Pages 396 through 399 are not included.

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- Description of quality control procedures.
- Identity of the source of product ingredients.
- Sales or other commercial/financial information.
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EPA: 68-02-4225
DYNAMAC No. 261-C
January 20, 1987

DATA EVALUATION RECORD

EL-107

Chronic 1-Year Feeding Toxicity Study in Rats

STUDY IDENTIFICATION: Lake S. G. An interim report on a chronic toxicity study of EL-107 administered in the diet to Fischer 344 rats for up to one year. (An unpublished study No. R01483, prepared by Lilly Research Laboratories, Eli Lilly and Co., Greenfield, IN; dated July 24, 1984.) Accession No. 073295.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 1-26-87

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1. CHEMICAL: EL-107.

2. TEST MATERIAL: EL-107 is a mixture of isomers consisting primarily of Lilly compound 121607, N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2, 6-dimethoxybenzamide; Lilly compound 135520, N-[3-(1,1-dimethylbutyl)-5-isoxazolyl]-2,6-dimethoxybenzamide; and Lilly compound 173490, N-[3-(1, 1, 2-trimethylpropyl)-5-isoxazolyl]-2, 6-dimethoxybenzamide. Lot No. Z10025 used in this study was [redacted] percent 121607, [redacted] percent 135520, [redacted] percent 173490.

See Appendix B

for chemical structures of components.

3. STUDY/ACTION TYPE: Chronic 1-year feeding toxicity study in rats.

4. STUDY IDENTIFICATION: Lake S. G. An interim report on a chronic toxicity study of EL-107 administered in the diet to Fischer 344 rats for up to one year. (An unpublished study No. R01483, prepared by Lilly Research Laboratories, Eli Lilly and Co., Greenfield, IN; dated July 24, 1984.) Accession No. 073295.

5. REVIEWED BY:

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EPA Section Head

Signature: Marcia Van Gemert
Date: 1/21/87

7. CONCLUSIONS:

- A. EL-107 was tolerated by rats without mortality when fed at dietary levels of 0.0125, 0.125, or 1.25 percent for up to 12 months. There were decreases in body weights, mean weight gains, and efficiency of food utilization in the high-dose females. In the mid- and high-dose groups, serum glucose was increased in females at 6 months and in males at 6 and 12 months. Hepatic microsomal p-nitroanisole O-demethylase activity was elevated in high-dose males and females and mid-dose females (6 and 12 months) and mid-dose males (3 and 6 months). The LOEL and the NOEL were 0.125 percent and 0.0125 percent EL-107, respectively, based on elevation of hepatic microsomal enzyme activity.
- B. The study was acceptable. It was not designed according to guideline protocol requirements as a chronic toxicity study but was a preliminary chronic toxicity study. See accession No. 073296, 2-Year Dietary Study in Rats with EL-107 (DER 1150).

Item 8--see footnote 1.

9. BACKGROUND:

A series of studies were conducted to establish dose levels using different lots of test material (3-month studies, R00182 and R12582; a 2-week pilot study, R08582; and a 3-month subchronic study with a 1-month reversibility phase, R12582). The latter subchronic study used the same test material as the present study at levels of 0, 0.05, 0.14, 0.42, or 1.25 percent EL-107. The results of this study are reviewed in a separate DER (accession Nos. 073293 - 073294).

Item 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

(See Appendix A for complete details. The following is a summary.)

Fischer 344 rats, approximately 3-4 weeks old (Harlan-Sprague Dawley), were acclimated for 7 days prior to study initiation and individually given a physical and ocular examination before assignment to test groups. At initiation the mean weights were 110.2 g for males and 67.7 g for females. The rats were individually caged in a temperature- and humidity-controlled room with a 12-hour light/dark cycle. Groups of 30 rats/sex were administered EL-107 at dietary levels of

¹ Only items appropriate to this DER have been included.

0, 0.0125, 0.125, or 1.25 percent. Diets were prepared every 2 weeks. Subgroups of 10 animals/sex/group were terminated after dosing for 3, 6, or 12 months.

Ophthalmologic examinations were performed prior to study initiation and at 3, 6, and 12 months. Rats were examined daily for physical condition and behavior and received detailed examinations weekly. Body weight and food consumption were determined weekly, and efficiency of food utilization values were calculated. Hematology and clinical chemistry determinations were performed on all rats at their respective termination times.

Enzyme induction was assessed at 3, 6, and 12 months on five animals/sex/group by determination of the level of hepatic p-nitroanisole O-demethylase. For this determination, liver samples (2 g) were collected at necropsy and homogenized.

At each necropsy, the following organs were weighed: adrenals, heart, kidneys, liver, ovaries, prostate, spleen, testes, thyroid, and uterus. 11 animals received a systematic gross examination. Approximately 33 tissues and all gross lesions from each animal were collected and fixed. Only tissues from the animals sacrificed at 1 year were examined microscopically.

Body weight data, weight gain, food consumption and efficiency data, hematology, clinical chemistry, and organ weight data were analyzed statistically using Bartlett's method for analysis of homogeneity of variance and Dunnett's method for pairwise comparison.

12. REPORTED RESULTS:

Test Compound and Dietary Analysis: The total EL-107 activity of the test compound was 95, 94.8, and 92.2 percent at 3, 6, and 12 months, respectively. The test compound was stable for at least 4 weeks in diets stored at 25°C or 37°C and was found to be homogeneously distributed in the diets. EL-107 was at or near theoretical levels in the diet at the four intervals of analysis: ranging from 0.012-0.013 for the 0.0125 percent dose, 0.121 to 0.132 for the 0.125 percent dose, and 1.17 to 1.35 for the 1.25 percent dose.

Ophthalmologic Examinations: At initiation of the study, 26 of 120 males and 26 of 120 females had minor ocular abnormalities (mostly small corneal scars). These animals were randomly distributed to all groups. No increase in abnormalities was found after 3, 6, or 12 months of dosing.

Clinical Observations: There were no compound-related toxic signs throughout the study. Chromodacryorrhea in the eyes was the most common observation, occurring in one to four animals/group; however, the incidence was not increased in dosed groups compared to controls. There were no mortalities during the study.

Body Weight and Food Consumption: There were significant decreases in body weights and mean weight gains in females receiving 1.25 percent EL-107 throughout the study and a decrease in weight gain in females receiving 0.125 percent for the first 3 months of the study. No significant changes in males receiving any EL-107 dosage were seen. Food consumption was similar in all groups of females, but food efficiency was decreased in high-dosed females throughout the study. Males receiving 1.25 percent had increased food consumption during the first 6 months of the study. Selected weight gain and food consumption and efficiency data are summarized in Table 1.

Hematology: There were no changes in hematologic parameters between controls and dosed animals that were considered to be of toxicologic importance since the significant changes ($p < 0.05$) were either of small magnitude or were not dose dependent. Values that were significantly different from control are presented in Table 2.

Clinical Chemistry: At the 3-month sacrifice there were no changes in clinical chemistry parameters that were considered toxicologically important. There were significant increases ($p < 0.05$) in glucose in mid- and high-dose males and in BUN and creatinine in high-dose females; there was also a significant decrease in mean creatinine in high-dose males and alkaline phosphatase in mid- and high-dose males ($p \leq 0.05$). The changes were either of small magnitude or not dose dependent.

At the 6-month sacrifice there were significant increases ($p \leq 0.05$) in mean glucose in mid- and high-dose males and females that were considered toxicologically important (Table 3). Other significant ($p \leq 0.05$) changes were either not dose related or not of a large magnitude, therefore, considered unimportant.

