006559

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

Caswell
#419F
006559

10/15/87

DEC 15 1987

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: EL-107 (Isoxaben®), Toxicology Chapter of the New Chemical Registration Standard

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

From: Margaret L. Jones M. L. Jones 10/Dec/87
Chemist
Review Section III
Toxicology Branch, HED (TS-769)

Through: Robert P. Zendzian, Ph.D. 12/10/87
Registration Standard Coordinator
Toxicology Branch

and William Burnam, Deputy Chief W.H. Burnam 12-10-87
Toxicology Branch

Attached is the Toxicology Chapter of the New Chemical Registration Standard for EL-107(Isoxaben®). The following portions of this chapter are available on Lexitron disk. You may obtain a copy from this reviewer.

A. Toxicology Summary
B. Toxicology Profile
C. Data Gaps
D. ADI Assessment
E. Toxicological Issues
F. Toxicology Summary Tables
G. Bibliography
H. One Liners
I. Data Evaluation Reports

cc A. Rispin, SIS
R. Zendzian
R. Cobertly

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Toxicology Chapter

of the

Isoxaben

Registration Standard

Prepared by

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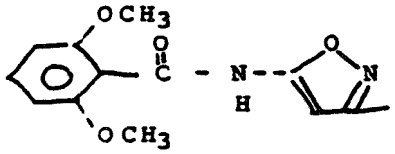
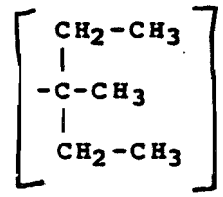
A. Toxicology Summary

Technical Isoxaben is a new pre-emergent herbicide for control of broadleaf weeds. Petitions for experimental use on wheat and barley and for permanent use on ornamentals are under consideration by the Agency. The data base is virtually complete for this chemical, allowing a thorough assessment of its toxicological properties.

The technical chemical consists of three isomers which differ in the isoxazolyl side chain. The chemical structure is shown below since no structural analogues were found and the compound does not appear to belong in any particular class.





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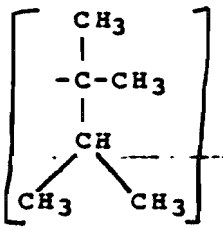
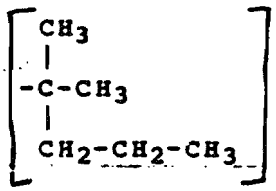


2.2%



8.3%

(-1,1,2- Trimethylpropyl) (-1-1-Dimethyl butyl)

The acute toxicity of technical Isoxaben is of low order. Acute oral toxicity in the mouse and the rat were category IV, acute dermal toxicity in rabbits was category III, and acute inhalation toxicity in rats was category II and III. Isoxaben does not appear to cause dermal or eye irritation or dermal sensitization. Subchronic testing in the rat demonstrated the target organ was the liver. Liver weight was elevated with concurrent liver hypertrophy. Liver enzyme induction was also found in the rat. In the dog, the target organ also appeared to be the liver, with increased liver weight as the effect of note.

Mutagenicity testing is marginally complete with several issues which need resolution. The category of chromosome aberration is incomplete since positive control data are needed to fully accept the dominant lethal study. The mouse micronucleus study was presumptively positive and needs to be confirmed by a repeat assay.

Teratogenicity testing in the rat demonstrated maternal toxicity in maternal bodyweight decrease throughout dosing and embryo/ fetotoxicity in increased preimplantation loss, increased resorptions, smaller litter size and increased numbers of runt fetuses. Significant numbers of teratogenic effects were not found in the rat. In the rabbit, no significant maternal toxicity, embryo/fetotoxicity or teratogenicity was found up to the limit dose for teratogenic testing (1000 mg/kg/day).

Reproductive testing in the rat showed parental effects including lowered mean bodyweight, lowered mean bodyweight gain, and lowered food efficiency. Reproductive toxicity was demonstrated by decreased numbers of viable pups in the F_{2a} and F_{2b} generations. Embryo/fetotoxicity was found in increased resorptions and post-implantation losses and a high dose effect in microphthalmia. The finding of microphthalmia was not demonstrated in the teratology studies.

Chronic/oncogenicity testing found evidence of oncogenicity in one species only. Both male and female mice developed significant numbers of hepatocellular adenomas at the high dose only. The neoplastic effects in the liver (adenomas) were supported by nonneoplastic histopathology in the liver as well (hepatocellular cytomegaly, hepatocellular vacuolation, and hepatocellular hyperplasia). In the rat, there was a significant trend toward the development of adrenal medullary tumors however, the numbers of tumors compared to controls were not significant. In the weight-of-the-evidence assessment, the Toxicology Branch Peer Review Committee classified Technical Isoxaben as a category C oncogen without a quantitative risk assessment.

Chronic testing in the dog also showed liver effects with hepatic microsomal enzyme induction and elevated alkaline phosphatase.

Metabolism data identified urinary metabolites of Isoxaben. Since recovery in males was slightly less than females (85% vs 92.6%), a concern for possible bioaccumulation was expressed.

B. Toxicology Profile

81 Series Acute Toxicity and Irritation Studies

81-1 Acute Oral

Sufficient data are available to show that Technical Isoxaben is not lethal to laboratory animals when taken by the oral route (MRID MJ031, MJ032). All test rats (10/sex) survived a 14 day study in which Technical Isoxaben was administered orally by gavage at a dose of 10,000 mg/kg in approximately 34 ml/kg dosage volume (category IV). In a study with mice (10/sex), all animals survived to 14 days after a dose of 10,000 mg/kg (category IV).

81-2 Acute Dermal

Sufficient data are available to show that Technical Isoxaben was not lethal when applied by the dermal route of exposure (MRID MJ001, core grade minimum). All test rabbits (5/sex) survived a 14-day study in which Technical Isoxaben was applied to the skin at a dose of 2000 mg/kg bodyweight. Toxicity category III. (Acute dermal LD_{50}>2000 mg/kg)

81-3 Acute Inhalation

Sufficient data are available to show that Technical Isoxaben was not lethal via the inhalation route of exposure (MRID MJ009, core grade minimum). When 10/sex rats were exposed to 2.55 $\pm$ 0.603(SD) mg/L [gravimetric exposure concentration] for 4 hours, there were no deaths. Toxicity category III. (Acute inhalation LC_{50} > 2.55 mg/L.) An earlier study demonstrated the LC_{50} was greater than 1.99 mg/L (MRID MJ003, core grade minimum).

81-4 Primary Eye Irritation

Sufficient data are available to show that Technical Isoxaben was a reversible eye irritant (irritation cleared within 7 days) when applied to one eye each of 3/sex rabbits at a dose of 28 mg in 0.1 cc (MRID MJ002, core grade guideline). Eye irritation category III.

81-5 Primary Dermal Irritation

Sufficient data are available to show that Technical Isoxaben was not a dermal irritant when applied to 3/sex rabbits at 200 mg/kg and observations carried out for 14 days (MRID MJ008, core grade minimum). Primary dermal irritation category IV.

81-6 Dermal Sensitization

Sufficient data are available to show that Technical Isoxaben does not produce dermal sensitization after induction (first application) followed by challenge (second application). Two trials were made, one using a 25% ethanol solution and a 1:1 aqueous solution of the Technical chemical using 12 guinea pigs per group and 6 per control group for each method (MRID MJ007, core grade minimum).

81-7 Acute Delayed Neurotoxicity

No data are available on the acute neurotoxic effects of Technical Isoxaben. This test is required only for compounds which are organophosphate inhibitors of cholinesterase, or related to such inhibitors or metabolites of such compounds Technical Isoxaben is not an organophosphate, therefore a study is not required.

82 Series Subchronic Testing

82-1 Subchronic Oral

Sufficient data are available for Technical Isoxaben to satisfy the data requirements of subchronic oral testing in the nonrodent. Only core grade supplementary data are available for the category of subchronic testing in the rodent.

Rats (25/sex/dose) were dosed with Technical Isoxaben orally at levels of 0, 0.001, 0.01, 0.14, or 1.25% for three months (MRID MJ011, core grade supplementary). Ten/group were held for a one-month recovery period and 15/group were sacrificed at three months. At three months, elevated liver weights with liver hypertrophy were found in males dosed with 0.14 and 1.25% Technical Isoxaben. Females showed elevated liver weight without concurrent hypertrophy at these doses. The no observed effect level (NOEL) was 0.01%. Liver enzyme induction was stimulated at 0.14 and 1.25% in males and females. NOEL = 0.01%. Food consumption data was not reported and compound intake could only be approximated, however, since adequate chronic data in the rodent are available for Technical Isoxaben another study will not be required at this time.

Dogs (4/sex/dose) were dosed with Technical Isoxaben by gelatin capsules at levels of 0, 25, 110, or 500 mg/kg/day for three months (MRID MJ010, core grade minimum). Mean and absolute liver weight were increased in males dosed with 500 mg/kg/day. A nonsignificant increase in relative liver weight of females at this dose was found showing a possible trend toward liver weight increases. NOEL= 110 mg/kg/day.

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82-2 Subchronic Dermal (21-day)

Sufficient data are available for Technical Isoxaben to satisfy the requirements for 21-day dermal testing.

Rabbits (5/sex/dose) were dosed with 0 and 1055 mg/kg/day Technical Isoxaben as well as 500 mg/kg and 1000 mg/kg of a 50% formulation (MRID MJ012, core grade minimum). A reversibility group was given 1000 mg/kg 50% formulation and held for a 14 day recovery period.

Only the results of the group administered Technical Isoxaben will be discussed in this registration standard. No overt signs of systemic toxicity or pathologic morphology abnormalities were noted in the study related to administration of Technical Isoxaben. Thyroid and parathyroid weight relative to bodyweight was increased in males and females, however, the increase was not statistically significant and was well within the range of control value ± standard deviation. The limit test of 1000 mg/kg was apparently achieved in this study and no further testing in this category will be required at this time.

82-3 Subchronic Dermal (90-day)

No data are available on the 90-day subchronic dermal toxicity of Technical Isoxaben. This study will not be required as long as existing acceptable end-uses do not result in repeated human skin contact.

82-4 Subchronic Inhalation (90-day)

No data are available on the 90-day subchronic inhalation toxicity of Technical Isoxaben. This study will not be required as long as existing acceptable end-uses do not result in repeated inhalation exposure.

82-5 Subchronic Neurotoxicity

No data are available on the subchronic neurotoxicity of Technical Isoxaben. Since an acute neurotoxicity study is not required and there is no evidence of neurotoxicity in mammalian species, this study is not required.

83 Series Chronic and Long Term Studies

83-1 Chronic Toxicity

Sufficient data are available on Technical Isoxaben to satisfy the data requirement for chronic testing in rodent and nonrodent species.

Mice (60/sex/dose) were administered Technical Isoxaben in the diet at levels of 0, 100, 1000, and 12500 ppm for 24 months (MRID MJ014, core grade minimum). Males and females showed an increase in hepatocellular adenomas and an increase in combined hepatocellular adenomas and carcinomas at the high dose. Liver hyperplasia and liver nodules were increased in males and females at the high dose. Other liver changes noted were increased absolute and relative liver weight at the high dose, hepatocytomegaly in high dose males and hepatocellular vacuolation in high dose males and females. Survival analysis showed no survival disparities in the study. NOEL = 100 ppm (liver vacuolation) For further discussion of this study, see the Toxicological Issues section and the report of the Toxicology Branch Peer Review Committee on Isoxaben (10/5/87)[a].

Rats (60/sex/dose) were administered Technical Isoxaben in the diet at levels of 0, 0.0125, 0.125, or 1.25 % for 24 months (MRID MJ016, core grade minimum). Liver and kidney were primarily affected. Mean liver weight in males was increased at the high dose. Liver/bodyweight and kidney/bodyweight ratios were increased in high dose males. Progressive glomerulonephrosis in the kidney was increased in high dose females and in mid and high dose males. Creatinine levels and blood urea nitrogen (BUN) were increased at the high dose in males and females, demonstrating kidney toxicity. NOEL = 0.0125% (5mg/kg for males, 6.2 mg/kg for females). For further discussion of this study, see the Toxicological Issues section and the report of the Toxicology Branch Peer Review Committee on Isoxaben (10/5/87).

Dogs (4/sex/dose) were administered Technical Isoxaben in gelatin capsules at levels of 0, 10, 100, or 1000 mg/kg/day for 12 months (MRID MJ015, core grade minimum). Hepatic microsomal enzyme induction was found in males receiving 1000 mg/kg/day. Alkaline phosphatase levels were elevated in mid and high dose groups (males and females) from three months onward. The levels were similar to those at the start, however, they did not undergo the expected age-related decrease as was seen in control and low dose groups. Liver/brain weight and liver/bodyweight ratios were increased in high dose males and females. NOEL = 10 mg/kg/day.

a. EPA memorandum. Peer Review of Isoxaben, Rinde, E. to Mountfort, R., October 5, 1987.

83-2 Oncogenicity Studies

Rat and mouse chronic/oncogenicity studies are discussed in section 83-1 (MRID MJ014 and MJ016, both studies were core grade minimum). No further chronic or oncogenicity testing is required at this time.

83-3 Teratogenicity Studies

Sufficient data are available for Technical Isoxaben to satisfy the requirements for teratology testing.

Rats (25 females/group) were mated 1:1 with males from the same colony and were dosed with 0, 100, 320, or 1000 mg/kg/day with Technical Isoxaben from gestation days 6 through 15 (MRID MJ017, core grade minimum). Decreased bodyweight gain was found at the 1000 mg/kg/day throughout dosing. (Maternal toxicity NOEL = 320 mg/kg/day.) Increased preimplantation loss, increased resorptions, smaller litter size, and increased incidences of runt fetuses were found at 1000 mg/kg/day. (Embryo/fetotoxicity NOEL = 320 mg/kg/day.) There were no significant numbers of compound-related teratogenic effects aside from the increase in numbers of runt fetuses. There were 4 runt fetuses in 241 fetuses examined, each occurring in a different litter.

Rabbits (20 females/group) were artificially inseminated with semen from male rabbits of the same strain and source and were dosed with 0, 100, 320, or 1000 mg/kg/day Technical Isoxaben from gestation day 6 through 18 (MRID MJ018, core grade minimum). No significant differences between controls and dosed animals were noted in the categories of maternal toxicity, embryo/fetotoxicity, or teratogenicity at any dose. The NOEL for each of these categories was greater than 1000 mg/kg/day, which satisfies the limit test (1000 mg/kg/day) for teratogenic testing even though maternal toxicity was not demonstrated.

83-4 Reproductive Toxicity

Sufficient data are available for Technical Isoxaben to satisfy the requirements for reproductive toxicity testing.

Rats (25/sex/dose) were administered 0, 500, 2500, or 12500 ppm (0, 25, 125, or 625 mg/kg/day) Technical Isoxaben in the diet in a three generation reproduction study (MRID MJ019, core grade minimum). Parental female toxicity was demonstrated in lowered mean bodyweight, lowered mean bodyweight gain, and lowered food efficiency during growth, gestation and lactation at 2500 ppm and 12500 ppm. Parental male toxicity was found in lowered mean bodyweight during the growth phases. Both parental males and females showed increased mean relative liver weight at 12500 ppm and females only at 2500 ppm. (NOEL = 500 ppm) Reproductive toxicity was demonstrated at 12500 ppm in significantly ($p<0.05$) decreased numbers of viable pups born in the F_{2a} and F_{2b} generations, and depression in mean bodyweight of progeny on postpartum day 21. (NOEL = 2500 ppm)

Embryo/fetotoxicity was demonstrated at 12500 ppm in decreases in numbers of viable fetuses per litter, increases in resorptions and postimplantation losses, and increases in hydroureter in

both F_1 and F_2 generations, as compared to controls. A possible teratogenic effect was found in microphthalmia at 12500 ppm, however the increase was found in 5 fetuses (3 litters) in the F_1 generation and 7 fetuses (3 litters) in the F_2 generation compared to one control fetus (1 litter) in the F_1 generation and no other observations of this abnormality at any other dose in either generation. There was, therefore, apparently no dose-response relationship for this effect which apparently occured at the high dose only. In addition, the effect was not observed in the rat teratogenicity study in which doses up to 1000 mg/kg/day by gavage were administered to the same strain (Wistar) of rats. (NOEL = 2500 ppm)

84 Series Mutagenicity

Three mutagenicity studies were reviewed for Technical Isoxaben. The category of other mechanisms of mutagenicity is a partial data gap and the category of chromosomal aberration is also a data gap. These deficiencies are discussed below.

84-2 Categories of Mutagenicity Testing

Gene mutation: Five tester strains of Salmonella typhimurium were used to test 0, 32, 62.5, 125, 250, and 500 ug/plate Technical Isoxaben for 48 hours (MRID MJ022, acceptable). The results were negative for induction of revertants in the five tester strains. Mouse lymphoma cells were used to test the potential for Technical Isoxaben to induce forward mutations at the thymidine kinase locus (MRID MJ029, acceptable). Technical Isoxaben was found not to induce an increase in mutation frequency with of without S-9 activation.

Chromosomal aberration: Male Wistar rats (25/dose) from a three-generation reproduction study in which Technical Isoxaben was administered at doses of 0, 500, 2500, and 12500 ppm were used in a dominant lethal test (MRID MJ021, provisionally acceptable). The study did not demonstrate dominant lethality up to the highest dose tested, however, positive control data were not included. Once these are provided, the study may be upgraded to acceptable status.

