03/08/90

PEER REVIEW FILES

007755

CHEMICAL NAME: Isoxaben
CASWELL NO.: 419F
CAS NO.: 82558-50-7
REVIEWER: Swentzel/Jones

CURRENT AGENCY DECISION

C (HED); D (SAP).

TUMOR TYPE / SPECIES

Hepatocellular adenomas; B6C3F1 mice (M & F).

REVIEWER PEER REVIEW PACKAGE	PEER REVIEW MEETING DATE	PEER REVIEW DOCUMENTS	PEER REVIEW CLASSIFICATION
5. / /	5. / /	5. / /	5.
4. / /	4. / /	4. / /	4.
3. / /	3. / /	3. / /	3.
2. / /	2. 09/29/88	2. 01/04/89	2. C
1. 06/10/87	1. 06/25/87	1. 10/05/87	1. C

SAP MEETING	SAP CLASSIFICATION
2. / /	2.
1. 09/07/88	1. D

QUALITATIVE/QUANTITATIVE RISK ASSESSMENT DOCUMENT	GENETIC TOXICITY ASSESSMENT DOCUMENT
2. / /	1. / /
1. 05/01/87	

MISCELLANEOUS:

Miscellaneous: Memoranda---Revision of Exposure Assessment for Children...6/1/89; Isoxaben Exposure Estimate...3/14/89; and Corrigendum: to the memo dated May 1, 1987...8/31/88. Stamped 2/7/90; #PR-007755; 275 p.; nha.

419F
Carwell ##

Isoxaben File Contents

01/09/90

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2. 01/04/89
1. 10/05/87

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1. 09/07/88

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1. 05/01/87

GENETIC TOXICITY DOCUMENT: / /

REVIEWERS PEER REVIEW PACKAGE FOR: MEETING 3. / /
MEETING 2. / /
MEETING 1. 06/10/87

MISCELLANEOUS:

Miscellaneous: Memoranda---Revision of Exposure Assessment for Children...6/1/89; Isoxaben Exposure Estimate...3/14/89; and Corrigendum: to the memo dated May 1, 1987...8/31/88.

Peer Review Documents
(Memo dates)

C07755

1/4/89

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

JAN 4

FILE COPY

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

Subject: Peer Review of Isoxaben - Reevaluation Following the September 7, 1988 Science Advisory Panel Review

From: K. Clark Swentzel
Section 2, Toxicology Branch 2 (TS-769C)

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

The Peer Review Committee met on September 29, 1988 to evaluate the comments from the Science Advisory Panel (SAP) regarding the Agency's classification of the carcinogenicity of Isoxaben.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

Theodore Farber — Theodore M. Farber

William Burnam — Wm F Burnam

Marcia van Gemert — Marcia van Gemert

Judy Hauswirth — Judith W. Hauswirth

Marion Copley — Marion Copley

Robert Beliles — Robert P Beliles

Esther Rinde — Esther Rinde

Retc Engler — Retc Engler

Kerry Dearfield — Kerry Dearfield

Lynnard Slaughter — L. J. Slaughter

2. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusion of the Committee.)

Richard Hill

Richard Levy — Richard A. Levy

Diane Beal

Jack Quest — Jack F. Quest

3. Reviewer: (Non-committee member responsible for data presentation; signature indicates technical accuracy of panel report.)

K. Clark Swentzel

B. Material Reviewed:

The SAP response (9/14/88) and liver tumor data from a 2-year feeding study in mice (Tables 1 & 2: attached). It should be noted that Table 1 includes revised data (see Peer Review Committee memorandum - Table I, Rinde to Mountfort, October 5, 1987), i.e., the incidences of hepatocellular adenomas and carcinomas, respectively, in high-dose males was 12/48 (not 14/48) and 5/55 (not 3/55).

C. Considerations:

1/ Initial Peer Review:

The Committee's initial classification of the carcinogenicity of Isoxaben (Group C) was based on a statistically significant increase in hepatocellular adenomas in male and female mice; a quantitative estimation of risk was not recommended since the weight of evidence was considered to be limited, based on a statistically significant increase in benign tumors in one species only.

2/ SAP Response:

The SAP recognized that the dose range in the mouse study was broad but indicated that the lack of a dose response between the mid- (1000 ppm) and high- (12500 ppm) doses made assessment of the oncogenic classification difficult. They concluded that "since significant hepato-carcinogenesis was observed only at the highest dose of Isoxaben, a level at which hepatotoxicity (elevated serum enzymes, nodular hyperplasia, fatty degeneration) occurred", the proper classification of this agent should be D.

3/ Peer Review - Reevaluation of the Agency Position:

The Committee recognizes that induced liver toxicity, which is sustained over an extended period of time, can cause histopathological alterations, however, it is the Committee's opinion that the mechanism involved in the compound-induced alterations should not be the primary issue of consideration. The Committee did not believe that the MTD had been exceeded just because there was target organ toxicity and concluded that the noted tumors were most likely compound-induced. Therefore, the Committee reiterated its original opinion that the weight of evidence is adequate to classify Isoxaben as a Group C carcinogen, based on criteria in the Agency's Guidelines for Carcinogen Risk Assessment.

TABLE 1 (revised)

ISOXABEN- $B_6C_3F_1$ MOUSE STUDY

Hepatocellular Carcinoma, Adenoma and Combined Carcinoma/Adenoma Rates[1]

Males				
Dose (ppm)	0	100	1000	12500
Hepatocellular carcinomas	9/56 (16)	5/49 (10)	5/55 (9)	5/55 (9)
Hepatocellular adenomas	3/44** (7)	1/41 (2)	3/47 (6)	12/48* (25)
Combined carcinomas and/or adenomas	12/56** (21)	6/49 (12)	8/55 (15)	17/55 (31)

1 Tumor bearing animals/animals at risk; (%) = percent
First tumor observed at 82 weeks in the control group.
(*$p<0.05$; **$p<0.01$)

Historical Control Data-$B_6C_3F_1$ Male Mice
(Lilly: 1982-1985: 10 Studies)

	Incidence (%)	Range (%)
Hepatocellular carcinomas	89/640 (13.9)	0/30-8/30 (0-26.7)
Hepatocellular adenomas	36/640 (5.6)	0/30-6/30 (0-20.0)
Combined carcinomas and/or adenomas	125/640 (19.5)	

Note: Significant trend analysis (Cochran-Armitage) indicated at Control. Significance of pairwise comparison with control (Fisher's Exact) indicated at Dose level.

TABLE 2

ISOXABEN - $B_6C_3F_1$ MOUSE STUDY

Hepatocellular Carcinoma, Adenoma
and Combined Carcinoma/Adenoma Rates[1]

Females

Dose (ppm)	0	100	1000	12500
Hepatocellular carcinomas	0/52 (0)	1/52 (2)	0/46 (0)	2/52 (4)
Hepatocellular adenomas	0/52** (0)	3/52 (6)	2/46 (4)	7/52** (13)
Combined carcinomas and/or adenomas	0/52** (0)	4/52 (8)	2/46 (4)	9/52** (17)

[1] Tumor bearing animals/animals at risk; (#) = percent
First tumor observed at 104 weeks in the 100 ppm group.
(*$p<0.05$; **$p<0.01$)

Historical Control Data-$B_6C_3F_1$ Female Mice
(Lilly: 1982-1985; 10 Studies)

	Incidence (%)	Range (%)
Hepatocellular carcinomas	13/637 (2)	0/30-2/30 (0-7)
Hepatocellular adenomas	7/637 (1)	0/30-2/30 (0-7)
Combined carcinomas and/or adenomas	20/637 (3)	

Note: Significant trend analysis (Cochran-Armitage) indicated at Control. Significance of pairwise comparison with control (Fisher's Exact) indicated at Dose level.

10/5/87

00755

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

OCT 5 1987

FILE COPY

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Peer Review of Isoxaben

FROM: Esther Rinde, Ph.D. Esther Rinde 8/7/87
Scientific Mission Support Staff (TS-769c)

TO: Richard Mountfort
Product Manager # 23
Registration Division (TS-767c)

The Toxicology Branch Peer Review Committee met on June 25, 1987 to discuss and evaluate the weight-of-the-evidence on Isoxaben with particular reference to its oncogenic potential.

A. Individuals in Attendance:

1. Peer Review Committee: (Signatures indicate concurrence with the peer review unless otherwise stated.)

William L. Burnam

Reto Engler

Robert Beliles

Richard Levy

Judith Hauswirth

Esther Rinde

2. Reviewers: (Non-committee members responsible for data presentation; signatures indicate technical accuracy of panel report.)

Margaret Jones (Toxicology) Margaret L. Jones

A. 3. Peer Review Members in Absentia: (Committee members who were unable to attend the discussion; signatures indicate concurrence with the overall conclusions of the Committee.)

Theodore M. Farber — Theodore M. Farber

Anne Barton — Anne Barton

Richard Hill/Don Barnes

Diane Beal

Jack Quest — John A. Quest

4. Other Attendees: C.J. Nelson and Linda Taylor (Tox. Branch) and Carole Gray (Registration Division).

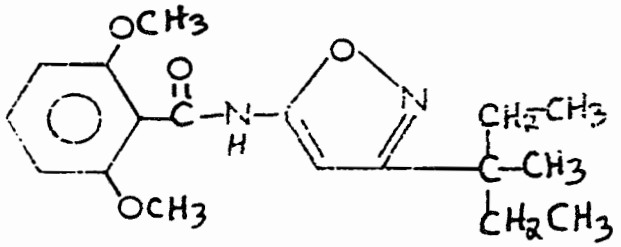
B. Material Reviewed:

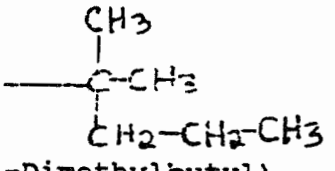
The material available for review consisted of DER's, one-liners, and other data summaries prepared by Ms. Jones. Tables and statistical data analyses for the mouse and rat studies were provided by C.J. Nelson [Memo, 5/1/87]. The material reviewed and the above memo are attached to the file copy of this report.

C. Background Information:

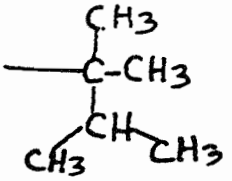
Isoxaben, a new chemical, is a pre-emergent herbicide which has been proposed for experimental use on wheat and barley, and for permanent use on ornamentals to control broadleaf weeds. Technical Isoxaben is 95.5% pure and consists of three isomers, differing only in the isoxazolyl side-chain.

N-[3-(1-Ethyl-1-methylpropyl)-
-5-isoxazolyl]-2,6-Dimethoxybenzamide





(-1,1-Dimethylbutyl)



(-1,1,2-Trimethylpropyl)

D. Evaluation of Oncogenicity Evidence for Isoxaben:

1. Mouse Oncogenicity Study

Reference: Chronic/oncogenicity in mice, MO0883, MO0983, Lilly Research, 11/85, Accession No. 265735, 265736.

Isoxaben (technical) was administered in the diet to groups of 30 male and 30 female B6C3F1 mice at 0, 100, 1000, or 12500 ppm for 24 months in two replicate studies (60/sex/dose, combined). (The Peer Review Committee considered only the combination of the two replicate studies in evaluating the results.)

In treated mice at the high dose (12500 ppm), when compared to concurrent controls, there was a significant increase in adenomas in both sexes, and in combined hepatocellular adenoma/carcinoma in females; there was also a significant dose-related trend for hepatocellular adenoma and for combined hepatocellular adenoma/carcinoma in both sexes. The incidences of liver neoplastic lesions seen in these animals are given in Table I.

Non-oncogenic effects included liver hyperplasia and nodules and hepatocellular vacuolation in high dose males and females, hepatocytomegaly in high dose males, elevated levels of alkaline phosphatase (in males only) and alanine transaminase at the high dose. Survival decreases in males at the low dose, and in females at the mid dose were not statistically different from concurrent controls; there were no dose-related survival disparities found for either sex [C.J. Nelson, Memo 5/1/87]. Body weight gain in high dose males was lower than in concurrent controls.