At the 12-month sacrifice the only toxicologically important change was an increase in glucose in mid- and high-dose males (Table 3). Other significant changes ($p \leq 0.05$) were increased BUN in low-dose males, increased creatinine in high-dose males and mid-dose females, and decreased mean alkaline phosphatase and alanine transaminase in high-dose males and females. These changes were small in magnitude and not considered toxicologically important.

Enzyme Induction: There were significant increases in hepatic p-nitroanisole O-demethylase activity in males and females receiving 1.25 percent EL-107 and in females receiving 0.125 percent at 6 and 12 months but no significant changes at 3 months (Table 4).

Organ Weights: Liver weights and liver-to-body weight ratios were consistently increased in high-dose males and females (3, 6, and 12 months). Mean liver weights and liver-to-body weight ratios were significantly increased in mid-dose males at 6 months (Table 5). Other changes in absolute and relative mean organ weights that were significantly different ($p \leq 0.05$) from control values were either

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TABLE 1. Mean Weight Gain and Food Consumption and Efficiency
in Female Rats Fed EL-107 in the Diet for 1 Year

Dietary Level (percent)	Months		
	0-3	0-6	0-12
	Mean Weight Gain (g) ^a		
0	123.9	152.2	188.2
0.125	116.3*	143.3	182.4
1.25	114.3**	139.4**	160.8**
	Mean Daily Food Consumption and (Efficiency) ^b		
0	11.7(11.7)	11.4(7.4)	11.5(4.5)
0.125	11.7(11.0*)	11.3(7.0)	11.6(4.3)
1.25	11.6(10.9*)	11.1(6.9)	11.3(3.9**)

^a For the 0-6 and 0-12 month intervals initial mean weights used to calculate weight gain did not include all animals on study but only initial values for animals remaining at the end of the interval. This table was derived from Tables 5-8 of the CBI.

^b Mean daily food consumption is in g/rat/day; efficiency = grams of body weight gained per 100 g food consumed.

* Significantly different from control value ($p \leq 0.05$).

** Significantly different from control value ($p \leq 0.01$).

TABLE 2. Mean Hematology Values in Rats Fed EL-107 that Differ Significantly from Control Values^a

Parameter/ Interval	Dietary Level (percent)			
	0	0.0125	0.125	1.25
Males				
<u>3 mo</u>				
RBC (10^6)	9.59±0.20	9.97±0.27*	9.92±0.23*	9.68±0.32
HGB (g/dL)	16.48±0.40	17.03±0.42*	16.78±0.33	16.23±0.45
PCV (%)	48.12±0.90	50.19±1.24*	49.57±0.79*	48.44±1.83
MCH (pg)	17.19±0.18	17.10±0.39	16.91±0.17*	16.78±0.27*
MCHC (%)	34.25±0.46	33.95±0.28	33.77±0.30	33.53±0.65*
<u>6 mo</u>				
RBC (10^6)	9.83±0.25	9.81±0.17	9.69±0.17	9.33±0.23*
HGB (g/dL)	17.03±0.24	16.73±0.34*	16.59±0.18*	16.22±0.30*
PCV (%)	49.42±1.29	48.54±0.78	48.11±1.02*	46.44±1.21*
<u>12 mo</u>				
RBC (10^6)	8.93±0.57	8.89±0.31	8.36±0.66*	8.57±0.41
HGB (g/dL)	16.27±0.86	15.94±0.43	14.95±1.11*	15.40±0.57*
PCV (%)	44.49±2.59	43.98±1.87	41.60±3.06*	42.48±1.51
Females				
<u>3 mo</u>				
MCHC (%)	33.77±0.79	34.44±0.69	34.44±0.51	34.55±0.59*
<u>6 mo</u>				
RBC (10^6)	9.06±0.16	8.79±0.25*	8.90±0.21	8.89±0.18
HGB (g/dL)	16.74±0.37	16.28±0.32*	16.46±0.33	16.49±0.31
PCV (%)	47.89±1.04	46.71±1.35	47.18±1.18	46.33±1.09*
MCV (pL)	53.00±0.67	53.00±0.47	52.90±0.32	52.30±0.82*
MCHC (%)	34.95±0.48	34.88±0.57	34.90±0.50	35.58±0.54*

^aMean ± standard deviation of 10 animals/group.

*Significantly different from control value ($p \leq 0.05$).

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TABLE 3. Mean Glucose Levels (mg/dL) in Rats Feed EL-107 for 1 Year

Dietary Level (percent)	6 months		12 months	
	Males	Females	Males	Females
0	171±24	156± 9	171±38	127±34
0.0125	184±12	170±15	187±22	152±22
0.125	199±24*	179±25*	201±15*	164±22*
1.25	201±26*	181±18*	200±15*	150±25

*Significantly different from control value ($p < 0.05$).

TABLE 4. Hepatic p-Nitroanisole O-Demethylase Activity in Rats Receiving EL-107 in the Diet for 1 Year^a 005732

	Percent EL-107 in the Diet			
	0	0.0125	0.125	1.25
Males				
3 months	22.5±1.8	23.1±1.9	18.5±1.5	22.3±1.0
6 months	27.5±1.8	26.3±1.1	29.7±1.6	36.9±1.7*
12 months	18.1±1.2	18.4±0.8	18.5±0.6	24.4±1.2*
Females				
3 months	14.1±1.1	13.5±0.8	16.5±1.3	18.0±1.9
6 months	20.1±0.9	21.1±1.1	27.3±1.7*	37.7±1.6*
12 months	14.2±0.7	15.1±1.4	18.3±0.4*	22.3±1.5*

^aMean values ± standard error (nmol p-nitrophenol/mg protein/hour).

*Significantly different from control value ($p \leq 0.05$).

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TABLE 5. Mean Liver Weights and Liver-to-Body Weight (BW) Ratios in Rats Fed EL-107 in the Diet for 1 Year^a

Interval	Percent EL-107 in the Diet			
	0	0.0125	0.125	1.25
Males				
<u>3 mo</u>				
g	7.80±0.72	8.13±0.69	8.57±0.95	9.52±0.84*
g/100 g BW	2.39±0.11	2.56±0.12*	2.61±0.18	2.86±0.11**
<u>6 mo</u>				
g	9.47±0.47	10.27±0.92	10.94±0.89**	12.17±0.68**
g/100 g BW	2.44±0.09	2.55±0.11	2.69±0.20**	2.98±0.13**
<u>12 mo</u>				
g	11.31±0.91	12.14±0.64	11.88±1.04	12.73±1.21**
g/100 g BW	2.51±0.14	2.62±0.13	2.57±0.07	2.88±0.19**
Females				
<u>3 mo</u>				
g	4.28±0.23	4.36±0.32	4.49±0.39	5.34±0.33*
g/100 g BW	2.32±0.11	2.40±0.15	2.47±0.12*	2.97±0.12**
<u>6 mo</u>				
g	5.24±0.51	5.33±0.34	5.32±0.33	5.72±0.40*
g/100 g BW	2.35±0.17	2.43±0.14	2.51±0.11	2.75±0.17**
<u>12 mo</u>				
g	6.01±0.57	6.14±0.69	6.13±0.53	6.45±0.54
g/100 g BW	2.40±0.11	2.34±0.10	2.46±0.10	2.79±0.29**

^a Mean ± S.D. of 10 animal/sex/group.* Significantly different from control value ($p \leq .05$).** Significantly different from control value ($p \leq .01$).

minimal or not time or dose correlated, and were therefore not considered to be toxicologically important. These included slight increases in mean kidney-to-body weight ratio but not absolute kidney weight in males receiving 1.25 percent EL-107 at 3 months, an increase in absolute but not relative kidney weight at 6 months, and no changes at 12 months. Spleen weights were slightly decreased compared to controls in mid- and high-dose females at 3 and 6 months and in mid-dose females at 12 months; spleen-to-body weight ratios were decreased in high-dose males and females at 3 months and in low-dose females at 12 months. Heart weight was increased in high-dose males only at 12 months.