Other mechanisms of mutagenicity: Adult male mice (10/group) were intubated with 5000 mg/kg/day Technical Isoxaben twice 24 hours apart and then sacrificed at 24, 48, or 72 hours after the second dose in a mouse micronucleus study testing for chromosomal aberrations in vivo (MRID MJ020, inconclusive results, presumptive positive). The reviewer concluded there was a slight but statistically significant increase in micronucleated polychromatic erythrocytes over controls at 24 hours. The reviewer further concluded there was no justification for testing males only, and requested a repeat assay to include females, a demonstration of cytotoxicity,

and sampling of bone marrow cells to begin as soon as 12 hours after dosing (Mauer, 1/7/87). Technical Isoxaben was tested for induction of unscheduled DNA synthesis (UDS) using primary rat hepatocyte cultures (MRID MJ030; acceptable). In two replication assays, results were negative.

The category of other mechanisms of mutagenicity is a partial data gap and a study must be provided to confirm the presumptive positive result in the mouse micronucleus test. The category of chromosomal aberration may be filled if additional data are provided, otherwise, a study to fulfill this category may be required.

85 Series Special Testing

85-1 General Metabolism

Nine studies on the metabolism of Technical Isoxaben (7 in rats and 2 in mice) were conducted and each will be described briefly No further metabolism testing in the laboratory animal will be required at this time.

Rats (5/sex/dose) were administered 10 or 250 mg/kg Technical Isoxaben in a single dose and biliary excretion at 24 hours was reduced in females receiving 250 mg/kg compared to females receiving 10 mg/kg (MRID MJ004, acceptable).

Rats (5/sex/dose) were administered 10, 100, 250, 500, or 1000 mg/kg ^{14}C-Technical Isoxaben in a volume of 10 ml/kg and the urinary and fecal excretion was measured for three successive 24 hour periods (MRID MJ005, acceptable). Fecal excretion was the major route of elimination and most of the excretion occurred within 24 hours. Gastrointestinal absorption appears to be rate-limiting, due to a decreasing ratio of urinary to fecal excretion with increasing dose.

Rats (5/sex/sampling time) were administered radiolabeled Technical Isoxaben at a dose of 250 mg/kg in 10 ml/kg volume and sacrificed at either 4 or 24 hours after sampling of blood from the abdominal aorta (MRID MJ006, acceptable). Tissues and organs were then analyzed. Increasing tissue/plasma ratios at 4 and 24 hours, were noted in adrenals and pituitary of males, and in adrenals, eyes, thyroid, pituitary, and ovaries of females. No conclusions could be made since the study was not carried beyond 24 hours or to maximum values for excretion of radiolabeled chemical.

Rats (5/sex/sampling time) were administered radiolabeled Technical Isoxaben at doses of 250 mg/kg in 10 percent acacia and followed to 24, 48, and 72 hours (MRID MJ023, acceptable). Excretion of the entire dose was virtually complete by 48

hours. After 72 hours, 99 percent of the administered dose was recovered unmetabolized in the feces. An estimated 20 percent was absorbed of which half was excreted in urine and half in feces. Fecal metabolites were not identified. Urinary metabolites demonstrated the major transformations were oxidation to produce an alcohol or a ketone, hydroxylation of the aromatic ring, and O-demethylation of the methoxy substituents. Tissue distribution was not examined.

Rats (5/sex) were predosed with Technical Isoxaben prior to administration of a radiolabeled dose (MRID MJ027, acceptable). The pattern of urinary and fecal excretion did not change significantly with predosing. Males excreted 80.59% of the radioactivity within 7 days, and females excreted 96.1% in combined urinary and fecal excretion. Significant amounts of radioactivity did not remain in tissues, however, in males, 0.4% remained in the carcass, and in females, 0.3% remained in the carcass.

Rats (5/sex) were placed into two groups, one with 2 males and 3 females and one with 3 males and 2 females to study excretion in expired air (MRID MJ025, acceptable). One group received a dose of 250 mg/kg Technical Isoxaben in 1 ml/100 gms and the second group received a second dose after one week. Sacrifice took place at 48 hours after dosing. The recovery of radioactivity was 85% for males and 92.6% for females including urine, feces, carcass, and expired air. Expired air accounted for a very small portion of the radioactivity administered (2.4% in males and 2.8% in females). The study was performed in order to find radioactivity which was not recovered in the urine and feces. There may be a possible concern for bioaccumulation since a very small portion of the radioactivity was found in the expired air, which does not account for the difference between males and females.

The remaining metabolism studies were supplementary (MRID MJ026, MJ024, and MJ028). A distribution study in rats suggests that significant radioactivity remains in tissues after 24 hours after dosing, most appearing to remain in the intestinal tract and tissue to plasma ratios also suggest accumulation in tissues. However, the study was not carried beyond 24 hours. An excretion study in mice and a study on the absorption and disappearance from plasma in mice were performed as range-finding studies to set doses for the mouse oncogenicity study. These studies found that the distribution of Technical Isoxaben is altered above 100 mg/kg and the plasma half-life was 8.3 to 8.9 hours for radioactivity. Most of the radioactivity was eliminated by 72 hours. The study indicates that absorption of Technical Isoxaben is limited to approximately 100 mg/kg plasma elimination is apparently unaffected by increasing doses.

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C. Data Requirements and Data Gaps

Technical Isoxaben is a new chemical which has no permanent tolerances to date. It is proposed for an Experimental Use Permit on wheat and barley and on ornamentals. As such, it will require data for food and nonfood uses.

81-1 Acute Oral
81-2 Acute Dermal
81-3 Acute Inhalation
81-4 Primary Eye Irritation
81-5 Primary Dermal Irritation
81-6 Dermal Sensitization

82-1 Subchronic Oral, two species rodent and nonrodent
82-2 Subchronic Dermal (21 day)

83-1 Chronic Toxicity, two species rodent and nonrodent
83-2 Oncogenicity Study, two studies, rat and mouse
83-3 Teratogenicity, two species, rodent and nonrodent
83-4 Reproduction

84-2 Mutagenicity Tests

85-1 General Metabolism

Based on this assessment of the toxicology data base for Technical Isoxaben, the following Guideline toxicology studies have been identified as data gaps and are required.

84-2 Mutagenicity.

The Agency has data to partially fulfill two of the required three categories of mutagenicity testing. The categories of "chromosomal aberration" and "other mechanisms of mutagenicity testing" require further data or additional testing. The dominant lethal test requires positive control data to be fully acceptable and the mouse micronucleus test requires a repeat assay to confirm the presumptive positive result.

D. ADI Assessment

The two year rat study (MRID MJ016, core grade minimum) was confirmed by the Toxicology Branch ADI (RfD) Committee as the study on which to base the acceptable daily intake (ADI). The study was verified by the Agency-wide RfD Committee. In the selected rat study, two replicates of 30/sex/dose (total 60/sex/dose) rats were administered 0, 125, 1250, or 12500 ppm Technical Isoxaben in the diet for two years. Actual intake differed between males and females (0, 5.0, 50.7, and 526.5 mg/kg/day for males, and 0, 6.2, 61.8, and 646.6 mg/kg/day for females). The no observed effect level (NOEL) based on nononcogenic effects was 125 ppm (5 mg/kg/day, males, and 6.2 mg/kg/day, females). Effects were observed in clinical chemistry values, relative organ weights, and food efficiency. Increased blood urea nitrogen (BUN) values (males and females), decreased alkaline phosphatase and aspartate aminotransferase (AST) in (males and females), increased heart/bodyweight ratio (males), and decreased food efficiency were found at the lowest effect level (1250 ppm).

The ADI (RfD) based on this study is 0.05 mg/kg/day. A safety factor of 100 was used to account for inter- and intraspecies differences[a].

Since several oncogenic effects were noted in the chronic testing of Technical Isoxaben (see Toxicological Issues Section, discussion of Peer Review for Isoxaben), some thought must be given to the need for a Section 409 tolerance in the event any tolerances are sought on commodities which could result in food or feed additives. Isoxaben has a virtually complete data base and has been designated a "Category C oncogen" by the Toxicology Branch Peer Review Committee[b].

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a. Reference Doses (RfDs) for Oral Exposure; Isoxaben; 9/22/87; EPA Contact: Reto Engler, FTS 557-7491.

b. EPA memorandum. Peer Review of Isoxaben, Rinde, E. to Mountfort, R., October 5, 1987.

E. Toxicological Issues

1. Oncogenicity

The Toxicology Branch Peer Review Committee has classified Technical Isoxaben as a Category C oncogen, without a quantitative risk assessment [a]. The evidence considered included the mouse oncogenicity study in which statistically significant increases in liver adenomas were found at the high dose (12500 ppm) in both males and females and a slight but not statistically significant increase in this lesion was noted in males only at the mid dose (1250 ppm). The incidence of hepatocellular adenomas was above that found in historical controls, however, a dose-response relationship was not found. Mutagenicity data was supportive, with a presumptive positive result in the mouse micronucleus test; however, a repeat assay has been required to confirm this result.

The oncogenic evidence was judged "limited" based on a statistically significant increase in benign tumors (hepatocellular adenomas) in one species only (mouse). The tumors were found in both sexes, were statistically significant at the high dose only, were of a common type, were predominantly benign, and occurred with no decrease in latency. In the rat, the incidence of adrenal and hepatocellular tumors was not statistically significant, the tumors were present in one sex only, and were benign and fairly common. Adrenal tumors in the rat were increased at the high dose only (were not statistically significant), showed a positive trend (according to statistical analysis), and liver tumors were neither statistically significant nor dose-related. Based on this reasoning, the evidence was not judged strong enough to warrant a quantitative risk assessment for Technical Isoxaben.

2. Metabolism

The evidence from the considerable body of metabolism data for Technical Isoxaben indicates a possible concern for bioaccumulation. There are adequate metabolism data to judge this concern for the present, however, this question should be reevaluated in the future to see that bioaccumulation does not occur from additional tolerances and uses of the pesticide.

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a. EPA memorandum. Peer Review of Isoxaben, Rinde, E. to Mountfort, R., October 5, 1987.

TABLE A
GENERIC DATA REQUIREMENTS FOR ISOXABEN

ata Requirement	Composition 1/	Use Patterns 2/	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)?3/
158.135 Toxicology					
ACUTE TESTING:					
81-1 - Acute Oral - Rat	TGAI		YES	MRID MJ031, MJ032	NO
81-2 - Acute Dermal -	TGAI		YES	MRID MJ001, MJ008	NO
81-3 - Acute Inhalation - Rat	TGAI		YES	MRID MJ003, MJ009	NO
81-4 - Eye Irritation - Rabbit	TGAI		YES	MRID MJ002	NO
81-5 - Dermal Irritation - Rabbit	TGAI		YES	MRID MJ008	NO
81-6 - Dermal Sensitization - Guinea Pig	TGAI		YES	MRID MJ007	NO
81-7 - Acute Delayed Neurotoxicity - Hen	TGAI		NO		NO 4/
SUBCHRONIC TESTING:					
82-1 - 90-Day Feeding -					
Rodent	TGAI		YES	MRID MJ011	NO
Non-rodent	TGAI		YES	MRID MJ010	NO

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ta Requirement	Composition 1/	Use Pattern 2/	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)?	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)?3/
58.135 Toxicology (Cont.)					
82-2 - 21-Day Dermal-	TGAI		YES	MRID MJ012	NO
82-3 - 90-Day Dermal-	TGAI		NO		NO 5/
82-4 - 90-Day Inhalation -	TGAI		NO		NO 6/
82-5 - 90-Day Neurotoxicity-	TGAI		NO		NO 7/
CHRONIC TESTING:					
83-1 - Chronic Toxicity -					
Rodent	TGAI		YES	MRID MJ014, MJ016	NO
Non-rodent	TGAI		YES	MRID MJ015	NO
83-2 - Oncogenicity Study -					
Rat	TGAI		YES	MRID MJ016	NO
Mouse	TGAI		YES	MRID MJ014	NO
83-3 - Teratogenicity -					
Rat	TGAI		YES	MRID MJ017	NO
Rabbit	TGAI		YES	MRID MJ018	NO
83-4 - Reproduction -	TGAI		YES	MRID MJ019	NO

TABLE A
GENERIC DATA REQUIREMENTS FOR ISOXABEN

ta Requirement	Composition 1/	Use Pattern 2/	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)? 3/
58.135 Toxicology (continued)					
MUTAGENICITY TESTING					
34-2 - Gene Mutation	TGAI		YES	MRID MJ022, MJ029	NO
34-2 - Chromosomal Aberration	TGAI		PARTIALLY	MRID MJ021	YES 8/
34-2 - Other Mechanisms of Mutagenicity	TGAI		PARTIALLY	MRID MJ020, MJ030	YES 9/
SPECIAL TESTING					
35-1 - General Metabolism	PAI or PAIRA		YES	MRID MJ004, MJ005 MJ006, MJ023, MJ028	NO
35-2 - Domestic Animal Safety	Choice		NO		NO 10/

1/ Composition: TGAI Technical Grade Active Ingredient; PAI = Pure Active Ingredient; PAIRA = Pure Active Ingredient, RAdiolabelled; Choice = Choice of several test substances determined on a case-by-case basis.
2/ The use patterns are coded as follows: A = Terrestrial, Food Crop; B = Terrestrial, Non-Food; C = Aquatic, Food Crop; D = Aquatic, Non-Food; E = Greenhouse, Food Crop; F = Greenhouse, Non-Food; G = Forestry; H = Domestic Outdoor; I = Indoor; IP = Industrial Preservative.
3/ Unless otherwise specified data must be submitted no later than six months after publication of this Standard
4/ This test is required only for compounds which are organophosphate inhibitors of cholinesterase, or related to such inhibitors or metabolites of such inhibitors. Isoxaben is not an organophosphate, therefore, a study is not required.
5/ This study is not required because existing acceptable end-uses should not result in repeated human skin contact.
6/ This study is not required because existing acceptable end-uses should not result in repeated inhalation exposure.
7/ Since an acute neurotoxicity study is not required for this compound and there is no evidence of neurotoxicity in mammalian species, this study is not required.
8/ Study was "provisionally acceptable" and can be upgraded to fully acceptable upon receipt of positive control data.
9/ Test was inconclusive. Presumptive positive results should be confirmed in a repeat assay.
10/ This study is not required under the existing use patterns.

TABLE B

PRODUCT SPECIFIC DATA REQUIREMENT. FOR MANUFACTURING-USE PRODUCTS CONTAINING ISOXABEN

ıta Requirement	Composition 1/	Does EPA Have Data To Satisfy This Requirement? (Yes, No or Partially)	Bibliographic Citation	Must Additional Data Be Submitted Under FIFRA Section 3(c)(2)(B)? 2/
58.135 Toxicology				
ACUTE TESTING				
81-1 - Acute Oral - Rat	MP	NO		YES
81-2 - Acute Dermal	MP	NO		YES
81-3 - Acute Inhalation - Rat	MP	NO		YES
81-4 - Primary Eye Irritation - Rabbit	MP	NO		YES
81-5 - Primary Dermal Irritation - Rabbit	MP	NO		YES
81-6 - Dermal Sensitization Guinea pig	MP	NO		YES

1/ Composition: MP = Manufacturing-use product.