Historical Controls:

The following control data are from 10 studies in B6C3F1 mice at Lilly Research Laboratories, 1982-1985:

	Incidence (%)		
	Hepatocellular Adenoma	Hepatocellular Carcinoma	Combined Adenoma/Carcinoma
Males	36/640 (5.6)	89/640 (13.9)	125/640 (19.5)
Females	7/637 (1.1)	13/637 (2.0)	20/637 (3.1)

The incidence of adenomas at the high dose* (12500 ppm) in treated mice of both sexes (29%, males; 13%, females) exceeded that of historical controls; the incidence of combined adenoma/carcinoma in females (17%) at the high dose* also exceeded that of historical controls.

*Only dose with statistically significant incidence, compared to concurrent controls.

TABLE I

Isoxaben-B6C3F1 Mouse Study

Hepatocellular Carcinoma, Adenoma and Combined Carcinoma/Adenoma Rates[1]

Dose (ppm)	Control 0	100	1000	12500
Males				
Hepatocellular carcinomas	9/56 (16)	5/49 (10)	5/55 (9)	3/55 (5)
Hepatocellular adenomas	3/44** (7)	1/41 (2)	3/47 (6)	14/48** (29)
Combined carcinomas and/or adenomas	12/56** (21)	6/49 (12)	8/55 (15)	17/55 (31)
Females				
Hepatocellular carcinomas	0/52 (0)	1/52 (2)	0/46 (0)	2/52 (4)
Hepatocellular adenomas	0/52** (0)	3/52 (6)	2/46 (4)	7/52** (13)
Combined carcinomas and/or adenomas	0/52** (0)	4/52 (8)	2/46 (4)	9/52** (17)

[1]Tumor bearing animals/animals at risk; ( ) = percent

First male tumor observed at 82 weeks in the control group.
First female tumor observed at 104 weeks in the 100 ppm group.

Cochran-Armitage Trend and Fisher Exact Test Results:

Significance of Cochran-Armitage Trend Test denoted at Control.
Significance of Fisher's Exact Test, of pairwise comparison with control, denoted at the Dose level.

(*p<0.05, **p<0.01)

D. 2. Rat Oncogenicity Study

Reference: Chronic/oncogenicity in rats, R01583, R01683, Lilly Research 11/85, Accession No. 265735, 265736.

Isoxaben (technical) was administered in the diet to 60 male and 60 female Fisher 344 rats at 0, 125, 1250, or 12,500 ppm.

Slight increases in hepatocellular adenomas were noted for males at the mid and high dose, which were not statistically significant or dose-related [C.J. Nelson, personal communication].

There was also an increase in benign adrenal pheochromocytomas in males at the high dose, which was not statistically significant when compared to concurrent controls, however a positive trend was detected. The incidences of these lesions are given in Table II.

Non-oncogenic effects included: dose-related increase in progressive glomerulonephrosis (statistically significant at high dose in females, and at mid and high dose for males); effects on the stomach mucosae and heart, considered to be sequelae of the kidney effects; parathyroid hyperplasia; increased BUN and creatinine, also reflecting the kidney changes. There was an increasing trend in mortality found for male rats, but not for females.

Historical Controls:

The following control data are from studies in Fischer 344 rats at Lilly Research Laboratories, 1982-1985.

	Incidence (%)			
	Hepatocellular Adenomas	Adrenal Pheochromocytomas		
		Benign	Malignant	Total
Males	3/359 (0.84)	22/359 (6.1)	4/359 (1.1)	26/359(7.2)
Females	3/360 (0.83)	10/360 (2.8)	1/360 (0.3)	11/360(3.1)

The incidence of hepatocellular adenoma in historical control data was never greater than 1/60 for any one study in both sexes.

The incidence of hepatocellular adenoma in treated females was zero; in treated males, it was 3/60 (5.0%) at mid dose, and 2/60 (3.3%) at high dose, which exceeded that in historical controls, as presented above.

The incidence of adrenal pheochromocytomas (predominantly benign) in treated high dose* (12500 ppm) males (31%) exceeded that in historical controls (6.1%).

*Only dose at which incidence exceeded that of concurrent controls.

TABLE II

Isoxaben - Fisher 344 Rat Study

A. Adrenal Medulla: Pheochromocytoma Rates[1]
Benign[2]

	Control			
Dose (ppm)	0	125	1250	12500
Dose (%)	0	.0125	0.125	1.25
Males	10/59* (17)	9/59 (15)	9/59 (15)	18/59 (31)
Females	3/49 (6)	2/45 (4)	4/49 (8)	1/47 (2)

[1]Tumor bearing animals/animals at risk; ( ) = percent
[2]There was one animal with a malignant tumor in control group and one in 125 ppm group only.

First male tumor observed at 66 weeks in 12500 ppm dose group.
First female tumor observed at 102 weeks in 1250 ppm group.

B. Hepatocellular Adenoma - Rates[1]

Males

	Control			
Dose (ppm)	0	125	1250	12500
	0/59 (0)	0/61 (0)	3/60 (5.0)	2/60 (3.3)

[1]Tumor bearing animals/animals at risk; ( ) = percent

Cochran-Armitage Trend and Fisher Exact Test Results:

Significance of Cochran-Armitage Trend Test denoted at Control. Significance of Fisher's Exact Test, of pairwise comparison with control, is noted at the Dose level.

(*$p<0.05$, **$p<0.01$)

D. 3. MTD:

The Committee agreed that the MTD was achieved at the high dose (12500 ppm) in the mouse oncogenicity study. In the rat oncogenicity study, it was noted that the kidneys were compromised (glomerulonephrosis) and that there was a greater than 10% decrease in body weight; however, neither the decrease in body weight, nor the glomerulonephrosis were directly related to the target organ (adrenal) response. The Committee determined that, even though the MTD was apparently slightly exceeded in the rat, these toxic effects did not compromise the relevance of the tumor data.

The Committee concluded that since the 90 day studies correctly predicted the MTD (refer to Table III) both oncogenicity studies were therefore acceptable.

E. Additional Toxicology Data on Isoxaben:

1. Metabolism

Major urinary metabolites (mostly alkyl side chain alcohols and ketones) of Isoxaben identified in the rat are shown in Figure 1. Fecal metabolites have not been identified. Metabolism in males and females was essentially identical. Isoxaben appears to bioaccumulate in various rat tissues, particularly in the male.

2. Non-Oncogenic Toxicologic Effects

Subchronic studies on Isoxaben are summarized in Table III.

3. Mutagenicity

Isoxaben was negative in the Ames Assay (strains not given) up to 500 ug/plate, with and without activation (acceptable). Isoxaben was negative for inducing dominant lethals in male rats fed levels up to 12500 ppm (provisionally acceptable).

Isoxaben was marginally positive in a mouse micronucleus assay (performed in males only) following a single-dose acute oral gavage at 5000 mg/kg. (The TOX reviewer concluded that this study was "Inconclusive. Presumptive positive results should be confirmed in a repeat assay ..." [I. Mauer]).

Isoxaben was negative in two acceptable unscheduled DNA synthesis assays, using rat hepatocytes.

4. Structure-Activity Correlations

No structurally related compounds of toxicological interest were identified in a search of several data bases (Chemline, Toxback, Toxnet, RTECS and Toxline).

TABLE III

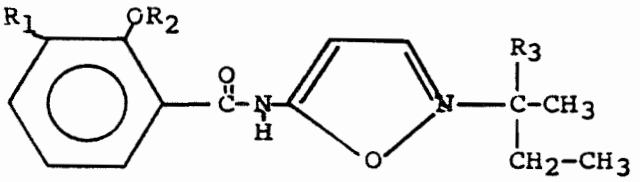
Subchronic studies on Isoxaben (EL-107)

		CORE
Rat Studies		
1.	0, 1.25, 2.5, 5.0% (0, 12500, 25000, 50000 ppm) increased absolute & relative liver weight relative kidney weight increase hepatic enzymes induced NOEL< 1.25%	minimum
2.	0, 0.05, 0.14, 0.42, 1.25% (0, 500, 1400, 4200, 12500 ppm) increased absolute & relative liver weight hepatic enzymes induced NOEL< 0.05%	supplementary
3.	0, 0.05, 0.14, 0.42, 1.25% (0, 500, 1400, 4200, 12500 ppm) increased absolute & relative liver weight/females, all doses increased liver/bodyweight in males 0.42, & 1.25% hepatic enzymes induced/males 0.42% /females 0.14, 0.42, 1.25% NOEL< 0.05%	guideline
Mouse Studies		
1.	0, 0.001, 0.01, 0.14, 1.25% (0, 10, 100, 1400, 12500 ppm) liver hypertrophy/males increased liver weight hepatic enzymes induced/males & females NOEL = 0.01 (100 ppm)	supplementary
2.	0, 0.01, 0.1, 1,25% (special liver) (0, 100, 1000, 12500 ppm) increased liver weight increased liver enzyme activity	supplementary
Dog Studies		
1.	0, 0.25, 0.5, 5.0 g/kg increased serum AP induced hepatic microsomal enzymes NOEL < 0.25% g/kg	minimum
2.	0, 25, 110, 500 mg/kg/day increased liver weight, liver/bodyweight NOEL = 110 mg/kg/day	minimum

FIGURE 1

Metabolism and Major Metabolites of Isoxaben

The metabolism study in rats (No. ABC-0153) identified the major urinary metabolites of EL-107 (Isoxaben). By 72 hours, 90 percent of the administered dose of technical chemical was recovered unmetabolized in the feces. Approximately 20 percent of the dose was absorbed and about half excreted in the urine and half in the feces as metabolites. The urinary metabolites were identified and most involved metabolism of the alkyl side chain to produce alcohols or ketones.



Amounts of parent and metabolites found in urine are as follows:

	R_1	R_2	R_3	Percent [^{14}C]in urine Males	Females
EL-107	-H	$-CH_3$	$-CH_2-CH_3$	1-1.5	0.3-0.5
Metabolites	-H	-H	$-\overset{O}{\overset{\|}{C}}-CH_3$	5-6	1-1.5
	-OH	$-CH_3$	$-\overset{O}{\overset{\|}{C}}-CH_3$	12-14	10-12
	-OH	$-CH_3$	$-\underset{OH}{\underset{\|}{CH}}-CH_3$	8-9	11-12
	-H	-H	$-\underset{OH}{\underset{\|}{CH}}-CH_3$	7-9	3-4

F. Weight of Evidence Considerations:

The Committee considered the following facts regarding the toxicology data on Isoxaben to be of importance in a weight-of-the evidence determination of oncogenic potential.

Isoxaben produced statistically significant increases in liver adenomas in both sexes of B6C3F1 mice, at the high dose (12500 ppm); the incidences were outside that in historical controls. No significant increase in progression to malignancy was noted at any dose level.

In the F344 rat, Isoxaben produced adrenal pheochromocytomas; the incidence in high dose males was not statistically significant when compared to concurrent controls, although it exceeded that of historical controls and showed a positive trend, compared to concurrent controls.

Slight increases in hepatocellular adenomas were noted for treated male rats at the mid (1250 ppm) and high dose, which exceeded that in historical controls, but which were not dose-related or statistically significant, when compared to concurrent controls.

In both studies, the MTD was reached or slightly exceeded at the high dose.

There is suggestive (but not conclusive) evidence of mutagenicity, based on a marginal increase in the mouse micronucleus test.

G. Classification of Oncogenic Potential:

Criteria contained in the EPA Guidelines [FR51: 33992-34003, 1986] for classifying a carcinogen were considered.

Based on the available evidence, Isoxaben was classified by the Peer Review Committee as Group C (possible human carcinogen). The evidence for Isoxaben was judged to be "limited", based on a statistically significant increase in benign tumors in one species only (mouse). The tumors in the mouse (liver adenomas) were present in both sexes; however, tumor incidence was statistically increased at the high dose only, the tumors were of a common type, were predominantly benign, and there was no decrease in latency. Tumors in the rat (adrenal and hepatocellular) were present in one sex only (male), were also predominantly benign, and fairly common. Adrenal tumors occurred with increased frequency only at the highest dose, and although they displayed a positive trend, the increased incidence was not statistically significant; increases in liver tumor incidences were neither statistically significant, nor dose-related.

Although Group C chemicals will generally be regarded as suitable for quantitative risk assessment, the Guidelines state that judgments in this regard may be made on a case-by-case basis. In this case, the evidence was not considered sufficient to warrant a quantitative estimation of risk for Isoxaben, for reasons summarized in the above paragraph.