Gross Pathology: There were no notable gross findings.

Histopathology: At the 12-month sacrifice, there were no compound-related histopathologic changes. Findings were of low incidence and were spontaneous. There was a fairly high incidence of interstitial cell hyperplasia in testes of all groups. There were no findings in liver, kidneys, or spleen that correlated with organ weight changes. Only tissues at the 12-month sacrifice were examined.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. There was no mortality in rats fed diets containing 0.0125, 0.125, or 1.25 percent EL-107 for 1 year. There were decreases in mean body weights, weight gains, and food efficiency in females receiving the high dose. There were significant increases ($p \leq 0.05$) in liver weights in high-dose males and females at 3, 6, and 12 months and in mid-dose males at 3 and 6 months. At 6 and 12 months there was an elevation of hepatic microsomal enzyme levels in high-dose males and females and mid-dose females. There were increases in serum glucose in mid- and high-dose males and females at 6 months and in mid- and high-dose males at 12 months. No important pathological changes were seen at 1 year. The NOEL was 0.0125 percent and the LOEL was 0.125 percent EL-107.

B. A quality assurance statement was dated August 1, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The study was adequately designed and conducted to fulfill the requirements intended. The protocol did not intend to conform to guideline requirements for a 2-year feeding study. We assess that the interpretations of the data by the study author were correct. Body weight gains were calculated to include only animal weights present in the study at 3-, 6-, or 12-month intervals. This treatment more adequately assesses effects on weights than comparing group mean body weights since the means were calculated from 30 animals at 0 time, 20 animals after 3 months, and 10 animals after 6

months. As the author noted, mean weights of control males remaining on study after 6 months were higher than those sacrificed and at 0.125 percent EL-107, mean weights of males remaining at 6 months were lower than those sacrificed at 6 months. 005732

Since the number of animals/group at each sacrifice was 10, the strength of the data on female organ weights may be less than would be expected in the 2-year chronic studies (accession No. 073296, see DER 1150). This latter study should also clarify if there was an adaptation to hepatic enzyme induction with long-term dosing.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Materials and Methods, CBI pp. 16-22, and Appendix B, Chemical Structures of Components.

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APPENDIX A
Materials and Methods

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EPA: 68-02-4225
TASK: 261-D
January 20, 1987

DATA EVALUATION RECORD

EL-107

Chronic Toxicity/Oncogenicity Study in Rats
Interim Report

STUDY IDENTIFICATION: Lake, S. G. A one year interim report on a chronic toxicity/oncogenicity study of EL-107 administered in the diet to Fischer 344 rats for two years. (Unpublished study Nos. R01583 and R01683 prepared by Lilly Research, Eli Lilly and Co., Greenfield, IN; dated July 24, 1984.) Accession No. 073296.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: _____

Date: _____

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

1. CHEMICAL: EL-107.

2. TEST MATERIAL: EL-107 is a mixture of isomers consisting primarily of Lilly compound 121607, N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide; Lilly compound 135520, N-[3-(1,1-dimethylbutyl)-5-isoxazolyl]-2,6-dimethoxybenzamide; and Lilly compound 173490, N-[3-(1,1,2-trimethylpropyl)-isoxazolyl]-2,6-dimethoxybenzamide. Lot Z10025, which was used in this study, was [redacted] percent 121607, [redacted] percent 135520, [redacted] percent 173490. Appendix A presents the results of a chemical analysis of the test material.

3. STUDY/ACTION TYPE: Chronic toxicity/oncogenicity study in rats.

4. STUDY IDENTIFICATION: Lake, S. G. A one year interim report on a chronic toxicity/oncogenicity study of EL-107 administered in the diet to Fischer 344 rats for two years. (Unpublished study Nos. R01583 and R01683 prepared by Lilly Research, Eli Lilly and Co., Greenfield, IN, dated July 24, 1984.) Accession No. 073295.

5. REVIEWED BY:

William L. McLellan, Ph.D.
Principal Reviewer
Dynamac Corporation

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Date: 1-20-87

Charles E. Rothwell, Ph.D.
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Chronic Toxicity
Technical Quality Control
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Date: 1-20-87

Marcia Van Gemert, Ph.D.
EPA Section Head

Signature: Marcia Van Gemert
Date: 1/21/87

7. CONCLUSIONS:

Definitive conclusions cannot be made because the study is an interim report covering only the first year of a 2-year study and the study contains only the in-life data. With these limitations, it can be tentatively established that based on toxicologically important decreased weight gain the LOEL in females is 1.250 percent EL-107 and the NOEL is 0.125 percent EL-107. There were no effects of dosing on clinical laboratory findings or survival of animals.

Item 8--see footnote 1.

9. BACKGROUND:

A previous report of a 1-year chronic feeding study using the same doses of EL-107 and same strain of rats was reviewed (DER 115C). This interim report covers the in-life portion during the first year of a 2-year study. This DER is abbreviated and mainly compares similarities and differences between the previous study (R01483, DER 115C) and the present replicate studies (R01583 and R01683).

Item 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):

A. Materials and Methods: (See Appendix B for complete materials and methods.)

1. Fischer 344 rats obtained from Harlan Sprague-Dawley and approximately 5-6 weeks old were used in the studies. In study R01583 the mean initial weights were 108 ± 1.2 g for males and 90.4 ± 0.9 g for females; in study R01683 the mean weights of rats were 88.6 ± 0.9 g for males and 85.1 ± 1.1 g for females.
2. Replicate groups of 30 rats/sex/group were fed EL-107 in the diets for 2 years at levels of 0, 0.0125, 0.125, or 1.25 percent (0, 125, 1250, or 12,500 ppm). Study R01683 (Feb. 10, 1983) was initiated 12 days after study R01583 (Feb. 22, 1983).
3. The materials and methods were similar to study R01483 (DER115C) with the following additions:
 - Clinical studies were performed at 6 and 12 months.
 - Hematologic analysis included measuring platelet counts.

¹ Only items appropriate to this DER have been included.

- Clinical chemistry included measuring Ca^{2+} , P, Cl^- , Na^+ , K^+ , triglycerides, aspartic amino transferase, and creatine phosphokinase for 10 animals/sex/group in study R01683. In addition, the parameters measured in study R01483 were measured at 12 months.
- No hepatic enzymes were measured.
- Urinalyses were included.
- No sacrifices were scheduled at the end of the first year of the study.

B. Protocol: See Appendix C.

12. REPORTED RESULTS:

Clinical Observations and Mortalities: There were no signs of toxicity in the first year of the study. There were no deaths and only two of 480 animals were sacrificed moribund (one male each in the 0.0125- and 0.125-percent groups).

Body Weights and Food Consumption: Table 1 summarizes weight gain data. There was a dose-related decrease in weight gain of females receiving 0.125 and 1.25 percent EL-107 and in the mean body weights of the female group receiving 1.25 percent. Mean weight gains in males receiving 1.25 percent were slightly lower (significant at $p = 0.01$ or 0.05) in study R01583 than in controls during the first year of the study; in study R01683 mean weight gain was lower only in the group receiving 1.25 percent at 3 months. Food consumption was slightly decreased in females receiving 1.25 percent in study R01683 but not study R01583. These changes in mean food consumption were not considered toxicologically important. Food efficiency was decreased in both males and females receiving 1.25 percent when compared to controls.