2/ Unless otherwise specified data must be submitted no later than six months after publication of this Standard

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Tox Chem No. 419F - EL 107 File Last Updated 12/8/87

Study/Lab/Study #/Date	Material	EPA 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 microphthalia) 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 (95.5%) technical Lot # Z10025 & formulation FN-7033	252915 00137849	NOEL (technical EL - 107) : < 1055 mg/kg/body wt (only level tested) NOEL (formulaton FN - 7033) : < 500 mg/kg/body wt (increases in thyroid weights per 100 grams of body weight. This study is not suficient 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

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ 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.; #R10582 & 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.; #R07282 & R08882; 12/82	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 absorbtion 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-O-5082 & R-O-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

Tox Chem No: 419F - EL 107

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Dermal sensitization - guinea pig; Eli Lilly Res. Labs.; no G00183; 12/83	EL - 107 technical and 1:1 aqueous dilution of suspenseor concentrate formulation (FN7033, 50% EL - 107)	252915 00137846	Not sensitizing		Minimum 004034
Primary dermal irritation - rabbit; Eli Lilly and Co.; #B-D-58-81; 9/81	EL-107 tech. Lot B31-72C-88 Purity 92.34%	250791 0013217	no irritation from 200 mg/kg.	IV	Minimum 003434
Acute dermal LD_{50} - rabbit; Eli Lilly & Co.; #B-D- 58-81; 9/81	EL-107 tech. Lot B31-72C-88 Purity 92.34%	250791	LD_{50} > 200 mg/kg (only dose tested)		Supplementary 003434
Primary eye irritation - rabbit; Eli Lilly & Co.; #B-E-61-81; 9/81	EL-107 tech. Lot B31-72C-88 Purity 92.34%	250791	Corneal dullness, mild iritis, and slight conjunctivitis. All treated eyes normal within 72 hrs.	III	Guideline 003434
Acute inhalation LC_{50}- rat; Eli Lilly & Co.; #R-H-37-81; 9/81	EL-107 tech. Lot B31-72C-88 Purity 92.34%	250791	LC_{50} > 1.99 $\pm$ 0.199 mg/L (11.5 mg/L nominal)	II	Minimum 003434
Acute LD_{50} - mice; Eli Lilly & Co.; #M-O-49-81 & M-O-50-81; 4/81	EL-107 25%; compound in 10% aqueous acacia Lot B31-72C-88 Purity 92.334%	250791	LD_{50}>10,000 mg/kg (only dose tested) compound - colored feces (beige) (M&F); poor grooming, tail erection. No mortalities at 10,000 mg/kg	IV	Guideline 003434
Acute intraperitoneal LD_{50} - mice; Eli Lilly & Co.; #M-P-18-81 & M-P-19-81; 4/81	EL-107 tech. 25% solution in 10% gum acacia Lot B31-72C-88 Purity 92.34%	250791	LD_{50} > 5000 mg/kg		Acceptable 003434

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Acute intraperitoneal LD_{50} - rat; Eli Lilly & Co.; #R-P-4-81 & R-D-5-81; 4/81	EL-107 25%	250791	LD_{50} > 2000 mg/kg (only level tested)		Acceptable 003434
Acute oral LD_{50} - rat; Eli Lilly & Co.; #R-0-48-81 & R-0-49-81; 4/81	EL-107 tech. 92.34% Lot B31-72C-88 30% solution in 10% aqueous acacia(34 mg/kg)	250 '91 00132126	LD_{50} > 10,000 mg/kg. No deaths observed at 10,000 mg/kg; leg weakness. Level tested: 10,000 mg/kg	IV	Guideline 003434
Acute inhalation LC_{50} - rat; Eli Lilly; #R-H-70-84; 10/84	EL-107-tech. (90.7%) Lot 270-EG-3	257774 00143285	LC_{50} = 2.55 mg/L/4 hrs ± 0.60	III	Minimum 004593
Dermal sensitization - guinea pig; Eli Lilly; #G00183; 12/83	TECH	257774	Negative for sensitization		Minimum 004593
2 Year feeding/oncogenic - rat; Eli Lilly; #R01583;	EL-107	258169	Levels tested in Fisher 344 strain- 0, 0.0125, 0.125 and 1.25 and 1.25% (approx 0, 5.6, 57 and 587 mg/kg) Study # 1		004593
2 Year feeding/oncogenic - rat; Eli Lilly; #R01583;	EL-107	258169 00164553	Levels tested in Fisher 344 strain- 0, 0.0125, 0.125 and 1.25 and 1.25% (approx 0, 5.6, 57 and 587 mg/kg) Study # 2		004593

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
2 Year feeding/oncogenic - mice; Eli Lilly; #M00883;	EL-107	258169 00164554	Levels tested in B6C3F$_1$ strain - 0, 0.01, 0.1 and 1.25%(approx- 0, 12, 118 and 1522 mg/kg) Study #1		004593 005732
2 Year feeding/oncogenic - mice; Eli Lilly; #M00983;	EL-107	258169 00164554	Levels tested in B6C3F$_1$ strain - 0, 0.01, 0.1 and 1.25%(approx- 0, 12, 118 and 1522 mg/kg) Study #2 (For results see Doc# 5732		004593 005732

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TDX Category	CDRE Grade/ Doc. No.
Mutagenic - gene mutation in bacteria (Ames); Lilly, Study #84100-1AMS1378, 10/84	EL-107 tech (95.5% ai)	073441	Negative for inducing revertants in S. Typhimurium w/without activation up to level of insolubility (500ug/plate)		Acceptable 005732
Mutagenic - chromosome aberrations in vivo (mouse micronucleus); CERTI(for Lilly), Study #071, 9/6/86	EL-107 tech (% ai not stated)	265739 0064555	Positive for inducing micronuclei following single-dose acute oral gavage at 5000 mg/kg; females not tested; no repeat assay		Inconclusive 005732
Mutagenic - chromosome aberrations in germinal tissue (rat DLT); Lilly, Study # MD1984; 10/85	EL-107 tech (95.5% ai)	265739 00164556	Negative for inducing dominant lethals in males fed levels up to 1.25% (12500 ppm providing intake of approximately 932 mg/kg/day). Data on positive controls required.		Provisionally Acceptable 005732
Acute dermal LD_{50} - rabbit; Lilly Research; Proj. No. B-D-124-84; 11/84	EL-107 tech (95.5% ai)	073293 00153112	LD_{50} > 2000 mg/kg bodyweight	III	Minimum 005732
90-Day feeding - mice; Lilly Research; MD2782; 5/85	EL-107 tech (93.7% ai) Batch No. MD2-2G6-118	265731 00164549	Systemic NOEL = 100 ppm Systemic LEL = 1400 ppm (Liver hypertrophy - males; increased liver wt. and liver enzyme induction-males and females). After a 30 day recovery period, males showed a dose related increase in MCHC at 0.01-1.25% plus a decrease in leukocyte count. Levels tested: 0,0.001,0.01,0.14, 1.25% (0, 10, 100, 1400, 12500 ppm) in $B_6C_3F_1$ strain.		Supplementary 005732

Tox Chem No. 419F

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE rade/ Doc. No.
90-Day oral - dog; Lilly Research; DO0783; 4/84	EL-107 tech (95.5% ai) Lot Z10025	073293 00153115	Systemic NOEL= 110 mg/kg/day Systemic LEL = 500 mg/kg/day (Increased liver weight, liver/ bodyweight ratio) Levels tested: 0, 25, 110, 500 mg/ kg/day by capsule in beagles		Minimum 005732
90-Day feeding - mice; Lilly Research: MO1083; 11/85	EL-107 tech (95.5% ai) Lot Z10025	265732	Special liver study NOEL = 100 ppm (15 mg/kg/day) LEL = 1000 ppm (Increased liver wt., incr. liver enzyme activity) Levels tested: 0, 100, 1000, 12500 ppm in $B_6C_3F_1$ strain		Supplemen- tary 005732
2-Year feeding/oncogenic - mice; Lilly Research MC0809; 11/85	EL-107 tech (95.5% ai) Lot Z 10025	265737 265738 00164554	Oncogenic LEL = 12500 ppm (Hepatocellular adenomas and carcinomas) Systemic NOEL= 100 ppm Systemic LEL - 1000 ppm (Lowered bodywt., bodyweight gain in males; hepatocellular vacuolation, hepatocellular hyperplasia, hep. cytomegaly) Levels tested: 0, 0.01, 0.1, 1.25% (0, 100, 1000, 12500 ppm) in $B_6C_3F_1$ strain combined report for replicated MO0883, MO0983.		Minimum 005732

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
2-Year feeding/oncogenic - rat; Lilly Research; R01583; 11/85	EL-107 tech (94.8% ai) Lot Z10025	073295 265735 265736 00164553	Oncogenic LEL =12500 ppm (Pheochromocytomas in males) Systemic NOEL= 125 ppm (5 mg/kg males, 6.2 mg/kg females) Systemic LEL= 1250 ppm (50.7 mg/kg males, 61.8 mg/kg females) (Lowered bodywt., bodyweight gain-high dose males and females, BUN, creatinine, cholesterol, alkaline phosphatase; heart, ovaries/bw, prostate/brain weight, progressive glomerulonephrosis at mid & high doses, hyperplasia in many organs examined.) Levels tested in Fischer 344 strain-0,0.0125,0.125 and 1.25% (=125,1250 and 12500 ppm)		Minimum 005732
3-generation repro. - rat; Lilly Res.; #R15382, R03783, R14183; 8/84	EL-107 tech (93.7-94.5% ai) Lot HD2-2G6-118	073297 073298 00153529	Maternal NOEL= 500 ppm Maternal LEL= 2500 ppm (Lowered bodywt., bodywt. gains; increased liver/bodywt. males and females) Reproduction NOEL= 2500 ppm Reproduction LEL= 12500 ppm Decreased # viable pups F_{2a}, F_{2b}, lowered bodyweight of progeny on postpartum day 21 Developmental NOEL= 2500 ppm Developmental LEL =12500 ppm (Decreases in viable fetuses/litter increased hydroureter, microphthalmia) Levels tested by diet: 0,500, 2500, 12500 ppm in Wistar strain		Minimum 005732

006559

006559

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Teratology - rat; (Hst: (WI)BR); Lilly Res.; #RO9483; 7/84	EL-107 tech (95.5% ai) Lot Z10025	073229 00153119	Maternal NOEL= 320 mg/kg/day Maternal LEL = 1000 mg/kg/day (Decreased bodyweight gain) Developmental NOEL = 320 mg/kg/day Developmental LEL = 1000 mg/kg/day (Increased preimplantation loss, increased resorptions, smaller litter size, increased number of runt fetuses). Levels tested: 0, 100, 320, 1000 mg/kg/day by gavage in Hsd:(WI)BR strain on gestation days 6-15 A/D = 1		Minimum 005732
Teratology - rabbit; Lilly; #RO3383; 5/84	EL-107 tech (95.5% ai) Lot Z10025	073299 00153118	Maternal NOEL >1000 mg/kg/day (HDT) Developmental NOEL > 1000 mg/kg/day Levels tested: 0,100,320,1000 mg/kg/day by gavage in Dutch Belted strain on gestation days 6-18.		Minimum 005732
Percutaneous absorption/ Rhesus monkey; Lilly, RO3983, RO4083; 11/83	EL-107 tech (97% ai) Lot 553-02V-094/553-1X7-018	073293	At 7 days, absorption of 11% of 2 mg/kg applied topically. Topical dose absorbed was 7.5% of IV dose (2 mg/kg given 1 month earlier).		Acceptable 005732
Metabolism/ rat; Lilly Research; ABC-01538/84	EL-107 tech (98.6% ai) Lot 553-3N1-056	073293	Single oral dose of 250 mg/kg almost entirely excreted at 48 hr.; at 72 hr.-90% recovered in feces, majority was unmetabolized; 20% of the dose was absorbed and 1/2 excreted in urine, 1/2 in feces as metabolites		Acceptable 005732

Tox Chem No. 419F | File Last Updated _____ | Current Date _____

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Excretion in expired air - rat; Lilly; MO2186; 6/86	EL-107 tech (93.3-94.8% ai) Lot Z10025	265740	Single oral dose of 250 mg/kg - 48 hr.- 85% recovery of radioactivity in males, 92.6% recovery in females; 2.4% expired air portion for males, 2.8% for females Males do not show complete recovery of radioactivity in 48 hr.; some residual radioactivity in carcass- possible bioaccumulation concern		Acceptable 005732
Distribution in - rat; Lilly R11285; 1/86	EL-107 tech (93.3-94.8% ai) Lot Z10025	265740	Approx. 1.0 ml/100g. bodyweight in 2 groups: 4 hr. and 24 hr.(approx. 1000 mg/kg final dose) Majority of radioactivity remains in intestinal tract, some tissue accumulation above plasma levels.		Supplementary 005732
Excretion pattern in mice/ICR mice; Lilly; M03082; 9/86	EL-107 tech (>85% ai) Batch 121607	265729	Levels tested: 1,15,100,200 mg/kg - males only were tested. Within 24 hrs. 79.4,88.7,91.7 81.0% of above respective doses were excreted. Distribution of EL-107 above 100 mg/kg is altered.		Supplementary 005732
Absorption and disappearance of plasma 14_C EL-107 mice; ICR mice; Lilly; MO3182; 9/86	EL-107 tech (>85% ai) Batch 121607	265729	Single gavage doses of 1,15,100, 200 mg/kg Plasma half-life 8.3-8.9 hr. for radioactivity. Most of plasma radioactivity eliminated in 72 hr. Amount absorbed apparently limited to 100 mg/kg. Plasma elimination not affected by increasing doses		Supplementary 005732
Repeated dose distribution in rat; Lilly; R13885; 1/86	EL-107 tech (93.3% ai) Lot Z10025	265740 00164558	Predosing with 250 mg/kg cold El-107 did not change pattern of urinary & fecal excretion. 80.59% (M) & 96.1% (F) within 7 days in combined urinary & fecal excret. No radioactivity detected in tissues after 7 da. Carcasses 0.3%(M) & 0.4% (F) radioactivity remained.		Acceptable 005732

005359

Tox Chem No. 419F EL-107 | File Last Updated 3/4/87 | Current Date

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
1-Year dog; Beagle; Lilly; D04783; 12/85	EL-107 tech (95.5% ai) Lot Z10025	275733 00164551	NOEL= 10 mg/kg LEL= 100 mg/kg Increased alkaline phosphatase; liver/brain weight ratio elevated in males and females, liver/bodyweight ratio elevated in females, some liver microsomal enzyme induction in high dose males Levels tested: 0, 10, 100, 1000 ppm in beagles		Minimum 005732
1-Year feeding - rat; Lilly; RO1483; 7/84	EL-107 tech (95.5% ai) Lot Z10025	073295	NOEL= 125 ppm LEL=1250 ppm Decreased bodyweight, weight gain, food efficiency in high dose females; liver microsomal enzyme induction- high dose males and females, mid dose females (6, 12 mos.) mid dose males (3,6 mos.); serum glucose incr. males and females at 6 mos. Levels tested: 0, 125, 1250, 12500 ppm (0, 0.0125, 0.125, 1.25%) in Fischer 344 strain.		Acceptable 005732
3-Month feeding - rat; Lilly; R12482; 4/84	EL-107 tech (94.2% ai) Lot HD2-, 2G6-118	073293 073294	No NOEL; LEL=0.05% (500 ppm) At 3 months: Increased liver wt. and liver/bodyweight ratio in all groups of females; increased liver/body-weight ratio in males at 0.42 and 1.25%; elevated hepatic microsomal enzyme activity in males at 0.42 and in females at 0.14, 0.42, and 1.25% At 4 months: Liver weights normal, enzyme activity normal Levels tested by diet: 0, 0.05, 0.14 0.42, and 1.25% (0,500,1400,4200,and 12500 ppm) with one month recovery		Guideline 005732

Tox Chem No. 419F | File Last Updated _____ | Current Date 5/4/87

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Risk assessment - rat; EPA	ISOXABEN	265735 265736 265737 265738	Qualitative Risk Assessment: 2-Year chronic oral study in male & female Fisher 344 rats & male & female B6C3F1 mice. Eli Lilly Rpt.; Nov. 85. (1) Significant related trends for male rat pheochromocytomas & both male & female progressive glomerulonephrosis. (2) Mid & high doses males & high dose females were significantly higher than controls. (3) Significant dose-related trends for hepatocellular adenoma & combined adenoma/carcinoma in both sexes of mouse. (4) High dose of both sexes had significantly more adenomas than controls. High dose females had significantly more combined adenomas/ carcinomas.		005889

Study/Lab/: #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, PIS, NOEL, LEL	TOX Category	CORE Grade/ Doc. No.
Mutagenic - genotoxicity Sister chromatid exchange in vivo/bone marrow of Chinese hamsters; Lilly; no study number; 10/82	EL-107 tech (94.2% ai) Lot H02-2G6-118	250793	No increase in SCE in bone marrow Levels tested by oral intubation: 12.5, 25, 50, 100 mg/kg		Unacceptable 005732
Mutagenic - Ames; Lilly; no study number; 12/82	EL-107 tech (94.2% ai) Lot H02-2G6-118	250793	Method used lacked appropriate sensitivity, could not adequately show whether EL-107 causes gene mutation in S. Typhimurium or E. Coli		Unacceptable 005750
Mutagenic - DNA repair in adult rat hepatocytes (UDS); Lilly; 82092-UDS1378, 821026UDS1378; 12/82	EL-107 tech (94.2% ai) Lot H02-2G6-118	250793	Not positive in 2 replicate assays; positive in positive control showing appropriate sensitivity		Acceptable 005750
Mutagenic - gene mutation-forward mutation in mouse lymphoma assay; Lilly; 820928-ML1378; 12/78	EL-107 tech (94.2% ai) Lot H02-2G6-118	250793 00132138	Forward mutation not induced, increased mutation frequency not found w/without S-9 activation; Positive control demonstrated sensitivity		Acceptable 005750
Risk assessment short term - mouse; EPA; 2/4/87			Regression analysis indicates a dose related trend for mouse liver wt. and liver hypertrophy (Memo - Misc. data analysis; H.Lacayo; 2/4/87)		005730

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Appended page 1 from Test Report R-0-48-81 R-0-49-81

MAID No. 31

006559

91-1

THE ACUTE ORAL TOXICITY OF COMPOUND 121607 (EL-107) IN FISCHER 344 RATS

Studies: R-0-48-81
R-0-49-81

Project Leader: S. G. Lake

Pathologist: S. G. Lake

Report Prepared by: M. I. Levitt

Toxicology Division
Lilly Research Laboratories
Division of Eli Lilly and Company
Greenfield, Indiana 46140

April, 1981

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Acc. No. 00-134

006559

91-1

TOXICOLOGY BRANCH DATA REVIEW

Study Type: Acute Oral Toxicity in Rats

Accession Number: 250791 (2)

MRID Number: MJ031

Sponsor: Eli Lilly and Company

Contracting Lab: Lilly Research Laboratories, Nos. R-O-48-81 (Females), R-O-49-81 (Males)

Date: April, 1981

Test Material: N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2, 6-dimethoxybenzamide, EL-107 (Lot 831-72C-88)

Protocol:

Ten male and 10 female rats were each given a single dose of 10,000 mg/kg by gavage (as a 30% suspension in 10% acacia) and observed for 14 days.

Results:

"No deaths occurred." "Signs of toxicity were limited to animals treated with EL-107 and consisted of generalized leg weakness and compound colored feces on the day of treatment. With the exception of soft stools which were observed in both treated and control animals after 8 days on test, all animals appeared normal 24 hours after dosing and for the remainder of the study." LD_{50}: >10,000 mg/kg for both males and females.

Conclusions: Acute oral toxicity category: IV.

Core Classification: Guideline

Updated 11/23/87

Attachments - Appended pages 1-4

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Pages 41 through 43 are not included.