Don —
Did you attend this one? I didn't. Send on its way.
Dick

PEER REVIEW LOG OUT LIST

		OUT	RETURN	
THEODORE FARBER	DATE:	8/27	9/2	
WILLIAM BURNAM	DATE:	9/2	9/8	
RETO ENGLER	DATE:	8/24	8/24	
JOHN QUEST	DATE:	8/10	8/10	
ESTHER RINDE	DATE:			
RICHARD LEVY	DATE:	9/9	9/15	
JUDITH HAUSWIRTH	DATE:	8/10 8/27	8/27	
ROBERT BELILES	DATE:	9/21	9/21	
MARGARET JONES	8/25 DATE:	8/10 9/11 RNH	9/13 with 8/26 Comments RNH	
RICHARD HILL	DATE:			
ANNE BARTON	DATE:	9/15	9/17	
DIANE BEAL	DATE:	9/22? 9/28	9/28	— I DISAGREE = LAST PARAGRAPH; A QUANTITATIVE RISK SHOULD BE DONE UNLESS DATA UNAVAILABLE

CHEMICAL NAME: ISOXABEN

PLEASE SIGN AND RETURN TO S. PENDARVIS WITHIN 2 DAYS TO 557-7351 (RM. 821 CM-2 TS-769)

[redacted]

007755

SAP Executive Summary; Meeting Date(s)

FEDERAL INSECTICIDE, FUNGICIDE, AND RODENTICIDE ACT

SCIENTIFIC ADVISORY PANEL

A Set of Scientific Issues Being Considered by the Agency in Connection with the Peer Review Classification of Isoxaben as a Class C Oncogen

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The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) has completed review of a set of scientific issues being considered by the Environmental Protection Agency's peer review classification of Isoxaben as a Class C oncogen. The review was conducted in an open meeting held in Arlington, Virginia, on September 7, 1988. All Panel members, except Dr. Thomas W. Clarkson, were present for the review.

Public notice of the meeting was published in the Federal Register on Monday, July 25, 1988.

Oral statements were received from staff of the Environmental Protection Agency and from Dr. J. L. Emmerson of Elanco Products Company and Dr. Joseph V. Rodricks of ENVIRON Corporation representing Elanco Products Company.

In consideration of all matters brought out during the meeting and careful review of all documents presented by the Agency, the Panel unanimously submits the following report.

REPORT OF PANEL RECOMMENDATIONS

Isoxaben

The Agency requested the Panel to focus its attention upon a scientific issue relating to the Peer Review of isoxaben. There follows the issues and the Panel's response to the issues:

Issue:

1. The Agency requests any comments the panel wishes to make regarding the assessment of the weight of evidence and classification of Isoxaben, with respect to Agency Guidelines for Carcinogen Risk Assessment.

Panel Response:

The Scientific Advisory Panel recognizes that the data base for evaluation of Isoxaben is unusual, i.e., the dose range used in the tumorigenesis studies was broad but the lack of a dose between 1,000 and 12,500 parts per million made assessment of the oncogenic classification difficult. Since significant hepatocarcinogenesis was observed only at the highest dose of Isoxaben, a level at which hepatotoxicity (elevated serum enzymes, nodular hyperplasia , fatty degeneration) occurred, the Scientific Advisory Panel believes that the proper classification of this agent should be D.

ISSUE:

2. The Agency requests any specific comments the Panel may have on the conclusion that the data on oncogenicity of Isoxaben does not warrant a conventional quantification of human risk? Alternatively, what methods can the Panel suggest to establish exposure levels which represent acceptable/minimal risks to humans?

Panel Response:

The Scientific Advisory Panel recommends the use of the RfD (reference dose) approach for the setting of human exposure levels.

FOR THE CHAIRMAN:

Certified as an accurate report of Findings:

Stephen L. Johnson
Executive Secretary
FIFRA Scientific Advisory Panel

Date: 9-14-88

Qualitative/Quantitative Risk Assessment

[redacted]

007755

5/1/87 Rec-Pw File

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

MAY 1 - 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Isoxaben, Rat and Mouse Study - Qualitative Risk Assessment of Combined Toxicity and Oncogenicity Study. Caswell #419F

FROM: C.J. Nelson, Statistician cjnelson 5/1/87
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Margaret Jones
Section III
Toxicology Branch
Hazard Evaluation Division (TS-769C)

THRU: Richard Levy, M.P.H., Leader-Biostatistics Team
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C) 5/1/87

and

[redacted]
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Summary:

In this two-year chronic oral study of male and female Fisher 344 rats and male and female B6C3F1 mice, a significant increasing trend in mortality with dose was found for the male rats. No survival disparities were found in the mice or female rats. That is there were no trends, no heterogeneity, and no pairwise differences with controls. Since most of the lesions in male rats occurred late, there was no need to adjust for mortality, since the table would collapse to one or two intervals.

For the rats, there was a significant increasing trend with dose for male pheochromocytomas, and both male and females progressive glomerulo nephrosis (PGN). The 0.125% and 1.25% male dose groups were significantly higher than controls as were the females at the 1.25% dose group.

2.1

For the mice, there was a significant increasing trend with dose for both male and female hepatocellular adenoma only and pooled hepatocellular adenoma and/or carcinoma. The high dose (125000) males and females had significantly more adenomas than the controls. The high dose (125000) females had significantly more adenomas and/or carcinomas than the controls. There were no trend or pairwise differences between dosed animals and control animals with hepatocellular carcinoma of either sex.

Background:

This study was conducted at Lilly Research Laboratories on male and female Fischer 344 rats and on male and female B6C3F1 mice. The study was done in two replicates, but were so close in time that the two studies were pooled. Technical Isoxaben was administered to 60 rats of both sexes at 0.0125%, 0.125%, and 1.25% in the diet (Study Numbers RO1583 and RO1683). Technical Isoxaben was administered to 60 mice of both sexes at 100ppm, 1000ppm, and 12500ppm in the diet (Study Numbers MO0883 and MO0983). There was also a concurrent control group of 60 animals for both sexes of both species.

Mortality Analysis:

The Thomas, Breslow, Gart Procedure (1977) was used to analyze the survival data. There was a significant trend ($p = .05$) for male rats with mortality increasing with increasing doses of Isoxaben (Table 1) using Cox's test (1972) for life table data. But there was no departure from trend. There were no survival disparities in female rats. There were no significant pairwise comparisons for either sex between the control and any treated group. The pairwise test of control versus high dose was nearly significant for male rats ($p = .09$ by Cox's test).

There were no survival disparities in male or female mice with increasing doses of Isoxaben (Table 2). The same procedure was used to analyze the mouse survival data as was used in the rat data. There were no significant pairwise comparisons for either sex between the control and any treated group.

Table 1. Isoxaben - Rat Study, Mortality Rates+ and Cox or Generalized K/W Test Results

A. Males

Dose	WEEKS				
(%)	0-26	27-52	53-78	79-105[a]	TOTALS
0	0/59	0/59	3/59	17/56	20/59 (34)*
.0125	0/60	1/60	0/59	25/59	26/60 (43)
.125	0/60	1/60	4/59	19/55	24/60 (40)
1.25	0/60	0/60	7/60	24/53	31/60 (52)

B. Females

Dose	WEEKS				
(%)	0-26	27-52	53-78	79-105[a]	TOTALS
0	0/61	0/61	0/61	14/61	14/61 (23)
.0125	0/60	0/60	4/60	16/56	20/60 (33)
.125	0/60	0/60	3/60	11/57	14/60 (23)
1.25	0/60	0/60	0/60	18/60	18/60 (30)

\+ Number of Animals Died/Number of Live Animals at the beginning of the interval.
() Percent
a Final sacrifice was at 104 or 105 weeks.

Note - The above survival tables are broken into aggregate time intervals for display purpose only.
Significance of Trend Analysis denoted at Control.
Significance of pairwise comparison with control denoted at Dose level. (* $p < .05$ ** $p < .01$)

Table 2. Isoxaben - Mouse Study, Mortality Rates[+] and Cox or Generalized K/W Test Results

A. Males

Dose (ppm)	WEEKS 0-26	27-52	53-78	79-105[a]	TOTALS
0	0/60	0/60	2/60	15/58	17/60 (28)
100	7/60	0/53	2/53	9/51	18/60 (30)
1000	2/60	1/58	1/57	9/56	13/60 (22)
12500	2/60	0/58	2/58	10/56	14/60 (23)

B. Females

Dose (ppm)	WEEKS 0-26	27-52	53-78	79-105[a]	TOTALS
0	0/60	1/60	0/59	7/59	8/60 (13)
100	1/60	1/59	1/58	5/57	8/60 (13)
1000	2/59	1/57	2/56	9/54	14/59 (24)
12500	0/60	0/60	2/60	6/58	8/60 (13)

\+ Number of Animals Died/Number of Live Animals at the beginning of the interval.
() Percent
a Final sacrifice was at 104 or 105 weeks.

Note - The above survival tables are broken into aggregate time intervals for display purpose only.
Significance of Trend Analysis denoted at Control.
Significance of pairwise comparison with control denoted at Dose level. (* $p < .05$ ** $p < .01$)

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Tumor Analysis:

Pheochromocytoma, Lymphosarcoma, and Progressive Glomerulo Nephrosis (PGN) was analyzed for the rat studies (Table 3, 4, and 5 respectively). Although there was a survival trend in the male rat, most of the lesions occurred late. Since there were no pairwise differences between control and dosed male rats, a time-adjusted analysis was not necessary. There were no survival disparities for the female rat or either sex of the mice. Therefor the Fisher's Exact Test was used for pairwise comparisons and the Cochran-Armitage Test was used to test for trends. There was a significant trend ($p = .014$) for Pheochromocytoma among male rats but no heterogeneity, and no pairwise differences were detected. There was no significant trend, heterogeneity, or pairwise comparisons for Pheochromocytoma among female rats. There were no significant trend, heterogeneity, or pairwise comparisons with control for Lymphosarcoma for either sex. There was a highly significant trend for both sexes ($p < .001$) for PGN. The heterogeneity Chi-square for males was significant ($p = .03$) but was not significant for females. There was significantly more PGN at the high dose (1.25%) than the controls (males $p = .001$, females $p = .007$). Also the mid dose (0.125%) males were significantly higher ($p = .005$) than controls.

Hepatocellular carcinoma, adenoma, and adenoma/carcinoma was analyzed for the mouse studies (Table 6, 7, and 8 respectively). There were no survival disparities in the mouse studies, hence the same tests were used in these analyses as those used in the rat study. There were no significant differences found for hepatocellular carcinoma for either sex. There was a significant trend for hepatocellular adenoma for both sexes (males $p < .001$, females $p = .004$). The high dose (12500ppm) mice had significantly more adenomas than the controls for both sexes (males $p = .005$, females $p = .006$). There was a significant trend for adenomas and carcinomas combined (males $p = .01$, females $p = .001$). The high dose (12500) female mice had significantly more combined tumors than the controls ($p = .001$) but the high dose males were not significant ($p = .18$).

Table 3. ISOXABEN - Rat Study, Adrenal Cortex Tumor (Pheochromocytoma) Rates[+] and Cochran-Armitage Trend Test and Fisher's Exact Test Results

Dose (%)	0	0.0125	0.125	1.25
Males	11/59 (19)*	10/59 (17)	9/59 (15)	18/59 (31)
Female	3/49 (6)	2/45 (4)	4/49 (8)	1/47 (2)

First male tumor observed at 66 weeks in 1.25% dose group.
First female tumor observed at 102 weeks in 0.125% dose group.

Table 4. ISOXABEN - Rat Study, Lymphosarcoma Rates[+] and Cochran-Armitage Test and Fisher's Exact Test Results

Dose (%)	0	0.0125	0.125	1.25
Males	1/59 (2)	2/60 (3)	4/60 (7)	3/60 (5)
Female	2/47 (4)	0/40 (0)	1/46 (2)	3/42 (7)

First male tumor observed at 46 weeks in 0.125% dose group.
First female tumor observed at sacrifice.

Table 5. ISOXABEN - Rat Study, Progressive Glomerulo Nephrosis Rates+, Cochran-Armitage Trend Test, and Fisher's Exact Test Results

Dose (%)	0	0.0125	0.125	1.25
Males	26/58 (47)**	33/59 (56)	40/57 (70)**	51/58 (88)**
Female	34/61 (56)**	27/59 (46)	35/60 (58)	47/60 (78)**

\+ Tumor Bearing Animals/ Animals at Risk
First male tumor observed at 70 weeks in 1.25% dose group.
First female tumor observed at week 70 in 0.125% dose group.