Hematology: The only compound-related changes were increases in platelets in high-dose males and females at 6 months, in high-dose females at 12 months, and in mid-dose males at 6 months (Table 2). Other changes were not considered toxicologically important because they were either of small magnitude or not dose dependent. These included a decrease in red cell count and increase in mean corpuscular hemoglobin (MCH) in high-dose males at 6 and 12 months, a decrease in packed cell volume in high-dose males at 12 months, a decrease in MCH in all dosed groups of females at 6 months, and a decrease in mean corpuscular volume (MCV) and increase in MCH in high-dose females at 12 months. Hematologic analysis was performed only in study R01683.

Clinical Chemistry: There were no changes in clinical chemistry parameters that were considered of toxicologic importance. Significant differences noted were either minimal in magnitude or not consistent with time or dose.

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TABLE 1. Mean Weight Gains (g) for Rats Fed EL-107 in the Diet

Dietary Level (percent)	Mean Weight Gain for Males at Month			Mean Weight Gain for Females at Month		
	3	6	12 ^a	3	6	12 ^b
<u>Study R01583</u>						
0	237	308	357	100	132	178
0.0125	240	312	371	103	130	183
0.125	233	306	360	97	126*	167*
1.25	224**	291**	341*	95*	120**	164**

<u>Study R01683</u>						
0	255	319	362	112	144	186
0.0125	258	328	375	114	144	190
0.125	251	323	371	103**	131**	180
1.25	243*	313	364	99**	124**	165**

^a In study R01583 the 12-month data for males are for day 385 and in study R01683 for day 378.

^b In study R01583 the data are for day 392 and in study R01683 for day 378.

*Significantly different from control value ($p \leq 0.05$).

**Significantly different from control value ($p \leq 0.01$).

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TABLE 2. Mean Platelet Counts in Rats Fed EL-107

Dietary Level (percent)	Platelets in Males at Month		Platelets in Females at Month	
	6	12	6	12
0	539±56	787±89	626±75	820±80
0.0125	553±67	807±82	598±57	823±145
0.125	626±63*	825±94	636±66	860±53
1.25	641±66*	872±56	723±64*	967±125*

* Significantly different from control value ($p \leq 0.05$).

Urinalyses: All parameters were similar among groups.

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13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Rats tolerated diets containing EL-107 at levels of 0.0125, 0.125, or 1.25 percent for 1 year without significant mortality or signs of toxicity. Slight decreases in mean body weights and mean weight gains were noted in high-dose females, and slight decreases in food efficiency were noted in mid- and high-dose females and high-dose males. Slightly increased platelet counts occurred in high-dose males and females and in mid-dose males at 6 months and in high-dose females at 1 year. The NOEL, based on data available after 1 year, is 0.0125 percent EL-107.

B. A quality assurance statement was signed and dated August 1, 1984.

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

This is an interim report containing data available on the in-life phase of the study after 1 year; there is, therefore, insufficient data to adequately assess the chronic effects of EL-107. However, it is useful to compare results of this study with those of study R01483 in which 30 rats/group/sex received the same doses and 10 rats/group/sex were sacrificed at 3, 6, and 12 months (accession No. 073295). In the present studies, body weight, food consumption, and food efficiency data are available for a larger sample; results of these studies support those of study R01483 for these parameters.

In study R01483, weight gains of high-dose males were similar to those of controls whereas weight gains of high-dose females were significantly lower than controls. In the present replicate studies, weight gains of high-dose males were significantly lower ($p = 0.05-0.01$) than controls in one replicate study (R01583) but not in the second replicate study (R01683). Weights in the first replicate study were only about 5 percent lower for high-dose males than controls (Table 1). Some lowering of weight gains was noted for mid-dose females (Table 1); however, we do not assess that this lowered weight gain is of toxicologic importance.

Clinical laboratory data are present for animals in study R01683 at 6 and 12 months; data for these parameters will be available for 10 rats/sex/group at 18 months and for all animals at termination. The protocol for study R01583 does not require clinical studies except on all survivors at termination.

In study R01483 (DER 115C) it was concluded that there were no toxicologically important changes in hematologic parameters between dosed animals and controls. Platelet counts were not included in this study. In the present study (R01683), the author concluded that the only compound-related changes were in platelet counts. Although

there were changes in other hematologic parameters in dosed animals when compared to controls, it is our assessment that they were not of toxicologic importance. Comparison of the data in both studies showed that there was no consistency between the two studies in the hematologic findings that were significantly different in dosed and control groups. Therefore, these findings are probably incidental.

Although decreases in alkaline phosphatase and alanine transaminase were noted in high-dose animals in both studies, none of the changes were considered of toxicologic importance by the report author. It is our assessment that any conclusions concerning these changes should be reserved until completion of the 2-year study.

Based on the available data a provisional LOEL based on decreased weight gain is 1.25 percent EL-107 and the NOEL is 0.125 percent EL-107.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Composition of Technical EL-107, Lot Z10025, CBI pp. 218-219; Appendix B, Materials and Methods, CBI pp. 14-19; and Appendix C, Protocol, CBI pp. 195-207.

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APPENDIX A

Composition of Technical EL-107, Lot Z10025

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EPA: 68-02-4225
TASK: 308-5b 005732
February 12, 1987

DATA EVALUATION RECORD

EL-107

Subchronic Oral Toxicity Study in Rats

STUDY IDENTIFICATION: Lake, S. G. A subchronic toxicity study of compound EL-107 (compounds 121607 and 135520) administered in the diet to Fischer 344 rats for three months with a one month reversibility phase. (Unpublished study No. R12582 prepared by Lilly Research Laboratory, Greenfield, IN, for Eli Lilly and Co.; dated April 1984.) Accession Nos. 073293-073294.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: _____

I. Cecil Felkner

Date: _____

2-12-87

005732

1. CHEMICAL: EL-107.

2. TEST MATERIAL: EL-107 is a mixture of isomers consisting primarily of Lilly compound 121607, N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide; Lilly compound 135520, N-[3-(1,1-dimethylbutyl)-5-isoxazolyl]-2,6-dimethoxybenzamide; and Lilly compound 173490, N-[3-(1,1,2-trimethylpropyl)-isoxazolyl]-2,6-dimethoxybenzamide. Lot H02-2G6-118, which was used in this study, was [redacted] percent 121607, [redacted] percent 135520, [redacted].

Appendix A presents the results of a chemical analysis of the test material.

3. STUDY/ACTION TYPE: Subchronic oral toxicity study in rats.

4. STUDY IDENTIFICATION: Lake, S. G. A subchronic toxicity study of compound EL-107 (compounds 121607 and 135520) administered in the diet to Fischer 344 rats for three months with a one month reversibility phase. (Unpublished study No. R12582 prepared by Lilly Research Laboratory, Greenfield, IN, for Eli Lilly and Co.; dated April 1984.) Accession Nos. 073293-073294.

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Date: 2-12-87

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Marcia Van Gemert, Ph.D.
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Date: _____

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MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

7. CONCLUSIONS:

When EL-107 was fed to Fischer 344 rats at dietary levels of 0.05, 0.14, 0.42, or 1.25 percent for 3 months, there were no mortalities, no signs of toxicity, and no biologically important effects on body weights, hematology, or clinical chemistry parameters. After 3 months there were significant ($p < 0.05$ or < 0.01) increases in absolute and relative liver weights in all dosed groups of females and liver-to-body weight ratios in the 0.42- and 1.25-percent groups of males. Hepatic p-nitroanisole O-demethylase was significantly elevated in males receiving 0.42 percent and females receiving 0.14, 0.42, and 1.25 percent. Enzyme activities returned to normal in all groups after a 1-month recovery period, and relative liver weights returned to normal in all groups except males receiving 1.25 percent EL-107. There were no histopathologic lesions that were compound related. The LOEL based on increase in liver weights was 0.05 percent EL-107 in the diet, the lowest dose tested. A NOEL was not established.

The study is Core Guideline.

Item 8--see footnote 1.