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81-1

Doc. No. 003434

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OL-81

TOXICOLOGY BRANCH DATA REVIEW

Study Type: Acute Oral Toxicity in Mice

Accession Number: 250791 (4)

MRID Number: 00132

Sponsor: Eli Lilly and Company

Contracting Lab: Lilly Research Laboratories, Nos. M-O-49-81 and M-O-50-81

Date: April, 1981

Test Material: N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide, EL-107

Tech. grade 92.34% Lot# B31-72C-88

Description: beige powder

Protocol:

The acute oral toxicity was evaluated in 10 male and 10 female mice. Animals were observed for mortality and signs of toxicity for 14 days following a single 10,000 mg/kg oral dose of test compound (25% in a 10% aqueous acacia). The obsrvation period was 14 days.

Results:

There were no deaths. "In females signs of toxicity were confined to compound-colored feces 1 to 3 days after dosing in animals treated with compound 121607. Compound-colored feces was also observed 1 to 3 days after dosing in males treated with the test compound. In addition, an isolated instance of poor grooming was seen 24 hours after treatment and 3 to 10 male mice exhibited tail erection after 4 days on test. All animals appeared normal 5 days after dosing and for the remainder of the studies."

Gross pathology revealed no compound related lesions.
LD_{50}: >10,000 mg/kg in mice

Comments:

Acute oral toxicity category: IV.

Core Classification: Guideline

Updated 11/23/87

Attachments: pp 1-5 Appended

Appended page 1

006559

THE ACUTE ORAL TOXICITY OF COMPOUND 121607 (EL-107) IN ICR MICE

Studies: M-O-49-81
M-O-50-81

Project Leader: S. G. Lake

Pathologist: S. G. Lake

Report Prepared by: M. I. Levitt

Toxicology Division
Lilly Research Laboratories
Division of Eli Lilly and Company
Greenfield, Indiana 46140

April, 1981

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81-2

006559

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

MRID 00J001

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

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

006559

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: [signature] for
Date: 1-20-87

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

Signature: [signature] for Paul Wennerberg
Date: 1-20-87

6. APPROVED BY:

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

Signature: [signature]
Date: 1/20/87

Margaret L. Jones
EPA Reviewer

Signature: [signature]
Date: 1-21-87

Marcia Van Gemert, Ph.D.
EPA Section Head

Signature: [signature]
Date: signed 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 LD_{50} 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 LD_{50} for EL-107 is greater than 2000 mg/kg, which corresponds to Toxicity Category III.

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

9. CLASSIFICATION: Core minimum.

Dermal LD_{50}: >2000 mg/kg body weight.

Toxicity Category: III.

81-3

006559

004593

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Acute Inhalation Toxicity (4-hours), rat

Accession Number: 257774

MRID Number: 251009

Sponsor: Eli Lilly No. R-H-70-84

Contracting Lab:

Date: October 29, 1984

Test Material: EL-107, Technical

Protocol: See Attached Materials and Methods.

Appendix E presents Shell's rationale for using EL-107 technical material instead of EL-107 DF in this acute inhalation study.

It is agreed that this substitution is acceptable.

Results:

The nominal concentration was 12.83 mg/L. The gravimetric exposure concentration was 2.55 ± 0.603 (SD) (range was 1.55 to 3.47 mg/L).

The mass median equivalent aerodynamic diameter was determined to be 40.49 µm with a geometric standard deviation of 4.28. The particle size distribution pattern is shown in Figure 2 of Appendix E.

All animals appeared normal throughout the study with the exception that five female rats displayed poor grooming in day 1.

There were no deaths and no compound-related lesions were found.

Conclusions:

Acute inhalation toxicity (4 hours): 2.55 ± 0.603 (SD) mg/L (HDT).

Acute inhalation toxicity category: III

Core Classification:

Minimum

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Doc. No. 003434

91-3

006559

TOXICOLOGY BRANCH DATA REVIEW

Study Type: Acute Inhalation Toxicity

Accession Number: 250791 (3)

MRID Number: 00[illegible]3

Sponsor: Eli Lilly and Company

Contracting Lab: Lilly Research Laboratories, No. R-H-37-81

Date: September, 1981

Test Material: N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2, 6-dimethoxybenzamide, EL-107

Protocol:

"The ten male and ten female rats were restrained in acrylic "nose only" exposure tubes which were fitted to the ports of a 41 L cylindrical plexiglass exposure chamber and exposed for one hour. The mass median equivalent aerodynamic diameter (MMEAD) was determined to be 13.10 um with a geometric standard deviation of 3.53. The nominal concentration was 11.5 mg of 121607/L. The acute exposure concentration was 1.99 ± 0.199 (SD) mg of 121607/L, with a range of 1.68 to 2.22 mg of 121607/L".

Results:

There were no deaths. "All animals appeared normal immediately postexposure. One male rat exhibited rales on day 1 postexposure only. All animals appeared normal throughout the remainder of the 14-day postexposure observation period."
LC_{50}: >1.99 ± 0.199 mg/l, actual; 11.5 mg/l, nominal

Conclusions:

Acute inhalation category: II.

Core Classification: Minimal

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Revised 11/24/87

Attachments: Appended pages 1-...

Appended page 1
from Report R-H-37-81

THE ACUTE INHALATION TOXICITY TESTING OF COMPOUND 121607 (EL-107) IN THE FISCHER 344 RAT

Study R-H-37-81
April 30-May 14, 1981

INTRODUCTION

The purpose of this study was to determine the toxicity resulting from the exposure of rats for one hour to a solid particulate aerosol of compound 121607 (EL-107).

MATERIALS AND METHODS

Personnel: The personnel responsible for the conduct of this study are identified in Appendix A.

Storage of Specimens, Raw Data and Final Report: The final report as well as the protocol and raw data for this study are stored at Eli Lilly and Company.

Test Animal: Ten male and ten female Fischer 344 rats, were obtained from Charles River Breeding Laboratories, Inc., Portage, Michigan. Prior to exposure the animals were housed as described below. The animals were acclimated for seven days before being assigned to the study. The initial body weights (mean ± SD) for males and females were 187 ± 5.1 g and 149 ± 4.1 g, respectively. The rats were 56-63 days of age at the initiation of the study. Body weights of animals were recorded prior to exposure, and at 1, 3, 5, 7, and 14 days postexposure. All animals were

Appended page 2
from Report R-H-37-81

APPENDIX B

006550

ANALYTICAL CHARACTERIZATION REPORT

Chemical Name: N-[3-(1-Ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide
Compound No.: 121607
Toxicology Lot: 831-72C-88
Lot Size: 17.4 kg

Identity and Potency

A. Quantitation

Assay Type	Method	Result	Assay Date
1. HPLC*	HMT-β	92.34 (n=2)	2-11-81
2. GLPC*	HMZ-β	95.25 (n=3)	2-10-81
3. Elemental Analysis (sample dried 4 hrs. 110°C.)		% Theory / % Found	2-12-81
	C:	65.04 / 65.30	
	H:	7.28 / 7.04	
	N:	8.43 / 8.50	
	O:	19.25 / 19.38	
4. Melting Range	USP	170-171°C. (sample dried)	2-11-81

B. Quantitation of Related Materials

1. Heavy Metals	USP	0 ppm	2-11-81
2. Moisture, K.F.	TKU-β	5.30%	2-10-81
3. Toluene	G.C.	0.12%	2-25-81
4. TLC*	JUV-β	Main spot has same Rf as ref. std. One faster-moving spot about 1.45% and two slower-moving spots each less than 1%; also POA material.	2-11-81

C. Identity

1. Spectroscopy

a. I.R.*	KBr pellet	Spectrum compares closely to that of ref. std.	2-09-81
b. NMR*	Methanol-d_4	Spectrum compares closely to that of ref. std.	2-09-81
c. Mass Spectrum		The spectrum is consistent with the structure.	
2. X-ray*		Pattern C810209-9 same as ref. std.	2-10-81

*vs. ref. std. lot 553-88C-257 at a potency of 95.0% "as is"

NOTE: HPLC assay HMT-β is more specific than GC assay HMZ-β, and the 92.3% result represents an accurate assessment of the purity of lot 831-72C-88.

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C. T. Doty
X4C86

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TOXICOLOGY BRANCH DATA REVIEW

Study Type: Acute Eye Irritation

Accession Number: 250791 (3)

MRID Number: MJ 002

Sponsor: Eli Lilly and Company

Contracting Lab: Lilly Research Laboratories, No. B-E-61-81

Date: September, 1981

Test Material: N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2, 6-dimethoxybenzamide, EL-107, Lot No 6 31-72C-88; 92.34% pure (HPLC) (See appendix B, attachment to DER MRID MJ003)

Protocol:

"One eye of each of six rabbits (3 per sex) was treated with 28 mg (equivalent in volume to 0.1 cc) of undiluted test material. Prior to administration the test sample was ~~triturated to a powder. The untreated~~ eye of each animal served as control.

The test material was applied over the corneal surface and into the conjunctival cul-de-sac. The eyelids were held closed for several seconds after treatment to prevent expulsion of test substance.

Eyes were examined and ocular reactions were graded. Eyes were scored at 1, 24, 48, and 72 hours after dosing and again at 7 days. To aid in the identification of corneal lesions, sodium fluorescein dye was applied 24 hours after exposure."

Results:

"Corneal dullness and mild iritis in two animals and slight conjunctivitis in all animals were observed one hour after exposure. Irritation to the cornea and iris cl red within 24 hours, conjunctivitis cleared by three days postexposure." All rabbits exhibited a negative response to sodium fluorescein dye administered 24 hours after exposure. All treated eyes returned to normal within 72 hours of exposure.

Conclusions:

Eye irritation category: III.

Core Classification: Guideline

66

Updated 11/25/87, See notes under "Test material"

81-5

TOXICOLOGY BRANCH DATA REVIEW

006559

Study Type: Acute Dermal Toxicity and Dermal Irritation

Accession Number: 250791 (3)

MRID Number: MRID MJ0008

Sponsor: Eli Lilly and Company

Contracting Lab: Lilly Research Laboratories, No. B-D-58-81

Date: September, 1981

Test Material: N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2, 6-dimethoxybenzamide, EL-107, Lot No. B31-72C-68, 92.34% pure (HPLC) (See MRID MJ003, appendix B)

Protocol:

Three rabbits per sex were treated. "The fur was clipped from the back of each animal and the exposed skin of one-half the animals in the test group was abraded with a stiff nylon brush. The abrasions were of sufficient depth to penetrate the stratum corneum but not to cause bleeding.

The test sample was applied undiluted to a gauze pad affixed to the treatment site with occlusive dressing and an adhesive sleeve. Occlusion was maintained for 24 hours."

"One hour after removal of the occlusive dressings, and twice daily for the subsequent 14 days, animals were examined for signs of toxicity. Individual body weights were recorded on the day the test substance was administered and weekly thereafter."

Results:

There was no evidence of dermal irritation during the study.
There was no evidence of systemic toxicity during the study.
LD_{50}: >200 mg/kg (HDT)

Conclusions:

Primary dermal irritation category: IV.
Acute dermal toxicity: dosage level inadequate.

Core Classification:

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Primary dermal irritation: minimal
Acute dermal toxicity: supplemental ~~* NOEL was not determined.~~

Updated 11/25/87; see notes under "Test material"

81-6

006550

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Dermal sensitization, guinea pig

Accession Number: 257774

MRID Number: 00157

Sponsor: Eli Lilly, No. G00183

Contracting Lab:

Date: December 1983

Test Material: EL-107, Technical (Lot H02-266-18) and a suspension concentrate formulation (FN-7033) containing 50% EL-107

Protocol:

Female guinea pigs of the Hartman strain were used.

Treatment groups and study duration were as follows.

"Group I: Induction and Challenge: 0.1% dinitrochlorobenzene (DNCB) in 70% ethanol

Group II: Challenge Control: 0.1% DNCB in 70% ethanol

Group III: Induction and Challenge: EL-107, technical material, at a concentration of 25% in 95% ethanol

Group IV: Challenge Control: EL-107, technical material, at a concentration of 25% in 95% ethanol

Group V: Induction and Challenge: A 1:1 aqueous dilution of the suspension concentrate formulation

Group VI: Challenge Control: A 1:1 aqueous dilution of the suspension concentrate formulation

Updated 1/24/87 - see notes under "Test material"

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"There were 12 animals in each induction and challenge group; each challenge control group contained 6 animals. The duration of the study was 24 days."

The suspension concentration formulation 50% contained EL-107. It was not the same formulation as EL-107 DF (75%).

"Animals were induced three times a week for 2 consecutive weeks in induction and challenge test groups (Groups 1, 3, and 5). Guinea pigs were prepared for treatment by clipping the hair in the nuchal area with Oster® clippers. Exposed skin was swabbed with acetone to remove extraneous lipid material that might inhibit percutaneous absorption. A dose of 0.2 ml of the test material was applied to the nuchal area of each animal. The application site was occluded with a 1 1/2 inch square patch (Band-Aid®) held in place with adhesive tape which was wrapped around the torso of the animal. The bandage was removed after 6 hours."

"Guinea pigs assigned to challenge control groups (Groups 2, 4, and 6) were left untreated during the induction period."

"Ten days following the last induction exposure, the challenge dose was administered to all test animals including the challenge controls. A previously untreated area in the center of the back of each animal was prepared for treatment by clipping the hair and swabbing the exposed skin with acetone. Each application site was treated and occluded for 6 hours as described for induction."

"During the induction and challenge phases of the study, treated areas were graded for dermal response 24 hours after each application of EL-107 according to the method outlined in Appendix D. Similar observations were conducted 24, 48, and 72 hours following challenge."

"Body weights were recorded at test initiation and weekly during the test period."

Results:

There was no sign of sensitization or dermal irritation in any of the animals challenged with either EL-107 technical or the suspension concentrate formulation (50% formulation).

The positive control gave positive results.

Conclusion:

Dermal sensitization was not found.

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Core Classification:

Minimum

82-1006559

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

MRID 00161010

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 Felkner, Ph.D.
Program Manager
Dynamac Corporation

Signature: I. Cecil Felkner

Date: 8-6-85

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

006559

1. CHEMICAL: EL-107 (Isomers 121607, [redacted] 121607 = N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide (85.0%)

[redacted]

2. TEST MATERIAL: EL-107, Technical, Lot No. 710025 with a purity of 95.5%. See Appendix C of ths 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
Dynamac Corporation

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

6. APPROVED BY:

Norbert P. Page, D.V.M.
Oncogenicity & Chronic Effects
Technical Quality Control
Dynamac Corporation

Signature: Ina Cecil Felton for
Date: 8-6-85

W. Thomas Edwards
EPA Reviewer

Signature: W. Thomas Edwards
Date: 5-15-86

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

Signature: Marcia Van Gemert
Date: 12/10/86

7. CONCLUSIONS:

A. When given orally to beagle dogs by gelatin capsules at doses of 0, 25, ~~100~~ 110 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 subchronic oral (capsule) non-isomeric study (D33582), and 3) a two-week pilot oral (capsule) isomeric 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. Athough 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
	3.00	2.32	2.80	5.06
MEAN	2.656	2.925	3.081	3.663

Reviewed by: Margaret L. Jones M. L. Jones 1/2/87 82-1
Section III, Tox. Branch (TS-769C)
Secondary reviewer: Marcia Van Gemert, Ph.D. M. VanGemert 1/6/87 006559
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.: M5011

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 B6C3F$_1$ 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: B6C3F$_1$ 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.

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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, [redacted] 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: $B6C3F_1$, 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.

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

B. STUDY DESIGN:

1. Animal assignment

Animals were assigned randomly to the following test groups:

Test Group	Dose in diet (ppm)	Main Study 3 months male	Main Study 3 months female	Sacrifice 3 months male	Sacrifice 3 months female	One Month Reversibility male	One Month Reversibility 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

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 $p \leq 0.05$. 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			
	% EL-107			
Males	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.

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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		X	
	Hematocrit (HCT)*		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.

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b. Clinical Chemistry

X		X	
	Electrolytes:		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		Total Serum Protein*
	Cholinesterase#		Triglycerides
	Creatinine phosphokinase*°		Serum protein electrophoresis
	Lactic acid dehydrogenase		
x	Serum alanine aminotransferase (also SGPT)*		
	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

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.

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6. Urinalysis°

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

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

* Required for chronic studies
° Not required for subchronic studies

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		X		X	
	Digestive system		Cardiovasc./Hemat.		Neurologic
	Tongue		.Aorta*	x	.Brain*†cerebrum,cerebellum, brain stem
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	xx	.Adrenals*w.kidneys
	.Cecum*	xx	.Kidneys*†w.adrenals		Lacrimal gland#
x	.Colon*	x	.Urinary bladder*	x	Mammary gland*#
	.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-nitroanisole0-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.[illegible]% EL-107 as indicated by increases in paranitrophenol (PNP) produced per miligram of protein per hour. The values are found in appended Table 10 from Stucy 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.

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

ISOXABEN

Page_____ is not included in this copy.

Pages 90 through 115 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.

X 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.

c. Microscopic pathology

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

Dose	Incidence
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.

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 scudy 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. Ophtnalmological 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.

Appendix b

006559

Title Test Article Characterization of Technical EL-107, Lot No. H02-2G6-118 for Use in Acute and Subchronic Toxicology Studies (I-EWD-82-30)

Author(s):	Date
B. S. Rutherford B. S. Rutherford, M. S. Assoc. Senior Analytical Chemist	12-7-83

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R. F. Sieck, Ph.D., Head,
Agricultural Analytical Chemistry — Dec 7, 1983

J. C. Walker
J. C. Walker, Ph.D., Director
Agricultural Chemistry and Development Division — 12/7/83

Agricultural Analytical Chemistry
Lilly Research Laboratories
Division of Eli Lilly and Company
Greenfield, IN 46140

006559

TEST ARTICLE CHARACTERIZATION OF TECHNICAL EL-107, LOT NO. H02-2G6-118, FOR USE IN ACUTE AND SUBCHRONIC TOXICOLOGY STUDIES

I-EWD-82-30

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Agricultural Analytical Chemistry

Lilly Research Laboratories
Division of Eli Lilly and Company
Greenfield, IN 46140

ISOXABEN

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Pages 121 through 135 are not included.