Note - Significance of Trend Analysis denoted at Control. Significance of pairwise comparison with control denoted at Dose level. (* p < .05, ** p < .01)

Table 6. ISOXABEN - Mouse Study, Hepatocellular Carcinoma Rates+, Cochran-Armitage Trend test, and Fisher's Exact Test Results

Dose (ppm)	0	100	1000	12500
Males	9/56 (16)	5/49 (10)	5/55 (9)	3/55 (5)
Female	0/52 (0)	1/52 (2)	0/46 (0)	2/52 (4)

First male tumor observed at 82 weeks in control group.
First female tumor observed at 104 weeks in 100ppm dose group.

Table 7. ISOXABEN - Mouse Study, Hepatocellular Adenoma Rates+, Cochran-Armitage Trend test, and Fisher's Exact Test Results

Dose (ppm)	0	100	1000	12500
Males	3/44 (7)**	1/41 (2)	3/47 (6)	14/48 (29)**
Female	0/52 (0)**	3/52 (6)	2/46 (4)	7/52 (13)**

First male tumor observed at 103 weeks in 12500 ppm group.
First female tumor observed at sacrifice.

Table 8. ISOXABEN - Mouse Study, Hepatocellular Adenoma and/or Carcinoma Rates+, Cochran-Armitage Trend test, and Fisher's Exact Test Results

Dose (ppm)	0	100	1000	12500
Males	12/56 (21)**	6/49 (12)	8/55 (15)	17/55 (31)
Female	0/52 (0)**	4/52 (8)	2/46 (4)	9/52 (17)**

+ Tumor Bearing Animals/ Animals at Risk.
First male tumor observed at 82 weeks in control group.
First female tumor observed at 104 weeks in 100ppm dose groups.

Note - Significance of Trend Analysis denoted at Control. Significance of pairwise comparison with control denoted at Dose level. (* p < .05, ** p < .01)

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Bibliography:

Thomas, D G, N Breslow, and J J Gart, Trend and Homogeneity Analyses of Proportions and Life Table Data. Computers and Biomedical Research 10, 373-381, 1977.

Cox, D.R. Regression Models and Life Tables (with discussion). J. Roy. Stat. Soc. Ser. B. 34, 187-220, 1972.

Reviewer's Peer Review Package for 1st Meeting

6/10/87

007755

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

FILE COPY

JUN 10 1987

MEMORANDUM

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Peer-Review of Isoxaben

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

TO: Addressees

Attached is a package on Isoxaben, prepared by Ms. Margaret Jones for your review.

A meeting to discuss the weight-of-the-evidence of the oncogenic effects is scheduled for Wednesday, June 24, 1987, at 11:00 AM in Dr. Farber's office (Rm. 821 CM-2).

Attachment

ADDRESSEES

T. Farber
W. Burnam
E. Rinde
J. Hauswirth
J. Quest
L. Kasza
R. Levy
M. Van Gemert
M. Jones
R. Beliles
D. Beal
A. Barton
R. Hill

#19 6/9/87 sp

MEMORANDUM

SUBJECT: Weight-of-the-evidence and oncogenicity of Isoxaben (EL-107)

TO: Peer Review Committee for Isoxaben
Toxicology Branch
Hazard Evaluation Division

FROM: Margaret L. Jones M. L. Jones 5/19/87
Review Section III
Toxicology Branch

THROUGH Marcia van Gemert, Ph.D., Head
Review Section III
Toxicology Branch M. van Gemert 5/19/87

Attached are a summary of issues and support documents to use in the assessment of the oncogenic evidence on Isoxaben. These include:

- Summary of toxicological issues
- Background
- Structure of the technical chemical
- Acute toxicity
- Reproduction and developmental toxicity
- Subchronic testing
- Metabolism and major metabolites
- Chronic and oncogenicity testing
- Mutagenicity
- "One liners"
- Data Evaluation Reports for key studies
  - Acute toxicity
  - Reproduction and developmental toxicity
  - Subchronic testing
  - Metabolism and major metabolites
  - Chronic/oncogenicity studies
  - Statistical Analysis of chronic/onco studies
  - Mutagenicity
- Historical Control data

Summary of Issues and Questions

1. Significance of hepatocellular adenomas and carcinomas in mice. Hepatocellular adenomas only were elevated in males and combined adenomas and carcinomas were elevated in females. Supporting evidence includes liver hyperplasia, hepatocytomegaly, hepatocellular vacuolation, induction of alkaline phosphatase.

2. Significance of pheochromocytomas and progressive glomerulonephrosis in the rat. Is there any significance in the observation of very few hepatocellular adenomas in the rat at the mid and high dose in males only.

3. Is further mutagenicity testing necessary. Mutagenicity was negative in in vivo dominant lethal and reverse mutation (Ames) and in unscheduled DNA synthesis but inconclusive in in vivo chromosome aberration (mouse micronucleus). The inconclusive study was positive for induction of micronuclei.

4. Reproduction study showed microphthalmia, but this was not confirmed in the developmental toxicity studies.

5. Metabolism shows possible concern for bioaccumulation in the rat. In the excretion in inspired air study (rat) males showed 85% recovery of radiation at 48 hours. There was some evidence of residues remaining in the carcass. Will further testing be necessary to confirm whether any tissue accumulation occurs?

7. Historical control data have been received. These data were forwarded to the statistical team for consideration along with the results of the mouse and rat chronic/oncogenicity studies. These data are attached for Peer Review consideration.

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.
Acute oral LD_{50} - rat; Eli Lilly; #R-0-01-85; 1/18/85	EL -107- DF 75% formulation Lot #Z-11245	257774	LD_{50} > 5000 mg/kg		Guideline 004593
Acute Dermal LD_{50} - rabbit; Eli Lilly; #B-D-196-84; 12/19/84	EL -107- DF 75% formulation	257774	LD_{50} > 5000 mg/kg		Minimum 004593
Primary dermal irritation-rabbit; Eli Lilly; #B-D-196-84; 12/19/84	EL -107- DF 75% formulation	257774	Moderate erythema and slight edema which cleared by day 5	III	Minimum 004593
Primary eye irritation - rabbit;	EL -107- DF 75% formulation	257774	Corneal dullness, slight iritis, moderate conjunctival hyperemia which cleared by day 7.	III	Guideline 004593
Acute inhalation LC_{50} - rat;	EL -107- DF 75% formulation	257774	Requirement waived [illegible]		
Acute inhalation LC_{50} - rat; Eli Lilly; #R-H-70-84; 10.	TECH	257774	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
Dermal sensitization - guinea pig;	EL -107- DF 75% formulation	257774	Requirement waived		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	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

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.
2 Year feeding/oncogenic - mice; Eli Lilly; #M00883;	EL-107	258169	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	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	TOX Category	CORE 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 #871, 9/6/86	EL-107 tech (% ai not stated)	265739	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 # RO1984; 10/85	EL-107 tech (95.5% ai)	265739	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	LD_{50} > 2000 mg/kg bodyweight	III	Minimum 005732
90-Day feeding - mice; Lilly Research; MO2782; 5/85	EL-107 tech (93.7% ai) Batch No. HO2-2G6-118	265731	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 Grade/ Doc. No.
90-Day oral - dog; Lilly Research; DO0783; 4/84	EL-107 tech (95.5% ai) Lot Z10025	073293	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	Oncogenic NOEL = 1000 ppm 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

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.
2-Year feeding/oncogenic - rat; Lilly Research; RO1583; 11/85	EL-107 tech (94.8% ai) Lot Z10025	073295 265735 265736	Oncogenic NOEL= 1250 ppm 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, RO3783, R14183; 8/84	EL-107 tech (93.7-94.5% ai) Lot HO2-2G6-118	073297 073298	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

Tox Chem No. 419F EL-107 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.
Teratology - rat; (Hst: (WI)BR); Lilly Res.; #RO9483; 7/84	EL-107 tech (95.5% ai) Lot Z10025	073229	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; #BO3383; 5/84	EL-107 tech (95.5% ai) Lot Z10025	073299	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, PO3983, PO4083; 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-0153 8/84	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; RO2186; 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 Within 24 hours, 79.4, 88.7, 91.7, 81.0% of the 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; M03182; 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; R12885; 1/86	EL-107 tech (93.3% ai) Lot Z10025	265740	Predosing with 250 mg/kg cold El-107 did not change pattern of urinary & fecal excretion. 80.59% (males) and 96.1% (females) within 7 days in combined urinary & fecal excret. No radioactivity detected in tissues after 7 da. Carcasses: 0.3%(males) and 0.4%(females) radioactivity remained.		Acceptable 005732

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	265733	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 HO2-2G6-118	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 femals 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

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Study/Lab/Study #/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	not in DER	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	not in DER	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	not in DER	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	not in DER	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

EPA: 68-02-4225
DYNAMAC NO. 261-63
January 20, 1987

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:

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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. 553-3N1-056), labeled at position 5 of the isoxazole ring (specific activity = 11.21 µCi/mg), had a radiochemical purity of 98.6 percent as determined by thin-layer chromatography.

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

4. STUDY IDENTIFICATION: Magnussen, J. D. and Rainey, D. P. Metabolism of ^{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. C73293.

5. REVIEWED BY:

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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 B-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 ou 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. 0E contained inordinately low levels (3.3 percent) of [^{14}C]).

B. Extraction of feces with methanol was essentially quantitative with 97.1 and 97.5 percent of the fecal [^{14}C] extracted from the male and female fecal samples, respectively. The major component in fecal extracts was EL-107, which represented 87.5 and 90.8 percent of the extractable fecal radioactivity in males and females, respectively. The remainder of the extractable fecal radioactivity (10-12 percent) consisted of highly polar [^{14}C] material (2-4 percent) that remained at the origin of the TLC plates, a band of radioactivity representing 1 to 2 percent of the radioactivity that chromatographed as a broad, diffuse band starting just above the origin, and two to three minor metabolite zones that as a group accounted for 2 to 4 percent of the extractable fecal radioactivity. No further characterization of this radioactivity was performed.

C. The 0- to 24-hour and the 24- to 48-hour urine collections for each sex were pooled. Extraction of urine with ethyl acetate removed 14.2 and 23.2 percent of the urinary [^{14}C] from male and female urine, respectively (this fraction of radioactivity was designated the neutral fraction or NF). The aqueous fractions remaining after the ethyl acetate extraction were treated with aryl sulfatase/β-glucuronidase to hydrolyze conjugated metabolites of EL-107. Following this hydrolysis procedure, 53.6 and 42.9 percent of the [^{14}C] in male and female urine, respectively, were extractable into ethyl acetate (this fraction of radioactivity was designated the aglycone fraction or AF). Of the remaining radioactivity, 10.3 and 14.9 percent was nonextractable from the male and female urine, respectively, following enzyme hydrolysis and approximately 15 to 20 percent could not be accounted for following enzyme hydrolysis. The cause for this lost radioactivity was not known, but could have resulted from incomplete phase separation due to emulsion formation.

D. The neutral and aglycone fractions from both male and female urine were each initially characterized by silica-gel column chromatography (Appendix D, Silica-gel Elution Profiles, CBI pp. 27-30, Figures 2-5). The authors stated that except for some minor differences, the elution profiles for the neutral fraction from male and female urine (Figures 2 and 4) were basically identical as were the profiles for the aglycone fraction from males and females (Figures 3 and 5).

E. The peak fractions eluted from the silica-gel columns were pooled as shown in Figures 2 to 5 and further analyzed by TLC. The distribution of metabolites in the neutral and aglycone fractions from female urine were essentially identical to the distribution found in the comparable fractions from male urine. With the exception of peaks MAF-2, FAF-1, and FNF-5, each of which contained a single radioactive component, all other peaks contained multiple components. In several cases, up to five metabolite zones were observed in a single column peak. Comparison of the 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.

Background

The herbicide Isoxaben is a new chemical for which there is a nearly complete data base. The toxicity issues uncovered in the course of data review are discussed in the following pages. The registrant objective appears to be to obtain an EUP for wheat and berley for this preemergent herbidcide and a permanent registration on ornamentals to control broadleaf weeds.