9. BACKGROUND:

The dose levels were chosen based on a 2-week pilot study (C08582) with Fischer 344 rats dosed at levels of 0.156 to 2.5 percent EL-107. In this study there were significant increases in relative liver weights in females receiving 1.25 and 2.5 percent and significant increases in hepatic p-nitroanisole O-demethylase activity in males receiving 0.625 and 2.5 percent and females receiving 1.25 and 2.5 percent EL-107.

Item 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):A. Materials and Methods: (See Appendix B for complete details.)

Fischer 344 rats, approximately 5-6 weeks old, were used in the study. Rats were acclimated for 7 days and caged individually in a temperature- and humidity-controlled room with a 12-hour light/dark cycle. Food (Purina Certified Rodent Chow #5002) and water were offered ad libitum. At initiation, the mean body weights (\pm standard error) of males and females were 96.0 ± 0.7

¹ Only items appropriate to this DER have been included.

and 86.9 ± 0.6 g, respectively. Groups of 25 rats/sex were administered EL-107 at dietary levels of 0, 0.05, 0.14, 0.42, or 1.25 percent. Diets were analyzed for concentration, homogeneity, and stability of EL-107. Animals received control diets or diets containing EL-107 for 3 months, and then a subgroup of 10 animals/sex/group was allowed a 1-month recovery period during which they received control diets.

Animals were examined daily for physical condition and behavior and received detailed examinations weekly. Body weights and food consumption were measured weekly. At termination of both the dosing and recovery phases, all rats were fasted overnight and blood was obtained for hematologic and clinical chemistry determinations.

Enzyme induction was assessed by determining the level of hepatic p-nitroanisole O-demethylase activity. This was performed on liver homogenates of five animals/sex/group at the end of dosing and at the end of the recovery period.

Fifteen animals/sex/group were sacrificed at 3 months and 10/sex/group at the end of the 1-month recovery period. The following organs were weighed: adrenals, heart, kidneys, liver, ovaries, prostate, spleen, testes, thyroids, and uterus. A complete gross examination was performed on each animal, and approximately 32 tissues/animal were fixed and examined microscopically.

Body weight data, weight gain data, food consumption and efficiency data, and organ weight data were analyzed statistically using Bartlett's method for analysis of variance and Dunnett's method for pairwise comparison.

12. REPORTED RESULTS:

Test Compound and Dietary Analysis: The test compound was stable when stored under normal laboratory conditions. However, there was a loss of 4.4 percent water from initiation to termination of the study. On initial analysis, combined activity of 121607 and its isomer 135520 was 86.7 percent and was 92.5 percent at termination.

The test material was stable in the diet at 25 or 37°C; analysis of a diet containing a theoretical concentration of 1.25 percent indicated 1.15 percent when prepared fresh and 1.12 percent and 1.16 percent when stored for 4 weeks at 25 or 37°C, respectively. Distribution in the diet was homogeneous. Analyzed concentrations of test material in all diets ranged from 90-104 percent of theoretical concentrations. The mean daily intake of EL-107 in mg/kg/day was 0, 32.34, 93.65, 284.96, and 852.59 for males and 0, 36.10, 104.06, 311.89, and 945.76 for females.

Clinical Observations: The report stated that there were no physical signs of toxicity during the dosing or recovery phase. There were no mortalities.

Body Weights and Food Consumption: There were significant decreases in mean body weights in males receiving 0.42 and 1.25 percent test material in the diet (Table 1). The decreases were noted after 8 days in the 1.25-percent group and after 36 days in the 0.42-percent group. There were no effects of dosing on mean body weights in females; however, the mean weight gains in females receiving 1.25 percent EL-107 were slightly but significantly ($p < 0.05$) lower than controls after 8 through 90 days. The magnitude of these differences in both sexes was minimal and not considered by the author to be toxicologically important. Mean food consumption was similar in all groups, but mean food efficiency was decreased in males and females receiving 1.25 percent EL-107 in the diet from days 8 through 90. Food efficiency was also slightly but significantly lower in both males and females receiving 0.14 and 0.42 percent in the diet at intermittent intervals (cumulative) during the study; however, the differences were small and not considered toxicologically important. During the recovery phase there was a reversal of the effect on body weights and food efficiency. Table 1 summarizes these data for the dosing period and the recovery period.

Hematology: No compound-related effects on hematology were seen in either phase of the study. All mean values in dosed animals at 3 months were similar to control values. At the 4-month sacrifice (after recovery) there were occasional sporadic values significantly ($p \leq 0.05$) different from control values but these were not consistent with dose, were within normal limits, and not considered of toxicologic importance.

Clinical Chemistry: There were no changes in clinical chemistry parameters that were considered to be of toxicologic importance. There was a significant increase ($p < 0.05$) in serum glucose levels in males receiving 1.25 percent EL-107 at 3 months and after the recovery period (Table 2). These changes were considered "treatment related," but minimal and not of toxicologic importance. Glucose was also significantly ($p < 0.05$) increased in females receiving 0.14 and 1.25 percent at 3 months but decreased in females receiving 0.05 and 0.14 percent after the 1-month recovery period when compared with controls. However, the changes were minimal and there were no dose-related trends. At the 3-month sacrifice there were sporadic clinical chemistry values in dosed groups that differed from control values, but there were no dose-related trends. After recovery there was a decrease in total bilirubin in males receiving 0.14 and 0.42 percent, a decrease in alanine transaminase in females receiving 0.05, 0.14, and 0.42 percent, and an increase in creatinine in all dosed females when compared to controls. These changes, although significant ($p < 0.05$), were minimal and not considered to be of toxicologic importance.

Enzyme Induction: After 3 months there was a significant increase ($p < 0.05$) in the mean level of hepatic p-nitroanisole O-demethylase activity in females receiving 0.14, 0.42, and 1.25 percent and in males receiving 0.42 percent EL-107 when compared to control values. There was a reversal of this effect after the 1-month recovery period (Table 3).

TABLE 1. Mean Body Weights, Food Consumption, and Food Efficiency in ⁰⁰⁵⁷³² Fed EL-107 for 3 Months and Allowed a 1-Month Recovery

Sex/Dietary Level (%)	Mean Body Weights (g)			Mean Food Consumption (g/rat/day)		Mean Food Efficiency ^b	
	Initial	3 mo ^a	4 mo ^a	0-3 mo ^a	0-4 mo ^a	0-3 mo ^a	0-4 mo ^a
	n=25	n=25	n=10	n=25	n=10	n=25	n=10
Males							
0.0	97.6	340	367	16.3	16.3	16.3	13.8
0.05	97.7	337	360	15.8	15.7	16.5	13.7
0.14	96.1	334	368	16.0	16.2	16.2	13.8
0.42	93.2	324*	352	15.8	15.7	15.9	13.7
1.25	95.5	322*	360	15.8	16.2	15.7**	13.5
Females							
0.0	85.6	194	210	11.2	11.4	10.6	8.7
0.05	86.7	196	212	11.4	11.4	10.5	8.9
0.14	87.4	194	212	11.7*	11.8	9.9*	8.7
0.42	86.1	191	205	11.4	11.6	10.0*	8.5
1.25	88.8	190	197	11.5	11.2	9.6**	8.3

*Significantly different from control value ($p \leq 0.05$).

**Significantly different from control value ($p \leq 0.01$).

^aThe report statistically analyzed cumulative values at weekly intervals.

^bGrams body weight gained/100 g food consumed.