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____ Sales or other commercial/financial information.

____ A draft product label.

____ The product confidential statement of formula.

____ Information about a pending registration action.

X FIFRA registration data.

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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.

TABLE I

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

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		X		X	
	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*
X	.Duodenum*	X	.Spleen*	X	Eyes (optic n.)*#
X	.Jejunum*		.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*††
X	Gall bladder*#	X	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.

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 lo 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.

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		X	
	Hematocrit (HCT)*	X	Leukocyte differential count*
X	Hemoglobin (HGB)*		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		X	
	Electrolytes:		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

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81. 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

C. METHODS AND RESULTS:

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. Ophthalmalogical 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 desplayed 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 phenomen.

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

A. MATERIALS:

1. Test compound: EL-107 is a mixture of isomers (Lilly compound 121607, 85%; [redacted]

Description; not given:
Batch # Z10025

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 (mg/kg) (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.

93-1

Reviewed by: Marcia 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)

006559

DATA EVALUATION REPORT

STUDY TYPE: 1-year dog study TOX. CHEM. NO.: 419F

ACCESSION NUMBER: 265733 MRID NO.: ? 00155?

TEST MATERIAL: EL-107 (Isomers 121607, [redacted]

SYNONYMS: Isoxaben technical IDENTITY OF PRODUCT IMPURITIES IS NOT INCLUDED

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 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)

"Pathology

There was no morphologic evidence of systemic toxicity or dermal irritation in rabbits following the dermal application of technical EL-107, or a suspension concentrate formulation containing 50% EL-107."

CONCLUSIONS:

Very slight dermal irritation in four of 20 animals exposed to 1000 mg formulation/kg body weight was observed.

The systemic effect of concern was increase in relative weights of the thyroid glands. Although there was much variation in thyroid plus parathyroid relative weights within groups, there was an increase in relative thyroid weight apparent in all treated groups except in the group which was allowed a two week recovery period.

LEL(technical EL-107): <1055 mg/kg bw

EL(formulation FN-7033): < 500mg/kg bw

This study was well performed but if considered alone is inadequate for regulatory purposes because a NOEL was not determined.

Core Classification:

Minimum

Clinical Chemistry

There were no toxicologically significant effects on glucose, urea nitrogen, creatinine, total bilirubin, alanine transaminase, or alkaline phosphatase determinations conducted pretest, at study termination, and 14 days after termination of treatment for the reversibility group."

"Organ Weights

Absolute and relative (per 100 g body weight) liver, kidney, heart, adrenal, ovary, testis, and spleen weights were not affected by technical EL-107 or the suspension concentrate containing 50% EL-107."

Increases in relative thyroid weights are shown in the following table which was adapted from information furnished.

TERMINAL BODY WEIGHT AND ORGAN WEIGHTS RELATIVE TO BODY WEIGHT

Treatment groups		Males		Females	
		Body Weights KG	Thyroids Para Thyroids MG per 100 G Body Weight	Body Weight KG	Thyroids Para Thyroids MG per 100 G Body Weight
Controls	MN	3.1	5.93	3.3	6.47
	SD	0.28	0.873	0.25	2.017
	SE	0.12	0.390	0.11	0.902
	OBS	5	5	5	5
1	MN	3.2	7.58	3.2	7.99
	SD	0.27	2.525	0.37	0.717
	SE	0.12	1.129	0.17	0.320
	OBS	5	5	5	5
2	MN	3.4	7.12	3.3	7.83
	SD	0.50	1.400	0.23	2.128
	SE	0.22	0.626	0.10	0.952
	OBS	5	5	5	5
3	MN	3.2	9.10	3.2	11.47**
	SD	0.43	4.143	0.50	2.410
	SE	0.19	1.853	0.23	1.078
	OBS	5	5	5	5
4	MN	3.1	5.59	3.4	6.35
	SD	0.19	0.816	0.51	1.482
	SE	0.09	0.365	0.23	0.663
	OBS	5	5	5	5

Group 4 had been allowed two weeks to recover.

Three sections of skin were collected: abdominal skin, treated skin from the back, and untreated skin from the back.

Histologic preparations of the tissue specimens collected at necropsy were examined microscopically by a certified veterinary pathologist with experience in evaluating laboratory animal tissues. The findings were recorded and tabulated. A summary of the important pathologic alterations was prepared.

Statistics: The statistical method described by Dunnett was used in the analysis of differences (at each time point) between control and treated group means for parameters for which data are generally distributed normally (body weight, weight gain, hematology, clinical chemistry, and organ weight data). The homogeneity of variances was tested by the method of Bartlett.[5] All references to statistical significance in the report represent a "p' value ≤0.05."

Results:

"Clinical Observations

There were no overt signs of systemic toxicity related to the administration of EL-107 or a suspension concentrate formulation containing 50% EL-107. No treatment related changes or abnormalities occurred between the pretest ophthalmic and physical examinations and those conducted at termination of study.

Body Weight

Terminal mean body weights were similar for control and treated animals.

Food Consumption

There were no treatment-related effects.

Dermal Irritation

The only evidence of dermal irritation in any treatment group was transient irritation which occurred in four of 20 animals exposed to 1000 mg formulation/kg body weight during the first week of treatment.

Hematology

There were no toxicologically important effects on erythrocyte and leukocyte counts, hemoglobin, packed cell volume, leukocyte differential count and erythrocyte morphology conducted pretest, at study termination, and 14 days after termination of treatment for the reversibility group.

a gauze pad, the torso of each rabbit was wrapped with an elastic bandage, which was held in place with strips of tape. Dressings were removed after six hours and the application sites were rinsed with tap water and dried with a towel. All rabbits were provided with collars to discourage ingestion of the test material."

"Survival, Clinical Observations, and Dermal Irritation: Twice each day all cages were inspected for dead or moribund animals. Each rabbit was removed from its cage once a day for treatment application and carefully examined for changes in behavior or appearance. Dermal irritation was graded daily using an eight point scale for erythema and edema according to the method of Draize.

Body Weight and Food Consumption: Rabbits were weighed once each week and doses were adjusted to correspond to changes in body weight. Food consumption was measured daily.

Hematology: Hematologic evaluations were conducted on each rabbit prior to study initiation, at termination of treatment, and with the reversibility group, two weeks after the last exposure. Blood samples were drawn from the medial artery of the ear. Hematologic parameters included: hemoglobin (HGB), mean corpuscular volume (MCV), erythrocyte morphology, erythrocyte count, packed cell volume (PCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Hematology methodology is contained in Appendix F.

Clinical Chemistry: Blood samples for clinical chemistry evaluations were collected concurrently with those for hematology. Serum samples were analyzed for glucose (GLU), blood urea nitrogen (BUN), creatinine (CREAT), total bilirubin (TB), alkaline phosphatase (AP), and alanine transaminase (ALT)."

"Organ Weights: At necropsy, the liver, kidney, heart, thyroids, adrenals, ovaries, testes, and spleen were trimmed and weighed. Organ weight relative to 100 g body weight was calculated for all animals.

Pathology: All animals were necropsied. The necropsy was a systematic gross examination of each animal's general physical condition, body orifices, external and internal organs and tissues. All necropsies were performed by pathologists whose findings were recorded. The following organs and tissues for histopathologic examination were collected and immersed in a fixative: kidney, liver, heart, lung, spleen, thymus, lymph node, salivary gland, pancreas, stomach, duodenum, jejunum, ileum, colon, ovary, uterus, adrenal, thyroid, testis, prostate, skin, mammary gland, skeletal muscle, urinary bladder, bone marrow, eye, cerebrum, cerebellum, brain stem, application site, gallbladder, gross lesions, and skin.

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82-2

006559

004034

TOXICOLOGY BRANCH
DATA REVIEW

Study Type: 21 Day Dermal Toxicity, Rabbit

Accession Number: 252915

MRID Number: MS012

Sponsor: Eli Lilly and Co.

Contracting Lab: Eli Lilly Res. Labs., no. B01783

Date: December 1983.

Test Material: EL-107 Technical (Lot Z-10025) and a suspension concentrate formulation (FN-7033) containing 50% EL-107

Procedure:

"Treatment Groups and Study Duration: The test rabbits were randomly distributed among one untreated control group and four treatment groups, each of which contained five males and five females." The groups were as follows:

0) Untreated Control (water rinse only)

1) 1055 mg EL-107/kg body weight (equivalent to 1000 mg active technical EL-107/kg)

2) 500 mg formulation/kg body weight

3) 1000 mg formulation/kg body weight

4) 1000 mg formulation/kg body weight (reversibility group received treatment for three weeks followed by a two week withdrawal period)

"All animals were treated daily for 21 consecutive days and then necropsied, except rabbits in the reversibility group, which were held an additional 14 days without further treatment in order to assess delayed systemic toxicity and reversibility of observed effects. The overall study duration was 35 days."

"Test Article Administration: The skin on the back of each animal was prepared for treatment by removing the fur with Oster clippers. The treatment site was reclipped a minimum of twice each week during the study.

Each dose of technical material was weighed on a Sartorius balance to the nearest 0.01 g. Technical test material was held in place on the back of each rabbit with a damp gauze pad. The suspension concentrate formulation was measured by volume to the nearest 0.1 ml and applied with a syringe to a gauze pad. Following application of either test material to

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006559

List of statistical questions on Study MO2782, Mouse Subchronic Study with EL-107(Isoxaben) technical.

1. Is there any relationship between dose and total Bilirubin values in females at 90 or at 120 days? or difference between controls and dose groups?

2. Is there any relationship between dose and uterine weight in females at 90 days or at 120 days? or difference between controls and dose groups? any difference when organ weight to body weight is compared for this organ?

3. Is there a dose/response for liver hypertrophy in males at 90 days and if so, what is the NOEL for this effect? Do liver weights support the dose/response effect? relative liver weights?

4. What is the relationship between body weight and test compound, if any? [redacted]

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(cont.) TABLE - Test for Dose Related Effects

Hyp. Dose nas no Effect on	Sig* Level	Remarks
Females (cont.)		
90 Days:		
Bili R	.20	Extremely weak evidence of an effect
Body Wt.	.08	Significant
Uter. Wt.	.32	Extremely weak evidence of an effect
Uter. Wt/Body	.67	No effect
Males		
90 Days:		
Liver Hypertrophy	.001	Significant - C-Armitage test
Body Wt.	.74	No effect
Liver Wt.	.001	Significant
Liver Wt./Body Wt.	.001	Significant

* The test stat. is based on the dose coef. term in a first order linear regression model unless otherwise indicated.

Note that applying an approximate t test to the female weight gains of 120 days between group 00 (wt gain 14.7 g) and group 03 (wt gain 9.3 g) gives significance at the .01 level. There appear to be no other significant differences.

Lastly we emphasize that due to exploratory nature of our testing (which induces experiment-wise error rate) we consider significant results as indicators of concern; and not as definative results.

#16 12/19/86 sp
rew: 1/20/87

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

006559

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Initial Data Analysis, Short-Term Mouse Study on EL-107 (Isoxaben) - Body Weight, Liver Weight, Bili R.

FROM: Herbert Lacayo, Statistician H. Lacayo 3/3/87
Scientific Mission Support Staff
Toxicology Branch/HED (TS-769)

TO: Margaret Jones
Pharmacologist, Section III
Toxicology Branch/HED (TS-769)

THRU: Richard Levy, Statistical Team Leader and R. Levy 3/3/87

Reto Engler, Chief
Scientific Mission Support Staff
Toxicology Branch/HED (TS-769)

~~This memo contains~~ the results of a partial data analysis on a short term mouse feeding study (EL-107/Isoxaben). The initial questions asked by M. Jones are appended to this memo. There appears to be weak evidence of a dose related effect on female mouse body weight; and strong evidence of a significant dose related effect on male mouse liver weight and liver hypertropny. There is very weak evidence that Bili R (female) and Utereine Wt. may be affected by dose and these should be closely examined in any subsequent study.

We summarize the statistical analysis in the following table.

TABLE - Test for Dose Related Effects

Hyp. Dose has no effect on	Sig* Level	Remarks
Females 120 Days:		
Bili R	.61	No effect
Body Wt.	.35	Extremely weak evidence of an effect
Uter. Wt.	.95	No effect
Uter. Wt. Body	.74	No effect

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83-3
MRID 407017

January 21, 1987

006559

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: Marcia Van Gemert

Date: 1.21.87

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

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. RO9483 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

006559

IDENTITY OF PRODUCT IMPURITIES NOT INCLUDED

1. CHEMICAL: EL-107: Lilly compound 121607, N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide (85%); [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:

Robin B. Phipps, B.S.
Principal Reviewer
Dynamac Corporation

Signature: G Millicovsky for
Date: 1/20/87

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

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Date: 1/20/87

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Teratogenicity and Reproductive Effects
Technical Quality Control
Dynamac Corporation

Signature: G Millicovsky
Date: 1/20/87

Margaret L. Jones
EPA Reviewer

Signature: _____
Date: _____

Marcia Van Gemert, Ph.D.
EPA Section Head

Signature: _____
Date: _____

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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.

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 OER 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 nonpregnant 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

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

[a]Corrected weight gain = total weight gain - gravid uterine weight.

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

TABLE 2. Mean Maternal Food Consumption (g/day ± 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±0.3	20.8±0.6	25.7±0.7	24.3±0.8
100	19.6±0.4	22.4±0.5*	25.4±0.8	24.5±0.7
320	19.7±0.4	21.3±0.4	25.5±0.7	24.6±0.8
1000	19.8±0.5	21.3±0.5	24.8±0.7	24.4±0.8

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

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

[a]Calculated by the reviewers from the individual animal data.

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

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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, distribu- tion, 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.

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

	Dosage (mg/kg/day)			
	0	100	320	1000
	Fetuses (Litters) Examined			
Examination Type				
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)
	Fetuses (Litters) Affected			
External				
Runt	1 (1)	1 (1)	2 (2)	4 (4)
Visceral				
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]
Skeletal				
Incomplete development of sternebrae	0	0	2 (2)	2 (2)
Incomplete development of/or absent vertebral centra	23 (13)	33 (16)	18 (10)	21 (13)

[a]Same 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

	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

[a]Includes fetuses with developmental variations.

[b]Includes runts and malformed fetuses.

APPENDIX A

Protocol

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83-3
MRID NO 00655J

EPA: 68-02-4225
DYNAMAC No. 261-F1
January 28, 1987

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. B03383 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 Corpcration

Signature: I Cecil Felkner

Date: 1-28-87

IDENTITY OF PRODUCT IMPURITIES IS NOT INCLUDED

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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 (85.0%). [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. 803383 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: 1-25-87

Guillermo Millicovsky, Ph.D.
Independent Reviewer
Dynamac Corporation

Signature: G. Millicovsky
Date: 1-28-87

6. APPROVED BY:

I. Cecil Felkner, Ph.D.
Teratogenic and Reproductive Effects
Technical Quality Control
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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 Min'mum.

Item 8--see footnote 1.

9. BACKGROUND: A preliminary study was conducted on nongravid 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):

A. 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 (85.0%), [redacted] 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

IDENTITY OF PRODUCT IMPURITIES 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 sternebrae, 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.

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	2.88 ±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

[a]Mean ± SD.

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

[a]Mean ± SD.

*Significantly different from controls at $p < 0.05$.

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

[a]Mean ± SD.

[b]Calculated by the reviewers from individual animal data.

[c]No. 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
Visceral Malformations:				
Hydrocephaly	1 (6.7)[a]	1 (6.7)	0 (0)	1 (6.3)
Skeletal Variants:				
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 sternebrae	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)

[a]Number 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.

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

Item 15--see footnote 1.

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

APPENDIX A

Protocol

ISOXABEN

Page_____ is not included in this copy.

Pages 194 through 202 are not included.

The material not included contains the following type of information:

____ Identity of product inert ingredients.

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____ Description of the product manufacturing process.

____ Description of quality control procedures.

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

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

IDENTITY OF PRODUCT IMPURITIES IS NOT INCLUDED 006559

1. CHEMICAL: EL-107 is a mixture of N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide (Lilly compound 121607). [redacted]

2. TEST MATERIAL: Technical EL-107 from lot No. H02-2G6-118 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.

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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 (PROTOCOLS):

A. Materials and Methods:

1. Test Material: The test material consisted of two lots of a mixture of three N substituted 5-isoxazolyl-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 F_0, F_1, and F_2 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 F_0 and F_1 generations and all final batches of the diets that were used during the F_1 and F_2 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 F_0, F_1, and F_2 parental generations, two, three, and one breeding trial, respectively, were conducted; litters from the last breeding trial of the F_1 generation (F_{2c}) and the F_2 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 F_1 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

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prior to the random selection of weanlings for the F_2 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 F_2 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 F_2 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 F_2 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 F_2 parental animals. After approximately 70 days on test diets, all animals were mated in the manner described in Section II.A.2a (Materials and Methods). To obtain F_{2c} litters, F_1 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 F_0 and two F_1 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.

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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 F_0, F_1, and F_2 males and F_0 females (Table 1). During the F_1 and F_2 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 F_0 and F_1 generation animals. However, during the F_2 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 F_2 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 F_1 males in the high-dose group (1.25%) were depressed at initiation of the growth period. Body weights of the F_2 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 F_0 or F_1 parental males exposed to the test material during the breeding trials. Body weights and body weight gains of F_1 parental females in the high-dose group and of F_2 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 F_0 generation, significant body weight decreases were observed in females in the high-dose group during the second breeding trial. During the F_1 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 F_0 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 F_0 generation, all males of the mid- and high-dose groups sired

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: 0	70	Mean Body Weight Gain (g)	Mean Daily Food Consumption (g)
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 ($p \leq 0.05$).