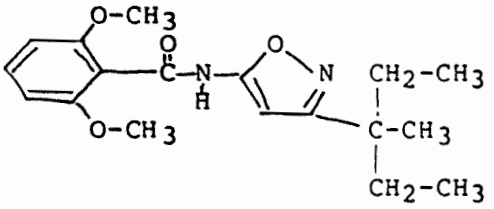
Open Literature Search, Structure/Activity Investigation

A search of the open literature revealed no toxicity information on Isoxaben. The data bases searched include TOXLINE (1981 forward), CHEMLINE, RTECS (NIOSH), TOXBACK 65 (1965-76),TOXNET, and TOXBACK 76 (1976-80). Metabolites and the component structures of the parent compound were also used in the search to pick up any existing information. There is apparently no relationship between this chemical and other known oncogens according to the results. A Chemical Information System search revealed the only related compounds were drugs which included the sulfur moiety. No compounds similar to Isoxaben were identified.

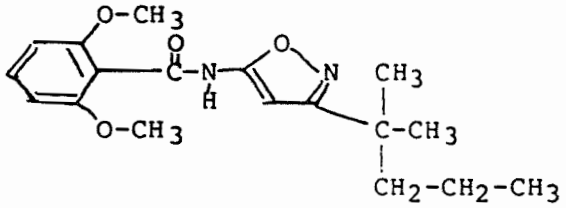
Structure of Technical Isoxaben

There are three isomers which total 95.5% purity in the technical chemical.

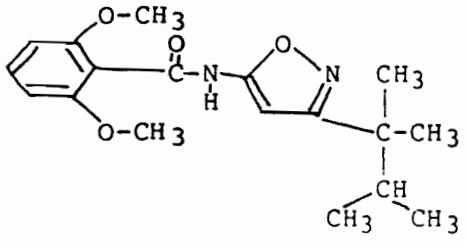
a. N-[3-(1-Ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide (85.0%)



b. N-[3-(1,1-Dimethylbutyl)-5-isoxazolyl]-2,6-dimethoxybenzamide (8.3%)



c. N-[3-(1,1,2-Trimethylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide (2.2%)



Acute Toxicity

Acute oral toxicity of the technical chemical is a data gap, according to Toxicology Branch records. Primary dermal irritation (category IV) showed no evidence of dermal irritation or systemic toxicity. The acute dermal LD_{50} was greater than 2 g/kg (Toxicity Category III) with slight edema and slight dermal irritation observed at day 4 and disappearing by day 11. Acute eye irritation (category III) tested 25 mg in each eye (0.1 cc). Corneal dullness and mild iritis were observed one hour after administration. Irritation of the cornea and iris cleared in 24 hours and conjunctivitis within 3 days after exposure. Acute inhalation (category II) LC_{50} was greater than 1.99 mg/l actual concentration and 11.5 mg/l nominal concentration with no toxic effects noted. Acute intraperitoneal testing of 25% Isoxaben used 5 g/kg (20 ml/kg) and produced no mortality. Recovery occurred after 3 days on test. Examination found fibrous adhesions in the liver and diaphragm but no evidence of compound-related systemic toxicity. There was evidence of unabsorbed compound in the abdominal cavity on the surfaces of intestine and liver (Acute IP LD_{50}>5 g/kg). Percutaneous absorption showed absorption of 11% of a 2 mg/kg topical dose at 7 days. The topical dose absorbed was 7.5% of the IV dose (2 mg/kg) applied one month earlier.

Reproduction and Developmental Toxicity

1. Three Generation Reproduction Study in Rats, R15382, RO3783, R14183, Lilly Research, 8/84, Accession No. 073297, 073298.

2. EL-107 Teratogenicity Study in Rats, RO9483, Lilly Research, 7/84, Accession No. 073229.

3. EL-107 Teratogenicity Study in Rabbits, BO3383, Lilly Research, 5/84, Accession No. 073299.

Three Generation Reproduction in the Rat

The three generation reproduction study (0, 0.05, 0.25, 1.25%) found maternal effects in reduced bodyweight, reduced bodyweight gain and efficiency of food utilization during growth, gestation and lactation. Relative liver/bodyweight ratio was also increased. Reproductive effects included decreased numbers of viable pups in the F_{2a} and F_{2b} and depressions in bodyweight of offspring on postpartum day 21. Developmental toxicity was demonstrated by decreaed numbers of viable fetuses per litter, increased resorptions and post-implantation losses and increased incidence of hydroureter in both generations. Microphthalmia was increased at the high dose.

Developmental toxicity in the Rat and Rabbit

In the rat, (0, 100, 320, 1000 mg/kg/day), developmental toxicity was demonstrated by increaed preimplantation loss, increased resorptions, smaller litter size and increases in runt fetuses at the high dose. Maternal toxicity was demonstrated by lower bodyweight gain during dosing (slight effect). The finding of microphthalmia in the reproduction study was not confirmed in the developmental toxicity study.

In the rabbit, (0, 100, 320, 1000 mg/kg/day), no evidence of maternal or developmental toxicity was found at any dose tested. The dose levels could not be verified, however, since analyses of content, stability and homogeneity were apparently not performed. The limit test was apparently satisfied.

Subchronic and Chronic Toxicity in Rodents

1. Subchronic Mouse/3 months administration with 1 month reversibility phase, MO2782, Lilly Research ,5/85, Accession No. 265731.

2. Subchronic Liver toxicity/3 months, MO1083, Lilly Research, 11/85, Accession No. 265732.

3. Subchronic Oral Toxicity in the Dog, DO0783, Lilly Research, 4/84, Accession No. 073293.

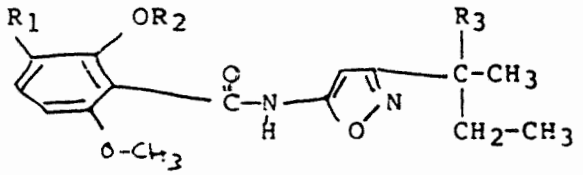
Studies in rodent species have shown effects apparently related to the liver. A subchronic mouse study (0, 0.001, 0.01, 0.14, and 1.25%) demonstrated liver hypertrophy and increased liver weight in males. Females also showed increased liver weights without hypertrophy. Liver microsomal enzyme induction occurred in both male and female mice. A subchronic rat study (0, 1.25, 2.5, and 5.0%) demonstrated a dose-related decrease in alkaline phosphatase. In addition, females showed decreased mean creatinine levels at all doses and decreased alanine transaminase at the high dose. Liver microsomal enzymes were elevated in males and females at all doses. Absolute and relative liver weights were increased in males and females at all doses. Absolute and relative liver weights were increased in males and females at all doses. Increased relative kidney weights were also noted in males and females. Another subchronic rat study (0, 0.05, 0.14, 1.25%) also showed increases in absolute and relative liver weight and induction of liver microsomal enzymes.

Other subchronic testing included studies in mice and dogs. Subchronic liver toxicity in mice (0, 0.01, 0.1, 1.25% was performed to identify a no observed effects level for liver toxicity. The study used the same doses as the chronic/oncogenicity mouse study and was started at the same time as the chronic study. It was used to evaluate the effects of the test compound on liver weight and enzyme induction at three months. A previous study in mice with doses ranging from 1.25% to 5% showed significant increases in liver microsomal enzymes at all levels and all treated males and females had increased liver weights. (The effects noted were statistically significant.) The liver toxicity study in mice found elevated liver weights at 1.25% and elevated liver-to-bodyweight ratios at 0.1 and 1.25%. In addition, liver microsomal enzyme induction occurred at the high dose.

A subchronic (3 months) toxicity study in dogs (0, 25, 110, and 500 mg/kg/day) found increases in absolute and relative liver weights in high dose males. Earlier studies using higher doses in dogs had shown liver microsomal enzyme induction at all doses in males and females.

Metabolism and Major Metabolites of Isoxaben

There are sufficient data on the metabolism of EL-107 to assess this category of toxicity. The metabolism study in rats (No. ABC-0153) identified the major urinary metabolites of EL-107. By 72 hours, 90 percent of the administered dose of technical chemical was recovered unmetabolized in the feces. Approximately 20 percent of the dose was absorbed and about half excreted in the urine and half in the feces as metabolites. The urinary metabolites were identified and most involved metabolism of the alkyl side chain to produce alcohols or ketones.



Amounts of parent and metabolites found in urine are as follows:

	R_1	R_2	R_3	Percent [^{14}C]in urine	
				Males	Females
EL-107	-H	$-CH_3$	$-CH_2-CH_3$	1-1.5	0.3-0.5
Metabolites	-H	-H	$-\overset{O}{\overset{\|}{C}}-CH_3$	5-6	1-1.5
	-OH	$-CH_3$	$-\overset{O}{\overset{\|}{C}}-CH_3$	12-14	10-12
	-OH	$-CH_3$	$-\underset{OH}{CH}-CH_3$	8-9	11-12
	-H	-H	$-\underset{OH}{CH}-CH_3$	7-9	3-4

The toxicity of these metabolites has not been tested.

Fecal metabolites were not identified. Metabolism in males and females was essentially similar, females producing slightly less than males of each metabolite except for one.

In an absorption study mice were given 1, 15, 100, or 200 mg/kg radiolabeled EL-107. This study showed plasma half-life to be 8-9 hours. Half life and plasma level did not appear to change with dose. The majority of radioactivity was eliminated by 72 hours in urine and feces. The amount absorbed is limited above 100 mg/kg according to the study.

Two distribution studies were done. In one, two groups of rats were given 1000 mg/kg of EL-107 by gavage and then sacrificed at 4 hours or 24 hours after collection of blood. The results showed that radioactivity which appeared in several tissues at 4 hours was still present at 24 hours even though the majority of radioactivity was confined to the intestinal tract at both times. Significant amounts of radioactivity remained at 24 hours indicating some tissue accumulation of the test substance in amounts above the

plasma level. The study was supplementary since it should have been carried out to elimination of 90% of the dose or to 7 days as prescribed in the Guidelines.

Another distribution study looked at the effects in rats of dosing with radiolabeled EL-107 after predosing repeatedly with "cold" EL-107. Five rats per sex were given daily doses of 250 mg/kg "cold" EL-107 for 14 days and then a single 250 mg/kg dose of radiolabeled EL-107. The predosing did not seem to change the pattern of excretion of EL-107 compared to the above studies. Within 7 days after dosing with radiolabeled EL-107 males excreted 80.59% and females excreted 96.1% of the administered dose, urinary and fecal elimination combined. Tissue levels were low at 7 days: 0.3 and 0.4 percent of dose in the carcass of males and females respectively, and 0.01 and 0.02 percent in the liver of males and females respectively.

Two studies looked at excretion in mice and excretion in expired air in rats. The mouse study demonstrated that as doses increased ($\geq$ 100 mg/kg) proportionately more of the radioactivity was excreted in feces than in urine whereas at lower doses proportions were roughly equal. Within 24 hours more than 81 percent of the administered dose was recovered in urine and feces.

In the study of expired air in rats the objective was to look at radioactivity in expired CO_2. The amounts eliminated over 48 hours in expired air were minimal, 2.4 and 2.8% for males and females respectively.

In summary, the metabolism data for EL-107 demonstrate a potential area for concern, which is bioaccumulation of the test substance in the carcass of rats, particularly males, as demonstrated in Study RO2186, "Excretion of ^{14}C EL-107 in expired air".

Chronic and Oncogenicity Testing

1. Chronic/oncogenicity in mice, MO0883, MO0983, Lilly Research, 11/85, Accession No. 265737, 265738.

2. Chronic/oncogenicity in rats, RO1583, RO1683, Lilly Research, 11/85, Accession No. 265735, 265736.

3. Memorandum, Isoxaben Rat and Mouse Study- Qualitative Risk Assessment of Combined Toxicity and Oncogenicity Study, Nelson to Jones, 5/1/87.

The chronic/oncogenicity study in B6C3F$_1$ mice (0, 0.01, 0.1, 1.25%) demonstrated that the test compound is capable of inducing abnormal cell proliferation and degeneration in the liver of B6C3F$_1$ mice. Observed neoplastic abnormalities were increases in hepatocellular adenomas at the high dose, a mild increase in hepatocellular carcinomas in females only at the high dose, and increases in combined adenomas and carcinomas at the high dose in males and females, as shown in the table below. Neoplastic findings were supported by such nonneoplastic findings as hepatocellular hyperplasia, hepatocellular vacuolation (increased in incidence and severity with dose), and hepatocellular cytomegaly. Additional liver toxicity was demonstrated by increased absolute and relative liver weight at the high dose, by elevated levels of alkaline phosphatase in males only and increased alanine transaminase at the high dose. The compound did not demonstrate decreased latency. The no observed effect level for accompanying systemic effects was 100 ppm (increased hepatocellular vacuolation).