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TABLE 2. Mean Serum Glucose Levels in Rats Fed EL-107 for 3 Months and after a 1-Month Recovery Period

Dose Level (percent)	Glucose (mg/dL)			
	Males		Females	
	3 mo ^a	4 mo ^b	3 mo ^a	4 mo ^b
0	162±20.4	154±18.9	160±21.6	174±19.7
0.05	179±22.4	152±20.9	164±11.3	149±22.6*
0.14	175±22.4	177±16.0	176±20.5*	150±15.5*
0.42	174±25.2	172±20.0	168±12.5	160±14.1
1.25	185±21.9*	184±23.2*	183±13.0*	157±13.4

*Significantly different from control value ($p \leq 0.05$).

^aMean \pm SD of 15 animals/group.

^bMean \pm SD of 10 animals/group.

TABLE 3. Mean Hepatic p-Nitroanisole O-Demethylase Activity in Rats Fed EL-107

Dietary Level (percent)	nmol p-nitrophenol/mg protein/hour	
	Males	Females
3 Months after Administration		
0	21.02±0.39 ^a	20.29±1.10
0.05	25.60±2.01	22.04±0.78
0.14	24.00±1.24	26.43±0.15*
0.42	29.63±1.12*	26.84±1.27*
1.25	24.68±1.70	27.03±0.98*

After a 1-Month Recovery		
0	24.18±0.90	15.03±1.12
0.05	22.28±1.32	16.10±0.57
0.14	20.14±0.75	15.83±0.56
0.42	20.00±2.09	14.39±0.90
1.25	17.48±1.06	16.36±1.03

*Significantly different from control value ($p \leq 0.05$).

^a Mean of five animals \pm standard error.

Organ Weights: There were significant increases in absolute and relative liver weights in all groups of dosed females and in mean liver-to-body weight ratios in males receiving 0.42 or 1.25 percent EL-107. With the exception of the liver-to-body weight ratio in males receiving 1.25 percent, all of the changes were reversed after the recovery period (Table 4). There was also a significant ($p < 0.05$) decrease in kidney-to-body weight ratio in males receiving 1.25 percent (0.5906 g/100 g body weight) compared to the control mean value (0.5687 g/100 g body weight), which was considered to be related to dosing. Other changes in organ weights were sporadic, minimal, and not considered related to dosing (increases in relative testes weights in 0.42- and 1.25-percent dose groups and decreases in spleen weights in dosed males).

Gross Pathology: There were no gross findings related to dosing. Only a few scattered incidental findings were reported.

Histopathology: There was minimal diffuse hepatocellular hypertrophy in one male and one female receiving 1.25 percent at the end of the treatment phase and in one female receiving 0.05 percent at the end of the recovery phase of the study. It could not be determined if these effects were related to dosing. Other findings were considered incidental. There was retinal degeneration in one female receiving 0.05 percent and two females receiving 0.14 percent and inflammation of the heart in three males in each of the groups receiving 0.05 and 0.42 percent EL-107 at the end of the treatment period. No neoplastic lesions were found in any animals.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

- A. Rats tolerated diets containing EL-107 at levels of 0.05, 0.14, 0.42, or 1.25 percent for 3 months without mortalities. There were increases in liver weights in all dosed groups of females and in male groups receiving 0.42 or 1.25 percent EL-107. Accompanying the changes in liver weights was an induction of p-nitroanisole O-demethylase in males receiving 0.42 percent and females receiving 0.14, 0.42, and 1.25 percent EL-107. Effects on the liver were reversible after a 1-month recovery period except for the increased liver weight in high-dose males. The author concluded that "a clear no-effect level was not established in this study."
- B. A quality assurance statement was signed and dated April 13, 1984.

TABLE 4. Mean Liver Weights and Liver-to-Body Weight Ratios
in Rats Fed EL-107

Dose Level (percent)	After 3 Months		After a 1-Month Recovery	
	Liver Weights (g)	Liver-to-Body Weight Ratios (g/100 g Body Weight)	Liver Weights (g)	Liver-to-Body Weight Ratios g/100 g Body Weight)
Males				
0	8.75±0.50 ^a	2.58±0.11	9.00±0.75	2.45±0.10
0.05	8.66±0.63	2.55±0.12	9.01±0.91	2.50±0.15
0.14	8.82±0.78	2.65±0.08	9.20±0.86	2.50±0.11
0.42	9.20±0.89	2.82±0.12**	9.17±1.11	2.60±0.14
1.25	9.25±1.36	2.88±0.26**	9.89±1.22	2.74±0.26**
Females				
0	4.38±0.28	2.29±0.14	5.00±0.22	2.39±0.12
0.05	4.92±0.54**	2.52±0.17**	4.97±0.44	2.34±0.14
0.14	4.87±0.47*	2.53±0.14**	5.05±0.36	2.39±0.10
0.42	4.99±0.41**	2.65±0.18**	4.86±0.31	2.37±0.11
1.25	5.40±0.55**	2.78±0.25**	4.77±0.49	2.42±0.23

^aMean ± standard deviation.

* Significantly different from control value ($p \leq 0.05$) using Dunnett's two-tailed test.

** Significantly different from control value ($p \leq 0.01$).

14. REVIEWERS' DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

The design and conduct of the study was acceptable for evaluating the subchronic toxicity of EL-107, and the summary data were supported by individual animal data. We assess that the author's conclusions that the decreased mean body weights in males receiving 0.042 and 1.25 percent EL-107 were not of toxicologic importance was correct since the effects were minimal; mean body weights in males at these dose levels were only about 5 percent lower than in controls. However, there were also slight decreases in mean food efficiency in males receiving 1.25 percent and females receiving 0.14, 0.42, and 1.25 percent (Table 1). We also agree with the author's interpretation of changes in hematologic and clinical chemistry parameters. There were no changes that we consider to be of toxicologic importance.

Interpretation of a LOEL based on increases in p-nitroanisole O-demethylase would have been more precise if the enzyme activity was measured in liver homogenates of all rats at the end of 3 months rather than on five/sex/group. The activity was increased in all dosed groups of males and females; however, not all increases were statistically significant. Inclusion of all animals may have increased the power of the statistical analysis.

Although the significant ($p < 0.01$) increase in absolute and relative liver weights in females receiving 0.05 percent EL-107 was not accompanied by an induction of hepatic p-nitroanisole O-demethylase or any histologic changes, it is our assessment that the report author's conclusion that a clear NOEL was not established is correct.

Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Test Article Characterization, CBI pp. 111, 117, and 118; Appendix B, Materials and Methods, CBI pp. 14-20.

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APPENDIX A

**Test Article Characterization
(CBI pp. 111, 117, and 118)**

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EPA: 68-02-4225
TASK: 308-5b
February 12, 1987

DATA EVALUATION RECORD

EL-107

Subchronic Oral Toxicity Study in Rats

STUDY IDENTIFICATION: Lake, S. G. A subchronic toxicity study of compound EL-107 (compounds 121607 and 135520) administered in the diet to Fischer 344 rats for three months with a one month reversibility phase. (Unpublished study No. R12582 prepared by Lilly Research Laboratory, Greenfield, IN, for Eli Lilly and Co.; dated April 1984.) Accession Nos. 073293-073294.

APPROVED BY:

I. Cecil Felkner, Ph.D.
Department Manager
Dynamac Corporation

Signature: _____

I. Cecil Felkner

Date: _____

2-12-87

1. CHEMICAL: EL-107.
2. TEST MATERIAL: EL-107 is a mixture of isomers consisting primarily of Lilly compound 121607, N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide; Lilly compound 135520, N-[3-(1,1-dimethylbutyl)-5-isoxazolyl]-2,6-dimethoxybenzamide; and Lilly compound 173490, N-[3-(1,1,2-trimethylpropyl)-isoxazolyl]-2,6-dimethoxybenzamide. Lot H02-266-118, which was used in this study, was [redacted] percent 121607, [redacted] percent 135520, [redacted]

Appendix A presents the results of a chemical analysis of the test material.