[a]Taken from Table 6 of Test Report for Studies R15382, R03783, R14183.

[b]Corresponds 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: 0	Mean Body Weight (g) on GD: 20	Mean Body Weight Gain During Gestation (g)	Mean Body Weight (g) on Postpartum Day 21
F_{1a}	0	260	382	122	317
	0.05	264	374	110	314
	0.25	252	366	114	307
	1.25	257	363	107*	300
F_{1b}	0	312	429	117	346
	0.05	316	432	116	345
	0.25	304	420	116	338
	1.25	289*	381*	92*	318*
F_{2a}	0	291	396	106	319
	0.05	292	399	107	317
	0.25	267*	366*	99	300
	1.25	251*	336*	86*	273*
F_{2b}	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 ($p \leq 0.05$).

[a]Taken from Table 8 of Test Report.

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

Generation	Dietary Concentration (%)	No. Females Mated	No. Females Pregnant	Fertility Index (%)	No. Live Litters Born	Mean No. Live Pups/Litter	Gestation Survival (%)[a]
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	98.5
F_{1b}	0	25	23	92	23	12.8	97.9
	0.05	25	22	88	22	12.6	98.5
	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

[a]Percentage of newborn progeny alive on postpartum day 0.

[b]Reported value was 20.

[c]Taken 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 F_1 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 F_1 males sired at least one litter and at least one pregnancy occurred for every F_1 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 F_1 and F_2 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 F_{1a} 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 0-4	Pup Survival (%) on Postpartum Days 4-21[b]	Pup Body Weight (g) on Postpartum Day 4	Pup Body Weight (g) on Postpartum Day 14	Pup Body Weight (g) on Postpartum Day 21
F_{1a}	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*
F_{1b}	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*
F_{2a}	0	87.6	99.5	9.2	24.7	36.5
	0.05	92.0	98.3	9.3	24.5	36.1
	0.25	93.7	98.6	9.1	22.6*	33.6
	1.25	95.5	99.3	8.4	20.1*	27.2*
F_{2b}	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*

[a]Taken from Tables 12 and 14 of Test Report.

[b]Litters were culled on day 4 to a maximum of 10 pups (5 males and 5 females, when possible).

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

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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 F_0 and F_1 parental male rats in the high-dose group (Table 6). The mean relative liver weights were significantly increased among F_0 females from the mid-dose group and in F_1 females from the mid- and high-dose groups.

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)

[a]Taken 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 M	F_0 F	F_1 M	F_1 F	F_2 M	F_2 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 ($p \leq 0.05$).

[a]Taken from Table 23 of Test Report.

[b]Correspond 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 F_0 and F_1 adults, only tissues from the control and high-dose groups were examined microscopically.

In the F_2 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 (F_2 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 F_1 generation were significantly decreased in the high-dose group (Table 7). During the F_2 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 F_1 and F_2 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 F_1 animals.

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: 0	7	14	20	Mean Total Body Weight Gain (g)
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 ($p \leq 0.05$).

[a]Taken from Table 9 of Test Report.

[b]Corresponds to 0, 500, 2500, and 12,500 ppm.

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

Generation	Dietary Concentration (%)[g]	No. Females Mated	No. Females Pregnant	Pregnant Rate(%)[b]	Mean No. Corpora Lutea	Mean No. Implantations	Preimplantation Loss (%)[c]	Mean No. Live Fetuses	Post-implantation Loss (%)[d]	Mean No. Resorptions	Mean Fetal Body Weight (g) Male	Mean Fetal Body Weight (g) Female	Male Sex Ratio (%)
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[h]	12.1[h]	9.6	9.6[h]	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[h]	11.3[h]	12.2	9.1[h]	21.6	2.1	3.7	3.4	52
	1.25[e]	8	7	88	12.6[h]	11.1[h]	13.0	7.7[h]	30.8	3.4	3.9	3.6	44
	0[f]	10	10	100	14.0	13.6	5.8	12.6	6.9	1.0	3.8	3.7	49

[a] Taken from Tables 20 and 22 of Test Report.

[b] No. females pregnant/No. females mated x 100.

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

[d] Recalculated by the reviewers from individual animal data using (No. implantations - No. live fetuses)/No. implantations x 100.

[e] Additional animals with microphthalmia were mated to produced offspring.

[f] Additional animals with linear retinal dysplasia were mated to produceloffspring.

[g] Corresponds to 0, 500, 2500, and 12,500 ppm, respectively.

[h] Significantly different from controls ($p \leq 0.05$).

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. Geneology: The geneologies 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

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

[a] Taken from Table 22 of Test Report.

[b] Corresponds to 0, 500, 2500, and 12,500 ppm, respectively.

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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)

[a] Taken from Table 21 of Test Report.

[b] Included are additional groups with eye defects.

[c,d,e,f] Same superscript indicates same fetus.

[g] Corresponds to 0, 500, 2500, and 12,500 ppm, respectively.

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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 micropthalmia 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:

Item	Study Authors	Reviewers
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 F_0 and F_1 parental generations and final batches at the termination of the F_1 and F_2 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.

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.

APPENDIX A

Protocol

ISOXABEN

Page_____ is not included in this copy.

Pages 228 through 270 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.

X FIFRA registration data.

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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: Margaret Jones 1/16/87
Section III, Tox. Branch (TS-769C)
Secondary reviewer: Marcia Van Gemert, Ph.D. 1/21/87
Section III, Tox. Branch (TS-769C)

00655[illegible]

DATA EVALUATION REPORT

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

ACCESSION NUMBER: 265737, 265738 MRID NO.: 00[illegible]

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 B6C3F$_1$ Mice

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

REPORT ISSUED: November 1985

CONCLUSIONS: Isoxaben was administered for 24 months to B6C3F$_1$ 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)

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IDENTITY OF PRODUCT IMPURITIES NOT INCLUDED

A. MATERIALS:

1. Test compound: EL-107, a mixture of isomers consisting primarily of Lilly compound 121607, [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 24 months* male	female	Replicate Study M00983 24 months** 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 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.

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.

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)

	Males	Females
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.

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 B6C3F$_1$ Mice
(Summary of Antemortem Observations)

Dose (ppm)	0	100	1000	12500
Males (No. exam.)	60	60	60	60
Opacity - eye(s)	3	4	3	1
Eye or eyes swollen	3	5	3	2
Females (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		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 ($p \leq 0.05$). 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 ($p \leq 0.05$). 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 ($p \leq 0.05$) 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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Hematolcgy Effects in Mice Fed EL-107 for Two Years

Males	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*

Females	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

X		X	
	Electrolytes:		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($p \leq 0.05$). 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 ($p \leq 0.05$). Creatinine was significantly higher at 1000 ppm ($p \leq 0.05$).

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

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.

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.

X		X		X	
	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 were attached)	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

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.

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

Dose (ppm) Males	Glucose (mg/dl)	Creatinine (mg/dl)	T.Bili (mg/dl)	ALP (IU/L)	ALT (IU/L)
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*

Females	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.

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 B6C3F$_1$ Mice (MC0809)[a]

Dose (ppm)	0	100	1000	12500
Males				
No. examined	60	59	60	59
Hepatocellular-				
Cytomegaly	9	2	7	33
Hyperplasia	19	11	16	27
Vacuolation	5	5	10	22
Females				
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 B6C3F$_1$ Mice

Dose (ppm)	0	100	1000	12500
Males				
minimal vacuolation	3	0	1	1
slight vacuolation	2	3	4	13
moderate vacuolation	0	2	5	8
Total/males	5	5	10	22
Females				
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 B6C3F$_1$ mice. These findings support the following neoplastic observations.

Males- Terminal sacrifice- Kidney weight with adrenals was higher than controls at 100 ppm and 1000 ppm ($p \leq 0.05$ 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 ($p \leq 0.05$).

Females- Terminal sacrifice- Liver weight and relative liver weight were significantly higher than controls at 12500 ppm ($p \leq 0.05$). 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 $B6C3F_1$ Mice[a] fed EL-107 for Two Years

Dose (ppm)	0	100	1000	12500
Males				
No. examined	60	59	60	60
nodules	26	15	21	35
lesions	6	4	7	9
Females				
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).

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.

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 $B6C3F_1$ Mice[a]

Dose (ppm)	0	100	1000	12500
Males				
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
Females				
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).

ISOXABEN

Page_____ is not included in this copy.

Pages 286 through 311 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.

X 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.

IDENTITY OF PRODUCT IMPURITIES NOT INCLUDED 006559

A. MATERIALS:

1. Test compound: EL-107, a mixture of isomers consisting of Lilly compound 121607 (85%) [redacted]

Description- not given,
Batch # 710025, 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 %	RO 1583 24 months male	RO 1583 24 months female	RO 1683 24 months male	RO 1683 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 wre 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 93% 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.

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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. 003732
Chief, Tox. Branch (TS-769C) 1/15/87

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): RO 1583, RO 1663

SPONSOR: Elanco 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 61.8 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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3. Animals received food, mash feed of Purina Certified Rodent Chow No 5002, and water ad libitum.

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 deatns 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.

5. 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.

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	
Males:	BUN mg/Dl	Creatinine mg/dl	BUN mg/Dl	Creatinine mg/Dl
Group				
1	17.26	0.6	15.37	0.642
SD	2.87	0.17	2.24	0.124
2	22.54	0.732	17.5	0.767
SD	10.61	0.197	0.7	0.186
3	20.35*	0.712	16.9	0.8
SD	4.16	0.206	3.68	0.194
4	28.81*	0.015*	20.36*	1.043*
SD	7.86	0.426	4.19	0.294
Females				
1	22.8	0.535	13.26	0.569
SD	37.6	0.443	2.09	0.170
2	14.7	0.437	14.32	0.518
SD	4.4	0.154	2.73	0.117
3	14.4	0.433	15.51*	0.553
SD	1.7	0.102	2.19	0.106
4	19.8*	0.479	17.82*	0.662
SD	13.1	0.204	5.88	0.146

* $p > 0.05$

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

* Required for chronic studies

b. Clinical Chemistry

X	Electrolytes:	X	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 RQ 1583 appeared to have decreased alkaline phosphatase levels.

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

Group	Prostate g/100g
1	0.396
SD	0.137
2	0.449
SD	0.183
3	0.375
SD	0.190
4	0.297*
SD	0.098

Study RO 1683
Males

Group	Liver g/100g
1	12.469
SD	2.025
2	13.229
SD	3.983
3	13.135
SD	2.133
4	13.882*
SD	1.792

Table IV

TERMINAL ORGAN WEIGHTS RELATIVE TO BODY WEIGHTS

Study RO 1583

Males:

	Liver g/100g	Kidneys g/100g	Heart g/100g	Brain g/100g
Group 1	2.923	0.6569	0.3152	0.463
SD	0.857	0.0970	0.0744	0.069
2	2.887	0.6688	0.3026	0.444
SD	0.503	0.0914	0.0707	0.052
3	3.031	0.6608	0.3082	0.462
SD	0.511	0.1269	0.0739	0.095
4	3.692*	0.8181*	0.3608*	0.526*
SD	0.938	0.1410	0.0160	0.040

° 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		X		X	
	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*
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*#
	.Rectum*	XX	.Testes*†		.Parathyroids*††
XX	.Liver*†		Epididymides	XX	.Thyroics*††
	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-rodent 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 1583 showed a significant increase in liver weights. These data are presented on table III.

Organ/body weight ratios:

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High dose males in both replicates had increased organ/body weight ratios for liver, kidney, heart and brain. Replicate 1583 also showed an increase in high dose male adrenal/body weight ratios.

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Table V

TERMINAL ORGAN WEIGHTS RELATIVE TO BRAIN WEIGHTS

Study RO 1583, Males Group	Prostate g/g
1	0.199
SD	0.073
2	0.222
SD	0.089
3	0.188
SD	0.096
4	0.148*
SD	0.049

Study RO 1683 Males Group	Liver g/g	Kidneys g/g
1	6.100	1.4363
SD	0.955	0.1543
2	6.527	1.4939
SD	2.004	0.1605
3	6.461	1.4868
SD	0.989	0.1637
4	6.892*	1.6874*
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, 33/60, 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 33/60 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-1075 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 ongross examination. So it is unclear how these parathyrodis 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.

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Table IV - cont.

Study RO 1683

Males

	Liver g/100g	Kidneys g/100g	Heart g/100g	Brain g/100g	Adrenals g/100g
Group 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	8.82	31.23	0.570
SD	0.400	0.0909	3.44	7.97	0.063
2	2.636	0.6376	8.51	32.98	0.546
SD	0.315	0.0480	3.11	7.57	0.048
3	2.833	0.6603	9.35	33.34	0.592
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 VII

Neoplastic Findings

Groups		males 1	2	3	4	females 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 61.3 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 phenomen found in animals with progressive glomerulonephrosis, however, this should have been more vigorously investigated.

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 RO1583 and RO1683

Males					Females			
Groups	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

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Pages 325 through 347 are not included.

The material not included contains the following type of information:

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____ 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.

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84-2
MRID 00502c
genotoxicity 84-2

TB 7-0037
(7-0139)

TOXICOLOGY BRANCH: DATA REVIEW

006559

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

Accesion 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 polychromatic 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 sigificant 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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Primary Reviewer: Irving Mauer, Ph.D.
Toxicology Branch
Hazard Evaluation Division
Secondary Reviewer: [signature] 1/8/87

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 (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

Test Material: EL-107 technical (Lot #Z10025), 95.5% ai, a mixture of isomers (Lilly Compounds: 121607, 85%; [redacted] incorporated into feed for dietary administration.

TB Conclusions/Evaluation: IDENTITY OF PRODUCT IMPURITIES IS NOT INCLUDED

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

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

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* 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 [F_2] 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).

Although the HDT (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.

Attachments

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

TABLE 9. SUMMARY OF REPRODUCTION PARAMETERS FOR UNTREATED FEMALE RATS MATED WITH MALE RATS GIVEN DIETS CONTAINING EL-107. MALE EFFECT AND DOMINANT LETHAL STUDY R01984.

EL-107 Dietary Concentration (%)	Mating Week	Females with Implantations (n)	Females with Implantations (%)	Corpora Lutea (n)	Corpora Lutea Mean ± SE	Implantations (n)	Implantations Mean ± SE	Dead Implants (n)	Dead Implants Mean ± SE	Preimplantation Loss ± SE (%)[a]	Postimplantation Loss ± SE (%)[b]
0	1	18	72	313	17.4 ± 0.6	284	15.8 ± 0.6	14	0.78 ± 0.15	9.02 ± 1.98	4.94 ± 1.00
	2	14	56	257	18.4 ± 0.8	222	15.9 ± 1.2	16	1.14 ± 0.25	11.98 ± 5.99	6.84 ± 1.70
0.05	1	19	76	316	16.6 ± 0.7	281	14.8 ± 0.8	27	1.42 ± 0.25	12.31 ± 4.32	9.37 ± 1.70
	2	16	64	266	16.6 ± 0.8	232	14.5 ± 0.6	21	1.31 ± 0.49	11.84 ± 2.77	9.63 ± 3.62
0.25	1	24	96	426	17.8 ± 0.7	385	16.0 ± 0.8	32	1.33 ± 0.29	11.05 ± 3.65	8.36 ± 2.27
	2	21	84	327	16.4 ± 0.5	296	14.1 ± 0.9	28	1.33 ± 0.23	9.69 ± 2.94	14.16 ± 4.69
1.25	1	19	76	325	17.1 ± 0.6	308	16.2 ± 0.5	22	1.16 ± 0.21	6.03 ± 1.56	7.31 ± 1.41
	2	17	68	278	16.4 ± 0.5	264	15.5 ± 0.5	25	1.47 ± 0.31	5.54 ± 1.32	9.16 ± 1.91

[a] Preimplantation Loss (%) = $\frac{\text{Corpora Lutea} - \text{Implantation Sites}}{\text{Corpora Lutea}} \times 100$

[b] Postimplantation Loss (%) = $\frac{\text{Dead Implants}}{\text{Implantation Sites}} \times 100$

TABLE 10.1 ANATOMICAL ANOMALIES IN FETUSES FROM UNTREATED FEMALE RATS MATED WITH MALE RATS GIVEN DIETS CONTAINING EL-107.
MALE EFFECT AND DOMINANT LETHAL STUDY R01984. MATING WEEK 1.

	EL-107 Dietary Concentration (%) Male Treatment			
	0.0	0.05	0.25	1.25
	Fetuses (Litters)[a] Examined			
	249 (17)	254 (19)	317 (22)	268 (18)
	Fetuses (Litters) Affected			
External Examination	0	0	0	0
Visceral Examination (Heads Only)				
Microphthalmia (Unilateral)	0	0	1 (1)	0

[a] Litters with live fetuses.

TABLE 10.2 ANATOMICAL ANOMALIES IN FETUSES FROM UNTREATED FEMALE RATS MATED WITH MALE RATS GIVEN DIETS CONTAINING EL-107.
MALE EFFECT AND DOMINANT LETHAL STUDY RO1984. MATING WEEK 2.