Isoxaben-Mouse Study Hepatocellular Carcinoma, Adenoma, and Combined Carcinoma and/or Adenoma Rates

Dose (ppm)	0	100	1000	12500
Males				
Hepatocellular carcinomas	9/56[a] (16)	5/49 (10)	5/55 (9)	3/55 (5)
Hepatocellular adenomas	3/44** (7)	1/41 (2)	3/47 (6)	14/48** (29)
Combined carcinomas and/or adenomas	12/56** (21)	6/49 (12)	8/55 (15)	17/55 (31)

Isoxaben- Mouse Study (Cont'd)

Females

Hepatocellular carcinomas	0/52 (0)	1/52 (2)	0/46 (0)	2/52 (4)
Hepatocellular adenomas	0/52** (0)	3/52 (6)	2/46 (4)	7/52** (13)
Combined carcinomas and/or adenomas	0/52** (0)	4/52 (8)	2/46 (4)	9/52** (17)

a. Tumor bearing animals/animals at risk with percent in parentheses.
First male tumor observed at 82 weeks in the control group.
First female tumor observed at 104 weeks in the 100 ppm group.
The significance of Trend Analysis (Cochran-Armitage Trend Test) noted at the Control.
The significance of pairwise comparison (Fisher's Exact Test) noted at the Dose level.
(*p<0.05, **p<0.01)

Analysis of the results has shown no survival disparities in the mouse study. The statistical team found that time-adjusted analysis was therefore not necessary. The Fisher's Exact Test was used for pairwise comparisons (control versus dose groups) and the Cochran-Armitage Trend Test was used for trends. The analysis showed no significant differences in hepatocellular carcinoma in controls as compared to dose groups in either sex. There were significant trends for adenomas alone and adenomas and carcinomas combined in both sexes. High dose males and females had significantly more adenomas than controls. High dose females had significantly more combined adenomas and carcinomas than controls whereas males did not.

The chronic/oncogenicity study in rats (0, 0.0125, 0.125, and 1.25%) demonstrated toxicity of Isoxaben in the liver, kidney, and adrenal gland. Liver effects were hepatocellular adenomas found in very low incidence at the mid and high doses. Kidney findings included progressive glomerulonephrosis increasing in incidence and severity with dose in both males and females. This was analysed in the following table. Mineralization of the aorta, stomach mucosae, kidney, and heart are sequelae of the above condition and reflect the apparent renal failure. Parathyroid hyperplasia increased at the high dose in the few animals examined. Pheochromocytomas (adrenal gland) were found in males and in very low numbers in females. The histopathology results were analysed by the Toxicology Branch Statistics Team and are reported below along with the significance of the results. Clinical chemistry parameters reflected the observed histopathology. Increased blood urea nitrogen

(BUN) levels and creatinine reflect the kidney changes. Several organ weights and the ratio of kidney/bodyweight, liver/bodyweight, brain/bodyweight were elevated at the high dose in males and females.

Isoxaben-Rat Study Progressive Glomerulonephrosis Rates

Dose (%)	0	0.0125	0.125	1.25
Males	26/58**[a] (47)	33/59 (56)	40/57** (70)	51/58** (88)
Females	34/61** (56)	27/59 (46)	35/60 (58)	47/60** (78)

a. Tumor bearing animals/animals at risk with percent in parentheses.
First male observation made at 70 weeks in 1.25% dose group.
First female observation made at 70 weeks in 0.125% dose group.
Significance of Trend Analysis (Cochran-Armitage Trend Test) noted at Control.
Significance of pairwise comparison (Fisher's Exact Test) noted at Dose level. (*p<0.05,**p<0.01)

Isoxaben-Rat Study Adrenal Cortex Tumor (Pheochromocytoma) Rates

Dose (%)	0	0.0125	0.125	1.25
Males	11/59*[a] (19)	10/59 (17)	9/59 (15)	18/59 (31)
Females	3/49 (6)	2/45 (4)	4/49 (8)	1/47 (2)

a. Tumor bearing animals/animals at risk with percent in parentheses.
First male tumor observed at 66 weeks in 1.25% dose group.
First female tumor observed at 102 weeks in 0.125% dose group.
The significance of Trend Analysis (Cochran-Armitage Trend Test) noted at the Control. The significance of pairwise comparison (Fisher's Exact Test) noted at the Dose level. (*p<0.05, **p<0.01)

Analysis of the results found an increasing trend in mortality in male rats but not in females. Since most of the lesions in males occurred late it was not necessary to run a time-adjusted analysis in spite of the observed survival trend. The analysis showed a significant trend in pheochromocytomas among males but no pairwise differences between controls and dose groups. Females showed no trends or pairwise differences in pehochromocytoma. Analysis of lymphosarcoma data showed no significant trend or pairwise differences. There was a significant trend for progressive glomerulonephrosis (PGN) in males and females. At the high dose PGN was significantly greater than controls in both males and females and at the mid dose PGN was significantly greater in males.

Mutagenicity

1. Chromosome aberrations in vivo (dominant lethal test in rats), R01984, Eli Lilly Research, 10/85.

2. Gene mutation in bacteria (S.typhimurium, Ames Test), 841001AMS1378, Eli Lilly Research, 10/84.

3. Chromosome aberrations in vivo (mouse micronucleus), 871, CERTI, 9/6/84.

The three categories of mutagenicity testing are marginally complete for Isoxaben.

Under the category of "gene mutation", an adequate Ames test has been reviewed (Lilly Study 84100) and was found negative for inducing revertants in S. Typhimurium up to 500 ug/plate.

Under the category of "structural chromosome aberrations" one study (CERTI Study 871), a mouse micronucleus test, was inconclusive. The test was positive for inducing micronuclei after a single oral gavage dose of 5000 mg/kg. However, females were not tested; the dose was apparently not high enough to demonstrate cytotoxicity; and samples of bone marrow were taken at 24-hour instead of 12-hour intervals. In addition, the percent active ingredient tested was not stated in the test report. A rat dominant lethal test (Lilly Study R01984) was provisionally acceptable. The study was negative for inducing lethals in males fed levels up to 1.25% (12500 ppm). However, required positive control data were not included. A sister chromatid exchange (Lilly, no number) was unacceptable.

Under the category of "other genotoxic effects", the study of DNA repair in rat hepatocytes (unscheduled DNA synthesis) was acceptable. The study was negative in two assays but positive in positive controls.

The category of mutagenicity testing is marginally satisfied with one acceptable study in each of the three required categories. Once additional information is received for the mouse micronucleus study, the provisional qualification can be removed and the study can be considered fully acceptable.

Mutagenicity testing has shown that Isoxaben is apparently not a mutagen. However, although the mouse micronucleus test was inconclusive, there is some evidence that micronuclei were induced by the compound, as discussed above.

Tox Chem No. 419F - EL 107 File Last Updated 11/9/84

Study/Lab/Study #/Date	Material	EPA Accession No.	Results: LD_{50}, LC_{50}, 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 technical and formulation FN - 7033	252915	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

Tox Chem No. 419I - EL 107

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; 12/81	EL-107 92.4% spiked with 14C-EL-101 100%	250791	Doses: 10, 100, 250, 500 and 1000 mg/kg (single dosing only). Collections were made of urine and feces during 1st, 2nd, and 3rd 24 hr. periods. Decreasing ratios of urinary to fecal excretion indicates that gastrointestinal 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-0-5082 & R-0-6182; 12/82	EL-107 92.4% spiked with 14C-EL-107 100%	250791	Some animals were killed after 4 hrs., some after 24 hrs. Build up in some tissues was quite large at 24 hrs. Tissue distribution was shown but distribution and possible storage could be better elucidated by a longer-term study.		Acceptable 003434

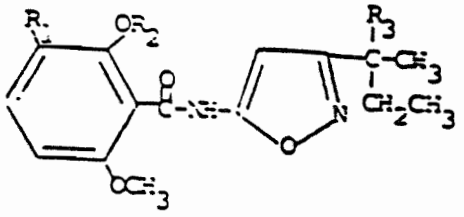
Tox Chem No. 419F - EL 107

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	Not sensitizing		Minimum 004034
Primary dermal irritation - rabbit; Eli Lilly and Co.; #B-D-58-81; 9/81	EL-107	250791	no irritation from 200 mg/kg.	IV	Minimum 003434
Acute dermal LD_{50} - rabbit; Eli Lilly & Co.; #B-D- 58-81; 9/81	EL-107	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	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	250791	LC_{50} > 1.99 + 0.199 mg/L (11.5 mg/L nominal)	II	Minimum 003434
Acute oral LD_{50} - mice; Eli Lilly & Co.; #M-O-49-81 & M-O-50-81; 4/81	EL-107 25%	250791	LD_{50} > 10,000 mg/kg (only dose tested)	IV	Guideline 003434
Acute intraperitoneal LD_{50} - mice; Eli Lilly & Co.; #M-P-18-81 & M-P-19-81; 4/81	EL-107 25%	250791	LD_{50} > 5000 mg/kg		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.
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 and Co.; #RO-545-79 & RO-546-79; 4/81	formulation-50% EL-107 (50W)	250791	Oral LD_{50} > 500 mg/kg (HDT)	III	Minimum 003434
Acute dermal LD_{50} - rabbit; Eli Lilly & Co.; BD-169-79; 4/81	formulation 50% EL-107 (50W)	250791	Dermal LD_{50} > 2000 mg/kg only dose tested	III	Minimum 003434
Primary dermal irritation - rabbit; Eli Lilly and Co.; #B-D-169-79; 4/81	formulation 50% EL-107 (50W)	250791	Dermal irritation slight.	IV	Minimum 003434
Primary eye irritation-rabbit; Eli Lilly & Co.; #B-E-186-79; 4/81	formulation 50% EL-107 (50W)	250791	Slight to moderate conjunctivitis, corneal dullness, mild iritis. One eye positive sodium fluorescein response at 24 hrs. All clear within 7 days.	II	Guideline 003434
Acute inhalation LC_{50}-rat; Eli Lilly and Co.; #R-H-88-79; 4/81	formulation 50% EL-107 (50W)	250791	LC_{50} > 2.42 mg/L (only level tested)	III	Minimum 003434
Acute oral LD_{50} - rat; Eli Lilly & Co.; #R-O-48-81 & R-O-49-81; 4/81	formulation 50% EL-107 (50W)	250791	LD_{50} > 10,000 mg/kg (only level tested)	IV	Guideline 003434

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 STUDY RESULTS:

This study was well conducted. The test material, animals, and methodology were adequately described, and the results supported the authors' conclusions. The authors concluded that virtually all of the EL-107 equivalents were excreted by the end of 3 days. Although this statement is generally correct, there are some points that should have been more thoroughly clarified. The sum of urinary and fecal [^{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

ISOXABEN 125851

Page_____ is not included in this copy.

Pages 70 through 85 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.

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

DATA EVALUATION REPORT

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

ACCESSION NUMBER: 265737, 265738 MRID NO.:

TEST MATERIAL: Isoxaben Technical

SYNONYMS: EL-107

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

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

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

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

A. MATERIALS:

1. Test compound: EL-107, a mixture of isomers consisting primarily of Lilly compound 121607, N-[3-(1-ethyl-1-methylpropyl)-5-isoxazolyl]-2,6-dimethoxybenzamide (85.0%), [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.

Hematology 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	Electrolytes:	X	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 CP
° 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.

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.

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.

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).

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.

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

Incidence of Neoplastic Lesions in the Liver of $B6C3F_1$ 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).

D. DISCUSSION:

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

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

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

2. Food and water consumption were not reported for this study. Food and water consumption should be measured for the first 13 months and then at monthly intervals according to the Pesticide Assessment Guidelines, Subpart F. Compound intake as reported must be considered approximate for this study. Food efficiency cannot be calculated from this information.

3. Hematology: Blood smears in 10/sex/dose were not taken at 12 and 18 months, as specified in the Guidelines. The effect of the test compound on blood cell morphology during the study is therefore unknown. Body weight measurements and clinical observations are the only measures of toxicity during the course of the test.

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Pages 100 through 127 are not included.

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____ Description of the product manufacturing process.