3. STUDY/ACTION TYPE: Subchronic oral toxicity study in rats.
4. STUDY IDENTIFICATION: Lake, S. G. A subchronic toxicity study of compound EL-107 (compounds 121607 and 135520) administered in the diet to Fischer 344 rats for three months with a one month reversibility phase. (Unpublished study No. R12582 prepared by Lilly Research Laboratory, Greenfield, IN, for Eli Lilly and Co.; dated April 1984.) Accession Nos. 073293-073294.
5. REVIEWED BY:

William L. McLellan, Ph.D.
Principal Reviewer
Dynamac Corporation

Signature: William L. McLellan
Date: 2-12-87

Charles E. Rothwell, Ph.D.
Independent Reviewer
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Date: _____

6. APPROVED BY:

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Subchronic Toxicology
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Dynamac Corporation

Signature: I. Cecil Felkner
Date: 2-12-87

Margaret L. Jones, M.S..
EPA Reviewer

Signature: M. L. Jones
Date: 2/12/87

Marcia Van Gemert, Ph.D.
EPA Section Head

Signature: _____
Date: _____

7. CONCLUSIONS:

When EL-107 was fed to Fischer 344 rats at dietary levels of 0.05, 0.14, 0.42, or 1.25 percent for 3 months, there were no mortalities, no signs of toxicity, and no biologically important effects on body weights, hematology, or clinical chemistry parameters. After 3 months there were significant ($p < 0.05$ or < 0.01) increases in absolute and relative liver weights in all dosed groups of females and liver-to-body weight ratios in the 0.42- and 1.25-percent groups of males. Hepatic p-nitroanisole O-demethylase was significantly elevated in males receiving 0.42 percent and females receiving 0.14, 0.42, and 1.25 percent. Enzyme activities returned to normal in all groups after a 1-month recovery period, and relative liver weights returned to normal in all groups except males receiving 1.25 percent EL-107. There were no histopathologic lesions that were compound related. The LOEL based on increase in liver weights was 0.05 percent EL-107 in the diet, the lowest dose tested. A NOEL was not established.

The study is Core Guideline.

Item 8--see footnote 1.

9. BACKGROUND:

The dose levels were chosen based on a 2-week pilot study (C08582) with Fischer 344 rats dosed at levels of 0.156 to 2.5 percent EL-107. In this study there were significant increases in relative liver weights in females receiving 1.25 and 2.5 percent and significant increases in hepatic p-nitroanisole O-demethylase activity in males receiving 0.625 and 2.5 percent and females receiving 1.25 and 2.5 percent EL-107.

Item 10--see footnote 1.

11. MATERIALS AND METHODS (PROTOCOLS):**A. Materials and Methods: (See Appendix B for complete details.)**

Fischer 344 rats, approximately 5-6 weeks old, were used in the study. Rats were acclimated for 7 days and caged individually in a temperature- and humidity-controlled room with a 12-hour light/dark cycle. Food (Purina Certified Rodent Chow #5002, and water were offered ad libitum. At initiation, the mean body weights (\pm standard error) of males and females were 96.0 ± 0.7

¹ Only items appropriate to this DER have been included.

and 86.9 ± 0.6 g, respectively. Groups of 25 rats/sex were administered EL-107 at dietary levels of 0, 0.05, 0.14, 0.42, or 1.25 percent. Diets were analyzed for concentration, homogeneity, and stability of EL-107. Animals received control diets or diets containing EL-107 for 3 months, and then a subgroup of 10 animals/sex/group was allowed a 1-month recovery period during which they received control diets.

Animals were examined daily for physical condition and behavior and received detailed examinations weekly. Body weights and food consumption were measured weekly. At termination of both the dosing and recovery phases, all rats were fasted overnight and blood was obtained for hematologic and clinical chemistry determinations.

Enzyme induction was assessed by determining the level of hepatic p-nitroanisole O-demethylase activity. This was performed on liver homogenates of five animals/sex/group at the end of dosing and at the end of the recovery period.

Fifteen animals/sex/group were sacrificed at 3 months and 10/sex/group at the end of the 1-month recovery period. The following organs were weighed: adrenals, heart, kidneys, liver, ovaries, prostate, spleen, testes, thyroids, and uterus. A complete gross examination was performed on each animal, and approximately 32 tissues/animal were fixed and examined microscopically.

Body weight data, weight gain data, food consumption and efficiency data, and organ weight data were analyzed statistically using Bartlett's method for analysis of variance and Dunnett's method for pairwise comparison.

12. REPORTED RESULTS:

Test Compound and Dietary Analysis: The test compound was stable when stored under normal laboratory conditions. However, there was a loss of 4.4 percent water from initiation to termination of the study. On initial analysis, combined activity of 121607 and its isomer 135520 was 86.7 percent and was 92.5 percent at termination.

The test material was stable in the diet at 25 or 37°C; analysis of a diet containing a theoretical concentration of 1.25 percent indicated 1.15 percent when prepared fresh and 1.12 percent and 1.16 percent when stored for 4 weeks at 25 or 37°C, respectively. Distribution in the diet was homogeneous. Analyzed concentrations of test material in all diets ranged from 90-104 percent of theoretical concentrations. The mean daily intake of EL-107 in mg/kg/day was 0, 32.34, 93.65, 284.96, and 852.59 for males and 0, 36.10, 104.06, 311.89, and 945.76 for females.

Clinical Observations: The report stated that there were no physical signs of toxicity during the dosing or recovery phase. There were no mortalities.

Body Weights and Food Consumption: There were significant decreases in mean body weights in males receiving 0.42 and 1.25 percent test material in the diet (Table 1). The decreases were noted after 0 days in the 1.25-percent group and after 36 days in the 0.42-percent group. There were no effects of dosing on mean body weights in females; however, the mean weight gains in females receiving 1.25 percent EL-107 were slightly but significantly ($p < 0.05$) lower than controls after 8 through 90 days. The magnitude of these differences in both sexes was minimal and not considered by the author to be toxicologically important. Mean food consumption was similar in all groups, but mean food efficiency was decreased in males and females receiving 1.25 percent EL-107 in the diet from days 8 through 90. Food efficiency was also slightly but significantly lower in both males and females receiving 0.14 and 0.42 percent in the diet at intermittent intervals (cumulative) during the study; however, the differences were small and not considered toxicologically important. During the recovery phase there was a reversal of the effect on body weights and food efficiency. Table 1 summarizes these data for the dosing period and the recovery period.

Hematology: No compound-related effects on hematology were seen in either phase of the study. All mean values in dosed animals at 3 months were similar to control values. At the 4-month sacrifice (after recovery) there were occasional sporadic values significantly ($p \leq 0.05$) different from control values but these were not consistent with dose, were within normal limits, and not considered of toxicologic importance.

Clinical Chemistry: There were no changes in clinical chemistry parameters that were considered to be of toxicologic importance. There was a significant increase ($p < 0.05$) in serum glucose levels in males receiving 1.25 percent EL-107 at 3 months and after the recovery period (Table 2). These changes were considered "treatment related," but minimal and not of toxicologic importance. Glucose was also significantly ($p < 0.05$) increased in females receiving 0.14 and 1.25 percent at 3 months but decreased in females receiving 0.05 and 0.14 percent after the 1-month recovery period when compared with controls. However, the changes were minimal and there were no dose-related trends. At the 3-month sacrifice there were sporadic clinical chemistry values in dosed groups that differed from control values, but there were no dose-related trends. After recovery there was a decrease in total bilirubin in males receiving 0.14 and 0.42 percent, a decrease in alanine transaminase in females receiving 0.05, 0.14, and 0.42 percent, and an increase in creatinine in all dosed females when compared to controls. These changes, although significant ($p < 0.05$), were minimal and not considered to be of toxicologic importance.