	EL-107 Dietary Concentration (%) Male Treatment			
	0.0	0.05	0.25	1.25
	Fetuses (Litters)[a] Examined			
	204 (13)	211 (16)	268 (20)	239 (17)
	Fetuses (Litters) Affected			
External Examination				
Edema	0	1 (1)[b]	0	1 (1)
Open Eyelid (Unilateral)	0	1 (1)	0	0
Visceral Examination (Heads Only)				
Microphthalmia (Unilateral)	1 (1)	1 (1)[b]	0	0

[a] Litters with live fetuses.

[b] Same fetus.

TABLE 11.1 FETAL PARAMETERS FROM UNTREATED FEMALE RATS MATED WITH MALE RATS GIVEN DIETS CONTAINING EL-107.
MALE EFFECT AND DOMINANT LETHAL STUDY R01984. MATING WEEK 1.

	EL-107 Dietary Concentration (%) Male Treatment			
	0	0.05	0.25	1.25
	Mean[a] ± S.E.			
Male sex ratio (%)	48.6 ± 3.92	50.2 ± 3.0	45.3 ± 6.4	48.2 ± 2.4
Female sex ratio (%)	51.4 ± 3.9	49.8 ± 3.0	54.7 ± 6.4	51.8 ± 2.4
Male fetal weight (g)	4.04 ± 0.07	4.07 ± 0.08	3.79 ± 0.10	4.11 ± 0.11
Female fetal weight (g)	3.83 ± 0.07	3.90 ± 0.08	3.75 ± 0.14	3.97 ± 0.10
Fetal runts (%)				
Male	0	0.9 ± 0.9	4.2 ± 3.2	0.6 ± 0.6
Female	0	0	3.8 ± 2.5	0
Normal fetuses (%)				
Male	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0	100.0 ± 0.0
Female	100.0 ± 0.0	100.0 ± 0.0	99.1 ± 0.9	100.0 ± 0.0
Variant fetuses (%)				
Male	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Female	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Abnormal fetuses (%)				
Male	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Female	0.0 ± 0.0	0.0 ± 0.0	0.9 ± 0.9	0.0 ± 0.0

[a] All values calculated on a per litter basis.

TABLE 11.2 FETAL PARAMETERS FROM UNTREATED FEMALE RATS MATED WITH MALE RATS GIVEN DIETS CONTAINING EL-107.
MALE EFFECT AND DOMINANT LETHAL STUDY R01984. MATING WEEK 2.

	EL-107 Dietary Concentration (%) Male Treatment			
	0	0.05	0.25	1.25
	Mean[a] ± S.E.			
Male sex ratio (%)	49.9 ± 3.5	47.3 ± 4.0	47.4 ± 4.1	51.4 ± 3.3
Female sex ratio (%)	50.1 ± 3.5	52.8 ± 4.0	52.6 ± 4.1	48.6 ± 3.3
Male fetal weight (g)	3.85 ± 0.05	4.37 ± 0.21	3.98 ± 0.06	3.86 ± 0.08
Female fetal weight (g)	3.64 ± 0.05	4.04 ± 0.18	3.75 ± 0.07	3.75 ± 0.08
Fetal runts (%)				
Male	0	0	0	2.1 ± 1.5
Female	0.8 ± 0.8	0	2.8 ± 1.1	0
Normal fetuses (%)				
Male	100.0 ± 0.0	99.0 ± 1.0	100.0 ± 0.0	99.2 ± 0.8
Female	98.9 ± 1.1	99.5 ± 0.5	100.0 ± 0.0	100.0 ± 0.0
Variant fetuses (%)				
Male	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Female	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Abnormal fetuses (%)				
Male	0.0 ± 0.0	1.0 ± 1.0	0.0 ± 0.0	0.8 ± 0.8
Female	1.1 ± 1.1	0.5 ± 0.5	0.0 ± 0.0	0.0 ± 0.0

[a] All values calculated on a per litter basis.

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006559 MRID 41522

gene mutation
84-2

TB 7-0037
(0337)

TOXICOLOGY BRANCH: DATA REVIEW

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: 07-7441

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 ug/plate (% survival = 109% and 113%, respectively, of solvent control), but dose-related increased precipitation was evident at doses of 500 ug/plate and above. Hence, 500 ug/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

01-07-87

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

[a] Mean ± 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] This plate was believed to have been inoculated twice; therefore, this value is the mean of two plates.
[f] Plate was counted due to presence of a chemical precipitate.

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TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Biliary excretion of radiocarbon in rats after single dose.

Accession Number: 250791(7)

MRID Number: mS cof

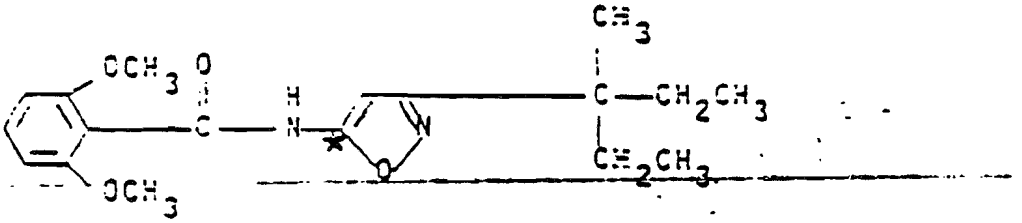
Sponsor: Eli Lilly and Company

Contracting Lab: Lilly Research Laboratories, Nos. R10582 & R11482

Date: December, 1981

Test Material: N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide, EL-107 (92.4%) and ^{14}C-EL-107 (100%)

Chemical Structure:



*Indicates position of radiocarbon atom.

Protocol: "Single oral doses of [EL-107 spiked with ^{14}C-EL-107] were given to male and female Fischer 344 rats with exteriorized bile duct cannulae and the amount of radioactivity excreted into bile at 24-hours after dosing was determined. Five rats per sex were studied at 10 and 250 mg/kg to determmine if biliary excretion was dose dependant."

Results: Percent-of-dose biliary excretions are shown in Table 1 which was excerpted from the Eli Lilly raport.

Discussion and Conclusion: Note the reduction in percent biliary excretion with increasing dose, especially in females. The decreased percent biliary excretion rates appear to be related to a rate-limiting gastro-intestinal absorption process, which is also indicated by the urine and fecal excretion studies (R07282 & R08882).

The biliary excretion difference between sexes is unexplained.

Core Classification: Acceptable

TABLE 1

BILIARY EXCRETION OF RADIOCARBON FROM FISCHER 344 RATS GIVEN A SINGLE ORAL DOSE OF ^{14}C-EL-107

STUDIES R10582 and R11482

Animal Number	Study Number	Dose Group	Sex	Percent of Dose 0-24 Hr.
1001	R10582	10.0	M	16.9
1002			M	13.5
1003			M	27.8
1004			M	44.1
1005			M	13.4
Mean				23.1
S.E.				5.9
1051	R10582	10.0	F	39.8
1052			F	13.7
1053			F	38.6
1054			F	51.9
1055			F	49.8
Mean				38.8
S.E.				6.8
2001	R11482	250.0	M	23.6
2002			M	28.4
2003			M	11.8
2004			M	11.8
2005			M	11.8
Mean				17.5
S.E.				3.6
2051	R11482	250.0	F	20.7
2052			F	8.5
2053			F	26.3
2054			F	14.0
2056			F	6.9
Mean				15.3[a]
S.E.				3.7

[a] Significantly different from 10 mg/kg female group. Students t-test ($P \leq 0.05$).

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TOXICOLOGY BRANCH
DATA REVIEW

Study Type: Radiocarbon excretion by rats after single oral doses.

Accession Number: 250791(8)

MRID Number: M5205

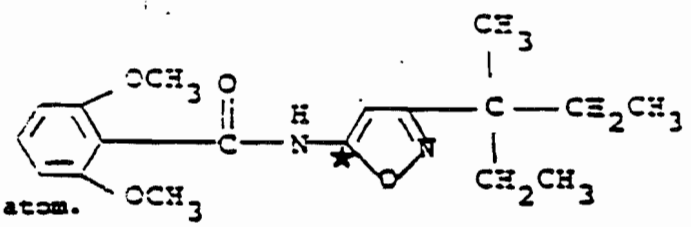
Sponsor: Eli Lilly and Company

Contracting Lab: Lilly Research Laboratories, Nos. R07282 & R08882

Date: December, 1981

Test Material: N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide, EL-107 technical (92.4%) and ^{14}C-EL-107 (100%)

Protocol: "A sample of lot B31-72C-88 with a purity of 92.4% was used in this study. ^{14}C-EL-107, labeled in the isoxazole ring, was also used and was shown to have a radiochemical purity of 100% by thin layer chromatography and a specific activity of 11.21 uCi/mg. The structure of EL-107 is shown below:



*Indicates position of radiocarbon atom.

The dosage levels and extent of radioisotope spiking are shown in the following table.

*Animal Number	Dose	^{14}C Dose	Dose Volume
1051-1055	10 mg/kg	14 uCi/kg	10 ml/kg
2051-2055	100	"	"
3051-3055	250	"	"
4051-4055	500	"	"
5051-5055	1000	"	"

Doses were given as microsuspensions in 10% acacia solutions.

The urinary and fecal excretion of radioactivity was measured for three successive 24-hour periods.

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Results:

Figures 1 (males) and 2 (females) attached present by bar graph the cumulative percentage excretion of radiolabeled EL-107. These figures were excerpted from Eli Lilly's report.

Discussions and Conclusions: It is noted, from each dosage level that most of the excretion of EL-107 occurred within 24 hours and that very little more was excreted by the 72nd hour.

Fecal excretion was the major elimination route.

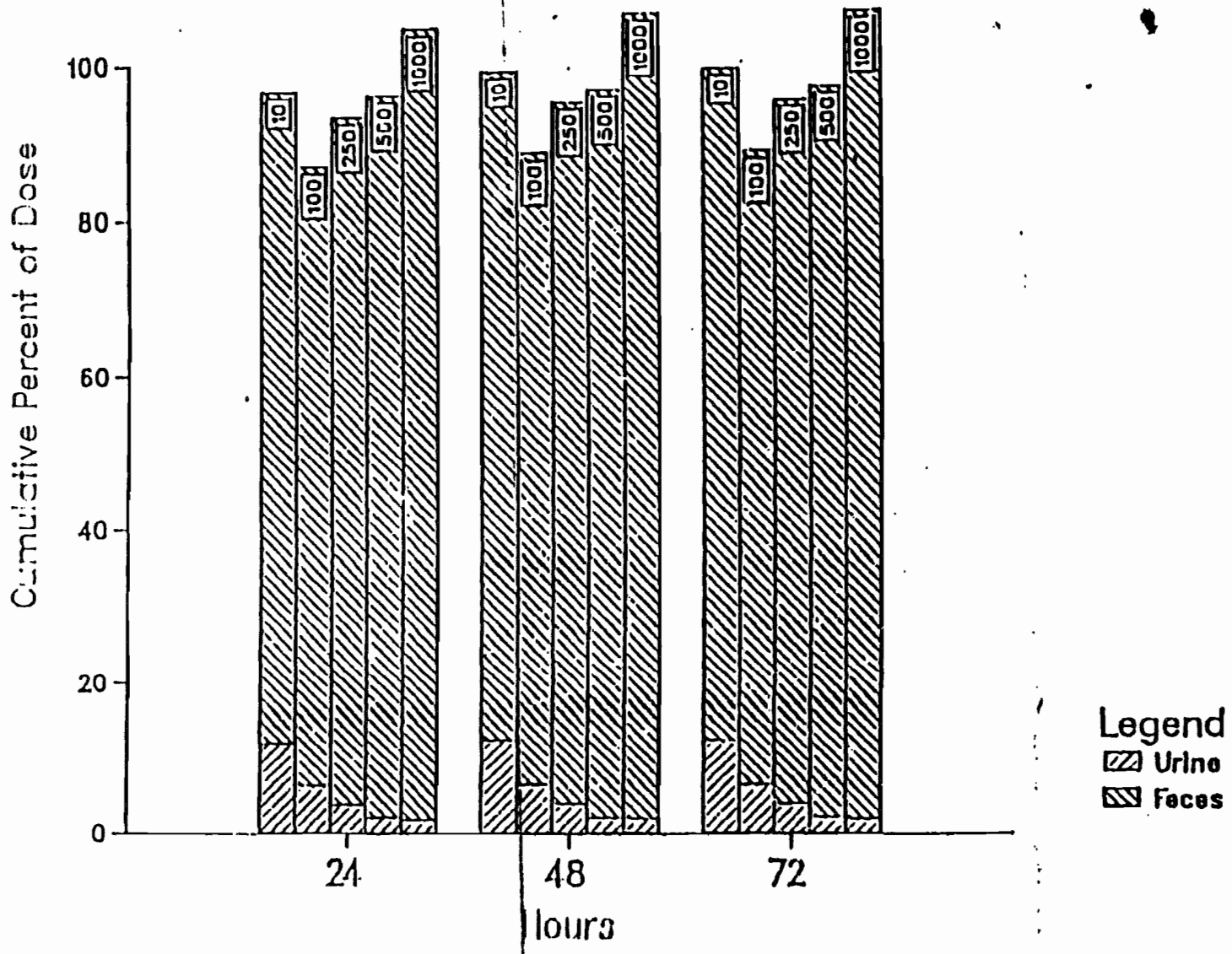
It appears that gastro-intestinal absorption is a rate-limiting process. This is indicated by dose related decreasing ratios of urinary excretion to fecal excretion.

"By the end of the collection periods, the mean percent of the radiolabeled dose excreted in urine and feces ranged from 70.8 to 94.9% in males and from 88.2 to 106.3% in females."

Core Classification: Acceptable

FIGURE 2

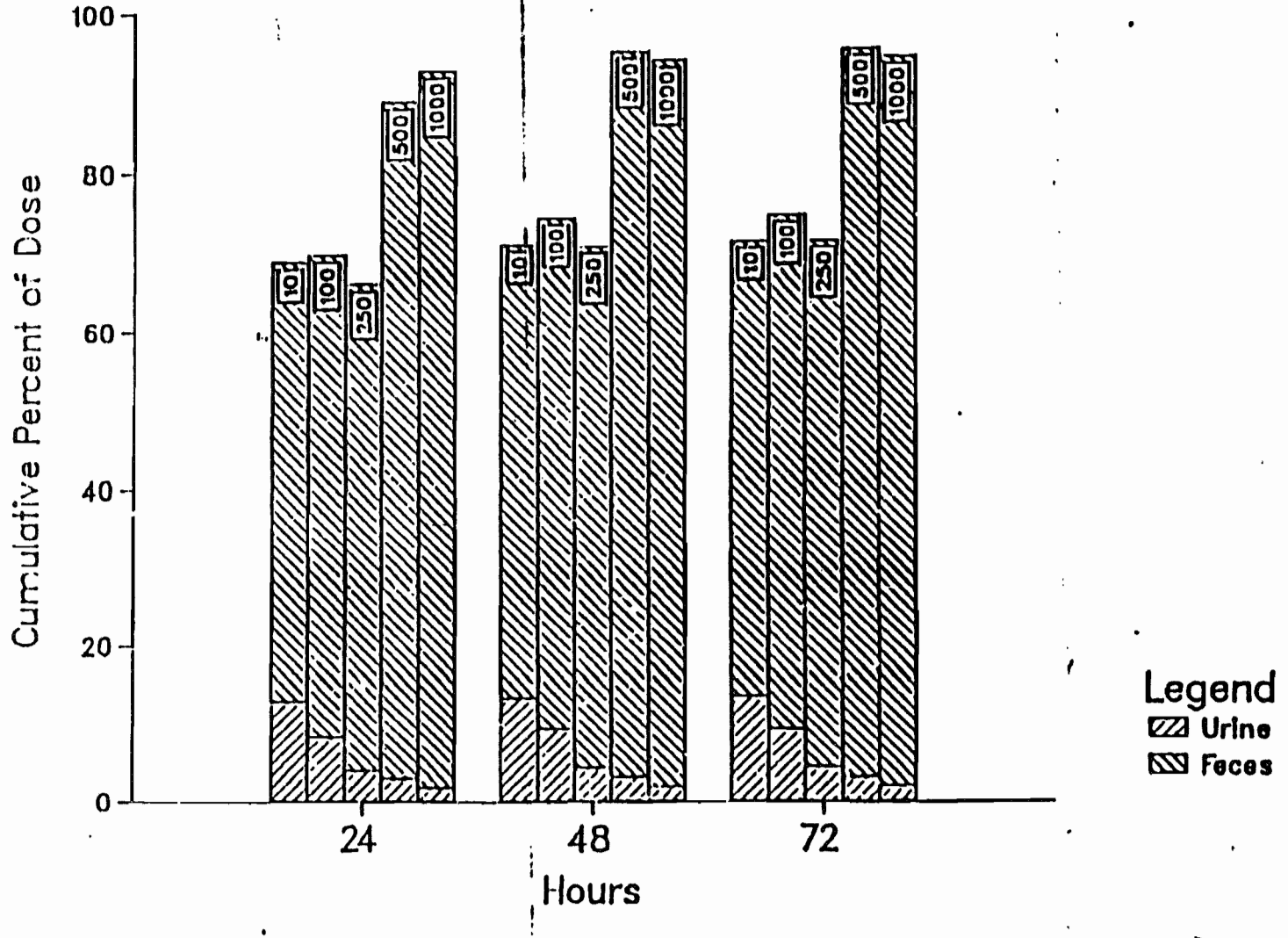
Cumulative Excretion of Radioactivity in Female Rats Receiving a Single Oral Dose of Radiolabeled EL-107. Study R08882.



* Doses Indicated Within Bars are in mg/kg

FIGURE 1

Cumulative Excretion of Radioactivity in Male Rats Receiving a Single Oral Dose of Radiolabeled EL-107. Study R07282.