____ Description of quality control procedures.

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Reviewed by: Marcia van Gemert, Ph.D. Marcia van Gemert 1/14/87 007755
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 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 92.6% for females of the dose administered. Expired air accounts for a very small amount of the total radioactivity. Males expired 2.4% and females 2.8% of the dose administered in 48 hours. There is not complete recovery at least in males at 48 hours of total radioactivity. There may be some interference with sample recovery, or incomplete recovery from the carcass. This 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

A. MATERIALS·

1. Test compound: EL-107, Batch # Z 10025,
EL-107 is a mixture of two predominant isomers designated 121607 and 135520. These are known chemically as N-[3-(1-ethyl-1-methylpropyl) -5-isoxazolyl[-2,6-dimethoxybenzamide and N-[3-(1,1-dimethylbutyl) -5-isoxazole[-2,6-dimethoxybenzamide. Initial lot analysis

[redacted]

Lot number 553-3nl-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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Pages 131 through 133 are not included.

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Reviewed by: Marcia van Gemert, Ph.D. M van Gemert 1.16.87 000755
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 component is ≥ 85%. The label is in the alpha position of the isoxazole ring.

Test Article preparation:

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

2. Test animals: Species: mouse, Strain: ICR, Age: not given
Source: not given
Weight: 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 cocktail (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 apopended 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.

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.

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

CAGE NUMBER	DOSE GROUP (MG/KG)	CUMULATIVE PERCENT OF DOSE								
		URINE			FECES			TOTAL URINE AND FECES		
		24 HR.	48 HR.	72 HR.	24 HR.	48 HR.	72 HR.	24 HR.	48 HR.	72 HR.
1001	1.0	16.1	16.3	16.4	38.4	41.0	41.4	54.5	57.3	57.9
1002		40.7	42.0	42.2	41.0	44.2	44.4	81.7	86.2	86.6
1003		50.5	50.9	51.0	35.2	36.1	36.3	85.7	87.0	87.4
1004		47.8	48.3	48.5	43.5	44.5	44.7	91.3	92.8	93.2
1005		36.5	37.3	37.3	47.2	49.1	49.5	83.7	86.4	86.9
MEAN		39.3	39.0	39.1	41.1	43.0	43.3	79.4	82.0	82.4
S.E.		6.1	6.1	6.2	2.1	2.2	2.2	6.4	6.3	6.2
2001	15.0	46.2	46.5	46.6	47.8	48.9	49.1	94.0	95.4	95.7
2002		40.9	41.5	41.6	46.4	47.7	47.8	87.2	89.2	89.4
2003		41.1	43.0	43.4	42.8	47.2	47.8	83.9	90.2	91.2
2004		34.7	37.6	37.8	48.1	51.2	51.6	84.7	88.7	89.3
2005		36.0	36.7	36.7	57.9	58.9	59.4	93.9	95.5	96.1
MEAN		40.2	41.0	41.2	48.6	50.8	51.1	88.7	91.8	92.3
S.E.		1.8	1.8	1.8	2.5	2.1	2.2	3.2	1.5	1.5
3001	100.0	21.5	21.9	21.9	67.4	68.7	68.9	88.9	90.6	90.8
3002		22.3	22.5	22.6	71.5	72.3	72.4	93.7	94.9	95.0
3003		18.6	18.9	19.0	71.1	72.1	72.3	89.8	91.1	91.3
3004		21.8	22.7	22.8	68.1	69.4	69.5	89.9	92.1	92.3
3005		25.2	25.5	25.6	71.3	73.0	73.3	96.4	98.5	98.9
MEAN		21.9	22.3	22.4	69.9	71.1	71.3	91.7	93.4	93.7
S.E.		1.0	1.1	1.1	0.9	0.9	0.9	1.4	1.5	1.5

TABLE 1. (CONTINUED)
STUDY M93082

CAGE NUMBER	DOSE GROUP (MG/KG)	CUMULATIVE PERCENT OF DOSE								
		URINE			FECES			TOTAL URINE AND FECES		
		24 HR.	48 HR.	72 HR.	24 HR.	48 HR.	72 HR.	24 HR.	48 HR.	72 HR.
4001	200.0	15.3	16.2	16.3	65.6	69.8	70.2	80.9	86.0	86.5
4002		17.1	18.5	18.8	53.7	66.7	68.0	70.8	85.2	86.8
4003		13.7	16.8	17.0	62.8	80.1	80.4	76.5	96.9	97.4
4004		13.9	14.7	14.8	74.1	75.6	75.9	88.0	90.3	90.7
4005		14.8	15.5	15.6	73.7	77.5	77.7	88.5	93.0	93.3
MEAN		15.0	16.3	16.5	66.0	73.9	74.5	81.0	90.3	91.0
S.E.		0.6	0.6	0.7	3.8	2.5	2.3	3.4	2.2	2.0

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

DATA EVALUATION REPORT

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

ACCESSION NUMBER: 265749 MRID NO.: ?

TEST MATERIAL: EL-107

SYNONYMS: Isoxaben

STUDY NUMBER(S): R11265

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 if one or more time periods beyond 24 hours were used for evaluation, since it appears that significant radioactivity remains in tissues. Section F Guidelines recommend that the study be carried out long enough to eliminate 90% of the radioactivity or that the study be carried out for 7 days, whichever comes first.

Classification: core-Supplementary

Special Review Criteria (40 CFR 154.7)

A. MATERIALS:

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

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 anaesthesia, samples of blood were taken and centrifuged for packed cell volume. Animals were exanguinated and organs were removed. Tissue preparation is on appended page 1. Appended page 2 details radioactivity quantification and packed cell volume determinations.

RESULTS:

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

hours. Many tissue/plasma concentrations were higher than 1.0 by 24 hours. Table 1 details these ratios.

TABLE 1

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

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

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

Table II
% of Administered Dose

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

DATA EVALUATION REPORT

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

ACCESSION NUMBER: 265740
MRID NO.: ?

TEST MATERIAL: EL-107

SYNONYMS: Isoxaben

STUDY NUMBER(S): R13665

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 N-[3-(1,1-dimethylbutyl) -5-isoxazole]-2,6-dimethoxybenzamide. [redacted] 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:
For the two weeks prior to radioactive EL-107 administration, unlabeled EL-107 was given at a dose of 25 mg/kg in 10% aqueous acacia and prepared daily. Each rat was given 1 ml/100 kg body weight by gavage which equals 250 mg/kg/day. The radioactive dose on day 15 consisted of both label and unlabeled EL-107 combined to yield 25 mg/ml solution with 0.13 uCi/mg specific activity. This solution was suspended in 10% acacia to yield 25 mg/ml and then evaporated.

2. Test animals: Species: rat, Strain:Fischer 344, Age: 9-10 weeks
Source: Harlan Sprague Dawley, Indianapolis Indiana.
Weight: males: 175.6 ± 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

RBC/Plasma ratios

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

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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 K11285. 80.59% of the radioactivity was excreted within 7 days for males and 96.1% excreted in females when combining urinary and fecal excretion. Significant amounts of radioactivity did not remain in the organs and tissues after 7 days post dosing with radiolabeled EL-107, with carcass radioactivity under 0.3% for males and 0.4% for females of the administered dose.

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Reviewed by: Marcia van Gemert, Ph.D. [illegible] 1/16/87 00,755
Head, Section III, Tox. Branch (TS-769C)
Secondary reviewer: Theodore M. Farber, Ph.D.
Chief, Tox. Branch (TS-769C)

005732

DATA EVALUATION REPORT

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

ACCESSION NUMBER: 265729 MRID NO.: ?

TEST MATERIAL: EL-107

SYNONYMS: Isoxaben

STUDY NUMBER(S): M03182

SPONSOR: Elanco

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

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

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

REPORT ISSUED: Sept. 1986

CONCLUSIONS:

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

Classification: core-supplementary

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:females: 17-22 grams

B. STUDY DESIGN:

1. Animal assignment

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

2. Quality assurance statement was not given.

3. Procedures:

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

4. Results:

Figure 2 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 3.

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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Section III, Tox. Branch (TS-769C)
Secondary reviewer: Theodore M. Farber, Ph.D.
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 1683

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)

A. MATERIALS:

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

Description- not given,
Batch # 710025, Purity 94.8%,

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

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 collectec at random. One-half of the samples were stored at 25°C and the other half at 37°C for 0,1, 2, and 4 weeks for stability measurements.

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

3. Animals received food, mash feed of Purina Certified Rocent 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 deaths are not an important consideration in the adjustment of the tumor incidence.

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

2. Body weight

Animals were weighed weekly for the duration of the experiment.

Results: Appended pages 2 and 3 give a summary of each replicate experiment's terminal body weight and body weight gain. In both replicates there was a significant drop in body weight and body weight gain in both males and females at the high dose ($p > 0.05$) throughout the course of the study there was intermittantly 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.

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 CP
° 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 hich 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 nigh dose, and mid dose males at 18 months actually showed an increase in alkaline phosphatase. At terminal sacrifice carciac puncture samples, only mid dose females in replicate experiment RO 1583 appeared to have decreased alkaline phosphatase levels.

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

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

TABLE I

Cardiac Puncture Terminal Blood Samples
Clinical Chemistry Parameters

	Study RO 1583		Study RO 1683	
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

* $p > 0.05$

6. 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

° 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	.Thyroids*††
	Gall bladder*#	XX	Prostate		Other
X	.Pancreas*		Seminal vesicle	X	Bone*#
	Respiratory	XX	Ovaries*†	X	Skeletal muscle*#
X	.Trachea*	XX	.Uterus*	X	Skin*#
X	.Lung*			X	All gross lesions and masses*
	Nose°				
	Pharynx°				
	Larynx°				

* Required for subchronic and chronic studies
° Required for chronic inhalation
In subchronic studies, examined only if indicated by signs of toxicity or target organ involvement
† Organ weights required in subchronic and chronic studies
†† Organ weight required for non-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 1683 showed a significant increase in liver weights. These data are presented on table III.

Organ/body weight ratios:

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

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

Organ/brain weight ratios:

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

Table III

TERMINAL ORGAN WEIGHTS

Study RO 1583 Males		Study RO 1683 Males	
Group	Prostate g/100g	Group	Liver g/100g
1	0.396	1	12.469
SD	0.137	SD	2.025
2	0.446	2	13.229
SD	0.183	SD	3.983
3	0.375	3	13.135
SD	0.190	SD	2.133
4	0.297*	4	13.882*
SD	0.098	SD	1.792

Table IV

TERMINAL ORGAN WEIGHTS RELATIVE TO BODY WEIGHTS

Study RO 1583

Males:

	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

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	0.391	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 V

TERMINAL ORGAN WEIGHTS RELATIVE TO BRAIN WEIGHTS

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

* = $p > 0.05$

b. Gross pathology

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

c. Non-neoplastic

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

There was an increase in parathyroid hyperplasia at the high dose. Very few animals were examined, but except for one control animal, every parathyroid examined showed hyperplasia. (see table II) The protocol did not specify examination of the parathyroids as routine histopathological tissue treatment, and enlarged parathyroids were not noted 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.

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

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 61.8 mg/kg for females.

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

ISOXABEN 125851

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007755

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

MAY 1 - 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Isoxaben, Rat and Mouse Study - Qualitative Risk Assessment of Combined Toxicity and Oncogenicity Study. Caswell #419F

FROM: C.J. Nelson, Statistician cjnelson 5/1/87
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

TO: Margaret Jones
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and

Reto Engler, Ph.D., Chief
Scientific Mission Support Staff
Toxicology Branch
Hazard Evaluation Division (TS-769C)

Summary:

In this two-year chronic oral study of male and female Fisher 344 rats and male and female B6C3F1 mice, a significant increasing trend in mortality with dose was found for the male rats. No survival disparities were found in the mice or female rats. That is there were no trends, no heterogeneity, and no pairwise differences with controls. Since most of the lesions in male rats occurred late, there was no need to adjust for mortality, since the table would collapse to one or two intervals.

For the rats, there was a significant increasing trend with dose for male pheochromocytomas, and both male and females progressive glomerulo nephrosis (PGN). The 0.125% and 1.25% male dose groups were significantly higher than controls as were the females at the 1.25% dose group.

For the mice, there was a significant increasing trend with dose for both male and female hepatocellular adenoma only and pooled hepatocellular adenoma and/or carcinoma. The high dose (125000) males and females had significantly more adenomas than the controls. The high dose (125000) females had significantly more adenomas and/or carcinomas than the controls. There were no trend or pairwise differences between dosed animals and control animals with hepatocellular carcinoma of either sex.