Enzyme Induction: After 3 months there was a significant increase ($p < 0.05$) in the mean level of hepatic p-nitroanisole O-demethylase activity in females receiving 0.14, 0.42, and 1.25 percent and in males receiving 0.42 percent EL-107 when compared to control values. There was a reversal of this effect after the 1-month recovery period (Table 3).

TABLE 1. Mean Body Weights, Food Consumption, and Food Efficiency in Rats Fed EL-107 for 3 Months and Allowed a 1-Month Recovery

Sex/Dietary Level (%)	Mean Body Weights (g)			Mean Food Consumption (g/rat/day)		Mean Food Efficiency ^b	
	Initial	3 mo ^a	4 mo ^a	0-3 mo ^a	0-4 mo ^a	0-3 mo ^a	0-4 mo ^a
	n=25	n=25	n=10	n=25	n=10	n=25	n=10
<u>Males</u>							
0.0	97.6	340	367	16.3	16.3	16.3	13.8
0.05	97.7	337	360	15.8	15.7	16.5	13.7
0.14	96.1	334	368	16.0	16.2	16.2	13.8
0.42	93.2	324*	352	15.8	15.7	15.9	13.7
1.25	95.5	322*	360	15.8	16.2	15.7**	13.5
<u>Females</u>							
0.0	85.6	194	210	11.2	11.4	10.6	8.7
0.05	86.7	196	212	11.4	11.4	10.5	8.9
0.14	87.4	194	212	11.7*	11.8	9.9*	8.7
0.42	86.1	191	205	11.4	11.6	10.0*	8.5
1.25	88.8	190	197	11.5	11.2	9.6**	8.3

*Significantly different from control value ($p \leq 0.05$).

**Significantly different from control value ($p \leq 0.01$).

^aThe report statistically analyzed cumulative values at weekly intervals.

^bGrams body weight gained/100 g food consumed.

TABLE 2. Mean Serum Glucose Levels in Rats Fed EL-107 for 3 Months and after a 1-Month Recovery Period

Dose Level (percent)	Glucose (mg/dL)			
	Males		Females	
	3 mo ^a	4 mo ^b	3 mo ^a	4 mo ^b
0	162±20.4	154±18.9	160±21.6	174±19.7
0.05	179±22.4	152±20.9	164±11.3	149±22.6*
0.14	175±22.4	177±16.0	176±20.5*	150±15.5*
0.42	174±25.2	172±20.0	168±12.5	160±14.1
1.25	185±21.9*	184±23.2*	183±13.0*	157±13.4

*Significantly different from control value ($p \leq 0.05$).

^aMean ± SD of 15 animals/group.

^bMean ± SD of 10 animals/group.

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TABLE 3. Mean Hepatic p-Nitroanisole O-Demethylase Activity in Rats Fed EL-107

Dietary Level (percent)	nmol p-nitrophenol/mg protein/hour	
	Males	Females
3 Months after Administration		
0	21.02±0.39 ^a	20.29±1.10
0.05	25.60±2.01	22.04±0.78
0.14	24.00±1.24	26.43±0.15*
0.42	29.63±1.12*	26.84±1.27*
1.25	24.68±1.70	27.03±0.98*

After a 1-Month Recovery		
0	24.18±0.90	15.03±1.12
0.05	22.28±1.32	16.10±0.57
0.14	20.14±0.75	15.83±0.56
0.42	20.00±2.09	14.39±0.90
1.25	17.48±1.06	16.36±1.03

*Significantly different from control value ($p \leq 0.05$).

^a Mean of five animals \pm standard error.

Organ Weights: There were significant increases in absolute and relative liver weights in all groups of dosed females and in mean liver-to-body weight ratios in males receiving 0.42 or 1.25 percent EL-107. With the exception of the liver-to-body weight ratio in males receiving 1.25 percent, all of the changes were reversed after the recovery period (Table 4). There was also a significant ($p < 0.05$) decrease in kidney-to-body weight ratio in males receiving 1.25 percent (0.5906 g/100 g body weight) compared to the control mean value (0.5687 g/100 g body weight), which was considered to be related to dosing. Other changes in organ weights were sporadic, minimal, and not considered related to dosing (increases in relative testes weights in 0.42- and 1.25-percent dose groups and decreases in spleen weights in dosed males).

Gross Pathology: There were no gross findings related to dosing. Only a few scattered incidental findings were reported.

Histopathology: There was minimal diffuse hepatocellular hypertrophy in one male and one female receiving 1.25 percent at the end of the treatment phase and in one female receiving 0.05 percent at the end of the recovery phase of the study. It could not be determined if these effects were related to dosing. Other findings were considered incidental. There was retinal degeneration in one female receiving 0.05 percent and two females receiving 0.14 percent and inflammation of the heart in three males in each of the groups receiving 0.05 and 0.42 percent EL-107 at the end of the treatment period. No neoplastic lesions were found in any animals.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

A. Rats tolerated diets containing EL-107 at levels of 0.05, 0.14, 0.42, or 1.25 percent for 3 months without mortalities. There were increases in liver weights in all dosed groups of females and in male groups receiving 0.42 or 1.25 percent EL-107. Accompanying the changes in liver weights was an induction of p-nitroanisole O-demethylase in males receiving 0.42 percent and females receiving 0.14, 0.42, and 1.25 percent EL-107. Effects on the liver were reversible after a 1-month recovery period except for the increased liver weight in high-dose males. The author concluded that "a clear no-effect level was not established in this study."

B. A quality assurance statement was signed and dated April 13, 1984.

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TABLE 4. Mean Liver Weights and Liver-to-Body Weight Ratios in Rats Fed EL-107

Dose Level (percent)	After 3 Months		After a 1-Month Recovery	
	Liver Weights (g)	Liver-to-Body Weight Ratios (g/100 g Body Weight)	Liver Weights (g)	Liver-to-Body Weight Ratios g/100 g Body Weight)
Males				
0	8.75±0.50 ^a	2.58±0.11	9.00±0.75	2.45±0.10
0.05	8.66±0.63	2.55±0.12	9.01±0.91	2.50±0.15
0.14	8.82±0.78	2.65±0.08	9.20±0.86	2.50±0.11
0.42	9.20±0.89	2.82±0.12**	9.17±1.11	2.60±0.14
1.25	9.25±1.36	2.88±0.26**	9.89±1.22	2.74±0.26**
Females				
0	4.38±0.28	2.29±0.14	5.00±0.22	2.39±0.12
0.05	4.92±0.54**	2.52±0.17**	4.97±0.44	2.34±0.14
0.14	4.87±0.47*	2.53±0.14**	5.05±0.36	2.39±0.10
0.42	4.99±0.41**	2.65±0.18**	4.86±0.31	2.37±0.11
1.25	5.40±0.55**	2.78±0.25**	4.77±0.49	2.42±0.23

^aMean ± standard deviation.* Significantly different from control value ($p \leq 0.05$) using Dunnett's two-tailed test.** Significantly different from control value ($p \leq 0.01$).

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Interpretation of a LOEL based on increases in p-nitroanisole O-demethylase would have been more precise if the enzyme activity was measured in liver homogenates of all rats at the end of 3 months rather than on five/sex/group. The activity was increased in all dosed groups of males and females; however, not all increases were statistically significant. Inclusion of all animals may have increased the power of the statistical analysis.

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Item 15--see footnote 1.

16. CBI APPENDIX: Appendix A, Test Article Characterization, CBI pp. 111, 117, and 118; Appendix B, Materials and Methods, CBI pp. 14-20.

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APPENDIX A

**Test Article Characterization
(CBI pp. 111, 117, and 118)**

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