* Doses Indicated Within Bars are in mg/kg

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TOXICOLOGY BRANCH

DATA REVIEW

Study Type: Tissue distribution of radiocarbon in rats after single oral doses

Accession Number: 250791 (9)

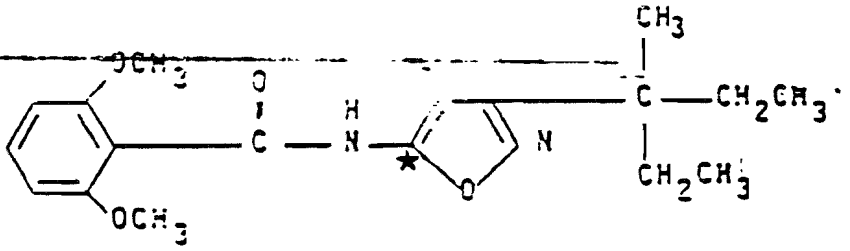
MRID Number: M5006

Sponsor: Eli Lilly and Company

Contracting Lab: Lilly Research Laboratories, Nos. R05082 & R06182.

Date: December 1982

Test Material: N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide, EL-107 (92.4%) and ^{14}C-EL-107 (100%)
The structure of EL-107 is shown below:



*Indicates position of radiocarbon atom.

Protocol:

"Rats were given single oral doses of radiolabeled EL-107 equal to 250 mg/kg. Each animal received a constant volume of dose suspension based on body weight (10 ml/kg). A total of five rats per sex per sampling time was utilized. After dosing, the rats were placed in individual cages and allowed free access to water. Food was withheld until four hours after dosing.

At 4 or 24 hours after dosing the animals were anesthetized with ether and after laparotomy a blood sample was obtained from the abdominal aorta. The animals were killed by cervical dislocation, and tissues and organs were removed."

"The microgram equivalents of [^{14}C]-EL-107 per gram of each tissue (or per ml for plasma) and the tissue to plasma ratios were calculated."

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Results:

Tissue levels of radiocarbon measured in male and female rats are presented in Tables 1 and 2 respectively (as excerpted from the report).

Discussions and Conclusions:

When comparing concentrations found after 24 hours with those found after 4 hours, it was noticed that there were large increases in ratios of tissue to plasma levels (whether expressed as MCG-EQ/G or as % increases) in adrenals, eyes, thyroid, pituitary, and ovaries. There were also increases of lesser amounts of concentration in some other organs. The largest increases were in female pituitaries, from a ratio of 1.75 to 20.55, an increase of 18.80 MCG-EQ/G or 1074%.

Differences between sexes were striking; the larger changes were found in females. For example the average relative increase in male pituitaries was from 12.09 to 14.71, 2.02 MCG EQ/G or 15.9% (refer to female results above.)

Twenty-four hours was not long enough for maximum values to be demonstrated in several organs or for decreases in the organs to be observed.

A longer-term distribution study could better elucidate persistence and storage in organs.

Core Classification: Acceptable

TABLE 1. CONCENTRATION AND TISSUE-TO-PLASMA RATIOS OF RADIOACTIVITY IN TISSUES FROM MALE RATS GIVEN ORAL DOSES OF 250 MG/KG OF RADIOLABELED 121607. STUDY R05052

	HOURS AFTER DOSING					
	4			24		
TISSUE	MCG-EQ/G		TISSUE/ PLASMA	MCG-EQ/G		TISSUE/ PLASMA
LIVER	23.75 ±	1.672	14.63	3.78 ±	0.272	7.37
KIDNEY	8.07 ±	0.925	4.93	2.06 ±	0.233	4.03
BRAIN	0.65 ±	0.033	0.41	0.16 ±	0.023	0.31
HEART	1.20 ±	0.037	0.75	0.30 ±	0.060	0.58
LUNG	1.93 ±	0.535	1.13	0.37 ±	0.030	0.71
ADRENALS	4.66 ±	0.386	2.91	1.70 ±	1.003	3.80
EYES	1.24 ±	0.193	0.77	0.43 ±	0.053	0.86
SPLEEN	1.08 ±	0.054	0.67	0.30 ±	0.030	0.59
PLASMA *	1.64 ±	0.146	1.00	0.52 ±	0.029	1.00
MUSCLE	0.74 ±	0.092	0.48	0.22 ±	0.018	0.43
FAT	2.00 ±	0.108	1.27	0.47 ±	0.030	0.91
DUODENUM	8.40 ±	0.592	5.22	1.30 ±	0.184	2.61
JEJUNUM	17.84 ±	3.931	10.96	1.93 ±	0.089	3.81
ILEUM	63.85 ±	19.066	40.37	3.34 ±	0.643	6.71
COLON	21.49 ±	11.414	13.19	4.09 ±	0.805	7.83
THYROID	27.03 ±	19.931	18.27	2.11 ±	0.316	4.15
THYMUS	0.69 ±	0.017	0.43	0.25 ±	0.021	0.49
PANCREAS	1.31 ±	0.061	0.82	0.36 ±	0.020	0.71
PITUITARY	18.02 ±	7.557	12.69	7.82 ±	3.903	14.71
PROSTATE	10.27 ±	5.730	5.69	1.15 ±	0.361	2.31
TESTES	0.51 ±	0.022	0.32	0.17 ±	0.009	0.34

* PLASMA VALUE EXPRESSED AS MCG-EQ/ML

EACH VALUE IS EXPRESSED AS THE MEAN ± S.E. FOR N=5 RATS

TABLE 2. CONCENTRATION AND TISSUE-TO-PLASMA RATIOS OF RADIOACTIVITY IN TISSUES FROM FEMALE RATS GIVEN ORAL DOSES OF 250 MG/KG OF RADIOLABELED 121607. STUDY R06102

	HOURS AFTER DOSING					
	4			24		
TISSUE	MCG-EQ/G		TISSUE/ PLASMA	MCG-EQ/G		TISSUE/ PLASMA
LIVER	25.40 ±	1.836	11.06	3.49 ±	0.561	8.04
KIDNEY	10.40 ±	0.800	4.49	2.20 ±	0.365	4.92
BRAIN	0.67 ±	0.051	0.29	0.48 ±	0.121	0.97
HEART	2.43 ±	0.426	1.09	0.61 ±	0.129	1.35
LUNG	1.86 ±	0.297	0.82	0.56 ±	0.112	1.19
ADRENALS	5.79 ±	1.262	2.48	2.87 ±	0.540	6.50
EYES	0.67 ±	0.066	0.29	1.05 ±	0.298	2.28
SPLEEN	1.31 ±	0.102	0.57	0.57 ±	0.087	1.37
PLASMA *	2.32 ±	0.132	1.00	0.47 ±	0.096	1.00
MUSCLE	3.30 ±	2.013	1.39	0.36 ±	0.060	0.80
FAT	5.98 ±	0.480	2.58	1.16 ±	0.212	2.65
DUODENUM	8.91 ±	0.557	3.89	1.63 ±	0.465	3.35
JEJUNUM	22.25 ±	7.391	9.17	2.50 ±	0.572	5.65
ILEUM	56.78 ±	7.577	25.02	3.64 ±	0.700	8.34
COLON	7.18 ±	2.892	3.23	5.73 ±	1.057	12.61
THYROID	3.22 ±	0.931	1.36	3.44 ±	0.284	8.54
THYMUS	1.48 ±	0.162	0.65	0.35 ±	0.050	0.79
PANCREAS	2.57 ±	0.230	1.11	0.42 ±	0.075	0.92
PITUITARY	4.28 ±	2.185	1.75	8.53 ±	2.389	20.55
OVARIES	2.24 ±	0.126	0.98	1.58 ±	0.347	3.53
UTERUS	1.43 ±	0.075	0.63	0.84 ±	0.130	1.92

* PLASMA VALUE EXPRESSED AS MCG-EQ/ML

EACH VALUE IS EXPRESSED AS THE MEAN ± S.E. FOR N=5 RATS

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EPA: 68-02-4225
DYNAMAC NO. 261-B3
January 20, 1987

M218 ONJ 223

DATA EVALUATION RECORD

EL-107

Metabolic Study in Rats

STUDY IDENTIFICATION: Magnussen, J. D. and Rainey, D. P. Metabolism of ^{14}C 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.

APPROVED BY:

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

Signature: [signature]

Date: 1-20-87

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

2. TEST MATERIAL: [^{14}C]-EL-107 (lot No. 553-341-056), 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 ^{14}C 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:

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Technical Quality Control
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Date: Jan 20, 1987

Marcia Van Gemert, Ph.D.
EPA Section Head

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Date: 1/21/87

7. CONCLUSIONS:

A. When adult rats were given single oral doses of 250 mg/kg of [^{14}C]-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 [^{14}C] (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.

8. 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 [^{14}C]-EL-107 (specific activity = 0.5 µCi/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. 005. If this rat was excluded as an outlier, the cumulative percent recovery after 72 hours would be 94.8 percent.

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/B-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 Rf values of the major metabolite zones in the various column peaks indicated that for the most part, the same metabolites were present in both

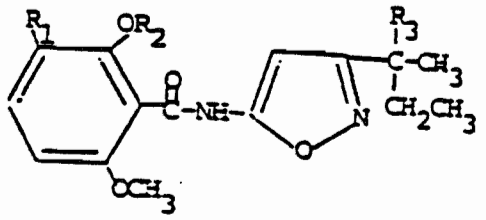
the neutral and aglycone fractions. The major exception to this was the two very polar zones of radioactivity observed in fractions MNF-6 and FNF-6. When these two column fractions were treated by enzyme hydrolysis as described previously for unextractable urinary radioactivity, TLC analysis of radioactivity 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 [^{14}C] now contained the same metabolite spectrum present in the comparable aglycone fraction.

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 [^{14}C].

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 [^{14}C] 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 B_1 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 B_2 represented approximately 16 percent of the [^{14}C] in fraction MAF-3 and 25 percent in FAF-3. It was also a minor component in MNF-1 and FNF-1. Metabolite B_3 represented 20 percent of the [^{14}C] 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 D_1 + D_2 constituted the major [^{14}C] zone in fractions MAF-6 and FAF-6. This major zone represented 70 percent of the [^{14}C] 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 [^{14}C]-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	$-CH_3$	$-CH_2CH_3$
Metabolite A	5-6	1-1.5	-H	-H	$-\overset{O}{\overset{\|}{C}}-CH_3$
Metabolite B_1 + B_2[b] (diastereomers)	7-9	3-4	-H	-H	$-\overset{OH}{\overset{\|}{C}}HCH_3$
Metabolite B_3[b]	2.5-3.5	< 0.2	-H	-H	$-CH_2CH_2OH$
Metabolite C	12-14	10-12	-OH	$-CH_3$	$-\overset{O}{\overset{\|}{C}}CH_3$
Metabolite D_1 + D_2 (diastereomers)	8-9	11-12	-OH	$-CH_3$	$-\overset{OH}{\overset{\|}{C}}HCH_3$
Metabolite E[a]	0.5-1.0	ND[c]	-H	$-CH_3$	$-\overset{O}{\overset{\|}{C}}HCH_3$
Metabolite F[a]	0.5-1.0	ND	-H	$-CH_3$	$-CH_2CH_2OH$
Metabolite G	ND	0.5-1.0	-H	-H	$-CH_2CH_3$
Metabolite H	ND	2.3	-OH	$-CH_3$	$-CH_2CH_3$

[a] Structures supported by comparison to authentic standards.

[b] Structures consistent with available data but not definitive.

[c] Not determined.

13. STUDY AUTHORS' CONCLUSIONS/QUALITY ASSURANCE MEASURES:

When administered to rats in single oral doses, [^{14}C]-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 ST OY 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 [^{14}C] was approximately 100 percent after 72 hours; however, it would have been more convincing had the authors measured the [^{14}C] 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 [^{14}C] 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 [^{14}C] (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 [^{14}C] during extraction could mean that minor differences may exist between the reported results and the true metabolic profile of EL-107.

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.

APPENDIX A

Metabolite Structures and Names

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006559

85-1

Reviewed by: Marcia van Gemert, Ph.D. M. van Gemert 1.16.87
Head, Section III, Tox. Branch (TS-769C)
Secondary reviewer: Theodore M. Farber, Ph.D.
Chief, Tox. Branch (TS-769C)

DATA EVALUATION REPORT

STUDY TYPE: Excretion pattern of EL-107 in mice
TOX. CHEM. NO.: 419F
ACCESSION NUMBER: 265729
MRID NO.: ?

TEST MATERIAL: EL-107

SYNONYMS: Isoxaben

STUDY NUMBER(S): M03082

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:

Within 24 hours 79.4, 88.7,91.7and 81.0% of the doses of 1, 15, 100 and 200 mg/kg respectively were excreted. The authors determined that distribution of EL 107 is altered above 100 mg/kg. This was a quick range-finding study for dose setting in the mouse oncogenicity study, and made no pretense as a full metabolism study.

Classification: core-supplementary

A. MATERIALS:

1. Test compound: EL-107, Batch # 121607, Isomeric complnent 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: males: 19-23 grams

B. STUDY DESIGN:

1. Animal assignment

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

2. Quality assurance statement was not given.

3. Procedures:

Animals were housed 3/cage in stainless steel metabolism cages. Urine and feces were collected once/day for three days. After the first day, cages were rinsed with 25 ml water and assayed for radioactivity. Urine was put directly into liquid scintillation coctail (Beckman ready-solv-MP) and feces homogenates were oxidized and $^{14}CO_2$ effluent was trapped in phenethylamine containing scintillation cocktail, and both urine and feces effluent were assayed for radioactivity in a Beckman liquid scintillation 9000 liquid scintillation spectrometer.

4. Results:

More than 89% of the radioactivity was excreted in the first day for all doses. In table I on appended pages 1 and 2, approximately the same percent was excreted in urine at the 1 and 15 mg/kg doses. However, as the doses increased, proportionately less of the compound was excreted in the urine and more was in the feces, calculated as a percent of the dose administered.

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The study was designed to determine above what dose would the distribution of EL-107 be altered. The authors concluded that this dose would be 100 mg/kg.

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MRID 00165552

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

S5-1

1/15/87

006559

DATA EVALUATION REPORT

STUDY TYPE: Repeated dose distribution study in rats
TOX. CHEM. NO.: 419F
ACCESSION NUMBER: 265740
MRID NO.: ?

TEST MATERIAL: EL-107

SYNONYMS: Isoxaben

STUDY NUMBER(S): R13885

SPONSOR: Elanco

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 ^{14}C 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

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 [redacted]
weight with a combined purity of 93.3%.
IDENTITY OF PRODUCT IMPURITIES IS NOT INCLUDED
Lot number 553-3n1-056 of compound 121607 was used to radiolabel. ^{14}C 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 specitic 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 ^{14}C-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

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

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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% of initial radioactive dose for females. Less than 0.02% of the total radioactivity can be accounted for by the cage washing procedure.

Discussion:

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 when compared to the previous rat metabolism studies ABC-0153 and R11285. 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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006559

85-1

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-769C)

MRID MSC 25

1/15/87

DATA EVALUATION REPORT

STUDY TYPE: Excretion of ^{14}C-EL-107 in expired air

TOX. CHEM. NO.: 419F

ACCESSION NUMBER: 265740

MRID NO.: ?

TEST MATERIAL: EL-107

SYNONYMS: Isoxaben

STUDY NUMBER(S): R02186

SPONSOR: Elanco

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

TITLE OF REPORT: Radiocarbon excretion of $^{14}CO_2$ in the expired air from Fischer 344 rats given single oral doses of EL-107 (121607)

AUTHOR(S): E.G. Gries

REPORT ISSUED: June 1986

CONCLUSIONS: Total radioactivity recovered from all sources, eg. urine and feces, carcass and expired air at 48 hours was 85% for males and 92.5% 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 phenomen 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

IDENTITY OF PRODUCT IMPURITIES 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 [redacted]
94.8%.
weight with a combined purity of 93.3%.

Lot number 553-3n1-056 of compound 121607 was used to radiolabel. ^{14}C 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:

Expired $^{14}CO_2$

Expired $^{14}CO_2$ 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 $^{14}CO_2$ 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 $^{14}CO_2$ 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 abe 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 phenomen found in the other metabolism studies that the firm decided to check expired air for residual $^{14}CO_2$ levels.

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MRID 00559

Reviewed by: Marcia van Gemert, Ph.D. 1/12/87
Head, Section III, Tox. Branch (TS-769C)
Secondary reviewer: Theodore M. Farber, Ph.D.
Chief, Tox. Branch (TS-769C)

95-1

006559

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
Greenfield, Indiana 46140

TITLE OF REPORT: Distribution of Radioactivity into tissues and organs from Fischer 344 rats given single oral doses of ^{14}C 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 ^{14}C- 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 it 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)

IDENTITY OF PRODUCT IMPURITIES IS OR NOT INCLUDED 006559

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 [redacted]

94.8%. The final analysis [redacted] weight with a combined purity of 93.3%.

Radiolabeled and unlabeled material were mixed to yield a 333 mg/kg solution at 0.03uCi/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 anaestnesia, 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		males	
Tissue	4 hrs	24 hrs	4 hrs	24 hrs
Carcass	39.9	19.3	65.62	34.94
Kidney	2.64	2.82	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	21.18	9.47	42.98	25.38
Ileum	185.65	13.99	177.31	37.83
Colon	4.66	11.67	10.70	15.03
Pancreas	1.85	1.38		
Prostate			1.69	1.67

Appenced 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		females	
Tissue	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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MRID 00655959

85-1

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

A. MATERIALS:

1. Test compound: EL-107, Batch # 121607. Isomeric compliment 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

3 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 valus 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.

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