Background:

This study was conducted at Lilly Research Laboratories on male and female Fischer 344 rats and on male and female B6C3F1 mice. The study was done in two replicates, but were so close in time that the two studies were pooled. Technical Isoxaben was administered to 60 rats of both sexes at 0.0125%, 0.125%, and 1.25% in the diet (Study Numbers RO1583 and RO1683). Technical Isoxaben was administered to 60 mice of both sexes at 100ppm, 1000ppm, and 12500ppm in the diet (Study Numbers MO0883 and MO0983). There was also a concurrent control group of 60 animals for both sexes of both species.

Mortality Analysis:

The Thomas, Breslow, Gart Procedure (1977) was used to analyze the survival data. There was a significant trend ($p = .05$) for male rats with mortality increasing with increasing doses of Isoxaben (Table 1) using Cox's test (1972) for life table data. But there was no departure from trend. There were no survival disparities in female rats. There were no significant pairwise comparisons for either sex between the control and any treated group. The pairwise test of control versus high dose was nearly significant for male rats ($p = .09$ by Cox's test).

There were no survival disparities in male or female mice with increasing doses of Isoxaben (Table 2). The same procedure was used to analyze the mouse survival data as was used in the rat data. There were no significant pairwise comparisons for either sex between the control and any treated group.

Table 1. Isoxaben - Rat Study, Mortality Rates[+] and Cox or Generalized K/W Test Results

A. Males

Dose (%)	WEEKS 0-26	27-52	53-78	79-105[a]	TOTALS
0	0/59	0/59	3/59	17/56	20/59 (34)*
.0125	0/60	1/60	0/59	25/59	26/60 (43)
.125	0/60	1/60	4/59	19/55	24/60 (40)
1.25	0/60	0/60	7/60	24/53	31/60 (52)

B. Females

Dose (%)	WEEKS 0-26	27-52	53-78	79-105[a]	TOTALS
0	0/61	0/61	0/61	14/61	14/61 (23)
.0125	0/60	0/60	4/60	16/56	20/60 (33)
.125	0/60	0/60	3/60	11/57	14/60 (23)
1.25	0/60	0/60	0/60	18/60	18/60 (30)

\+ Number of Animals Died/Number of Live Animals at the beginning of the interval.
() Percent
a Final sacrifice was at 104 or 105 weeks.

Note - The above survival tables are broken into aggregate time intervals for display purpose only.
Significance of Trend Analysis denoted at Control.
Significance of pairwise comparison with control denoted at Dose level. (* $p < .05$ ** $p < .01$)

Table 2. Isoxaben - Mouse Study, Mortality Rates[+] and Cox or Generalized K/W Test Results

A. Males

Dose (ppm)	WEEKS 0-26	27-52	53-78	79-105[a]	TOTALS
0	0/60	0/60	2/60	15/58	17/60 (28)
100	7/60	0/53	2/53	9/51	18/60 (30)
1000	2/60	1/58	1/57	9/56	13/60 (22)
12500	2/60	0/58	2/58	10/56	14/60 (23)

B. Females

Dose (ppm)	WEEKS 0-26	27-52	53-78	79-105[a]	TOTALS
0	0/60	1/60	0/59	7/59	8/60 (13)
100	1/60	1/59	1/58	5/57	8/60 (13)
1000	2/59	1/57	2/56	9/54	14/59 (24)
12500	0/60	0/60	2/60	6/58	8/60 (13)

\+ Number of Animals Died/Number of Live Animals at the beginning of the interval.
() Percent
a Final sacrifice was at 104 or 105 weeks.

Note - The above survival tables are broken into aggregate time intervals for display purpose only.
Significance of Trend Analysis denoted at Control.
Significance of pairwise comparison with control denoted at Dose level. (* $p < .05$ ** $p < .01$)

Tumor Analysis:

Pheochromocytoma, Lymphosarcoma, and Progressive Glomerulo Nephrosis (PGN) was analyzed for the rat studies (Table 3, 4, and 5 respectively). Although there was a survival trend in the male rat, most of the lesions occurred late. Since there were no pairwise differences between control and dosed male rats, a time-adjusted analysis was not necessary. There were no survival disparities for the female rat or either sex of the mice. Therefor the Fisher's Exact Test was used for pairwise comparisons and the Cochran-Armitage Test was used to test for trends. There was a significant trend ($p = .014$) for Pheochromocytoma among male rats but no heterogeneity, and no pairwise differences were detected. There was no significant trend, heterogeneity, or pairwise comparisons for Pheochromocytoma among female rats. There were no significant trend, heterogeneity, or pairwise comparisons with control for Lymphosarcoma for either sex. There was a highly significant trend for both sexes ($p < .001$) for PGN. The heterogeneity Chi-square for males was significant ($p = .03$) but was not significant for females. There was significantly more PGN at the high dose (1.25%) than the controls (males $p = .001$, females $p = .007$). Also the mid dose (0.125%) males were significantly higher ($p = .005$) than controls.

Hepatocellular carcinoma, adenoma, and adenoma/carcinoma was analyzed for the mouse studies (Table 6, 7, and 8 respectively). There were no survival disparities in the mouse studies, hence the same tests were used in these analyses as those used in the rat study. There were no significant differences found for hepatocellular carcinoma for either sex. There was a significant trend for hepatocellular adenoma for both sexes (males $p < .001$, females $p = .004$). The high dose (12500ppm) mice had significantly more adenomas than the controls for both sexes (males $p = .005$, females $p = .006$). There was a significant trend for adenomas and carcinomas combined (males $p = .01$, females $p = .001$). The high dose (12500) female mice had significantly more combined tumors than the controls ($p = .001$) but the high dose males were not significant ($p = .18$).

Table 3. ISOXABEN - Rat Study, Adrenal Cortex Tumor (Pheochromocytoma) Rates+ and Cochran-Armitage Trend Test and Fisher's Exact Test Results

Dose (%)	0	0.0125	0.125	1.25
Males	11/59 (19)*	10/59 (17)	9/59 (15)	18/59 (31)
Female	3/49 (6)	2/45 (4)	4/49 (8)	1/47 (2)

First male tumor observed at 66 weeks in 1.25% dose group. First female tumor observed at 102 weeks in 0.125% dose group.

Table 4. ISOXABEN - Rat Study, Lymphosarcoma Rates+ and Cochran-Armitage Test and Fisher's Exact Test Results

Dose (%)	0	0.0125	0.125	1.25
Males	1/59 (2)	2/60 (3)	4/60 (7)	3/60 (5)
Female	2/47 (4)	0/40 (0)	1/46 (2)	3/42 (7)

First male tumor observed at 46 weeks in 0.125% dose group. First female tumor observed at sacrifice.

Table 5. ISOXABEN - Rat Study, Progressive Glomerulo Nephrosis Rates+, Cochran-Armitage Trend Test, and Fisher's Exact Test Results

Dose (%)	0	0.0125	0.125	1.25
Males	26/58 (47)**	33/59 (56)	40/57 (70)**	51/58 (88)**
Female	34/61 (56)**	27/59 (46)	35/60 (58)	47/60 (78)**

\+ Tumor Bearing Animals/ Animals at Risk
First male tumor observed at 70 weeks in 1.25% dose group. First female tumor observed at week 70 in 0.125% dose group.

Note - Significance of Trend Analysis denoted at Control. Significance of pairwise comparison with control denoted at Dose level. (* $p < .05$, ** $p < .01$)

Table 6. ISOXABEN - Mouse Study, Hepatocellular Carcinoma Rates+, Cochran-Armitage Trend test, and Fisher's Exact Test Results

Dose (ppm)	0	100	1000	12500
Males	9/56 (16)	5/49 (10)	5/55 (9)	3/55 (5)
Female	0/52 (0)	1/52 (2)	0/46 (0)	2/52 (4)

First male tumor observed at 82 weeks in control group. First female tumor observed at 104 weeks in 100ppm dose group.

Table 7. ISOXABEN - Mouse Study, Hepatocellular Adenoma Rates+, Cochran-Armitage Trend test, and Fisher's Exact Test Results

Dose (ppm)	0	100	1000	12500
Males	3/44 (7)**	1/41 (2)	3/47 (6)	14/48 (29)**
Female	0/52 (0)**	3/52 (6)	2/46 (4)	7/52 (13)**

First male tumor observed at 103 weeks in 12500 ppm group.
First female tumor observed at sacrifice.

Table 8. ISOXABEN - Mouse Study, Hepatocellular Adenoma and/or Carcinoma Rates+, Cochran-Armitage Trend test, and Fisher's Exact Test Results

Dose (ppm)	0	100	1000	12500
Males	12/56 (21)**	6/49 (12)	8/55 (15)	17/55 (31)
Female	0/52 (0)**	4/52 (8)	2/46 (4)	9/52 (17)**

+ Tumor Bearing Animals/ Animals at Risk.
First male tumor observed at 82 weeks in control group.
First female tumor observed at 104 weeks in 100ppm dose groups.

Note - Significance of Trend Analysis denoted at Control. Significance of pairwise comparison with control denoted at Dose level. (* $p < .05$, ** $p < .01$)

Bibliography:

Thomas, D G, N Breslow, and J J Gart, Trend and Homogeneity Analyses of Proportions and Life Table Data, Computers and Biomedical Research 10, 373-381, 1977.

Cox, D.R. Regression Models and Life Tables (with discussion). J. Roy. Stat. Soc. Ser. B. 34, 187-220, 1972.

ISOXABEN 125851

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TB 7-0037
(7-0139)

TOXICOLOGY BRANCH: DATA REVIEW

Caswell: 419F
EPA Chem: 125851

Chemical: Isoxaben (EL-107)

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

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

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

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

01-07-87

Judith W. Hausenblas
1/9/87

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TB 7-0037
(0337)

TOXICOLOGY BRANCH: DATA REVIEW

125732

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: 073441

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-amino-acridine (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

J.W. Hamsworth
1/8/87

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] One of the plates was believed to have been inoculated twice; therefore, [illegible] at the mean of two plates
[f] One plate counted due to presence of a chemical precipitate.

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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 #210025), 95.5% ai, a mixture of isomers ([redacted], incorporated into feed for dietary administration.

TB Conclusions/Evaluation:

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

Procedures:

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

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.

ISOXABEN 125851

Page_____ is not included in this copy.

Pages 229 through 239 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.

007755

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

FEB 12 1987

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Staff Participation in the Development of Risk Assessment Guidelines for Non-Cancer Health Risks.

FROM: Douglas D. Campt, Director
Office of Pesticide Programs (TS-766C)

TO: Peter W. Preuss, Chairman
Risk Assessment Forum (RD-689)

This is in reply to your memo of December 31, 1986, requesting Dr. Reto Engler's participation in an inter-office Workgroup on risk assessment guidelines for non-cancer health effects.

I approve his participation in this important Agency effort. Dr. Engler's experience in long-term non-cancer risk assessment makes him well suited to represent OPP on the workgroup. We also have appointed Drs. Esther Rinde and John Quest (both of the Toxicology Branch) to assist on the subcommittees of the Workgroup dealing with "less than lifetime exposure/risk" and "severity of effects" respectively.

cc: Pam Bassford (RD-689)
Reto Engler TOX
John Quest
Esther Rinde
John Melone
Theodore Farber

Miscellaneous

6/1/89

007755

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

JUN

OFFICE OF
PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: REVISION OF EXPOSURE ASSESSMENT FOR CHILDREN PLAYING ON RESIDENTIAL TURF TREATED WITH ISOXABEN

TO: Larry Schnaubelt
Product Manager 23
Registration Division (H7505C)

FROM: David Jaquith
Review Section 1
Non-Dietary Exposure Branch
Health Effects Division (H7509C)

THRU: Michael Firestone, Ph.D., Head
Review Section 1
Non-Dietary Exposure Branch
Health Effects Division (H7509C)

THRU: Charles L. Trichilo, Ph.D., Chief
Non-Dietary Exposure Branch
Health Effects Division (H7509C)

Please find below the NDEB review of ....

HED Project #: 9-1469A

RD of SRRD Record #: 245254, 245255

Registration #: _____

Caswell #: 419F

Company Name: Elanco Products

Date Received: 5/17/89 Action Code: 116

Date Completed: 5/31/89 Review Time: 20 days

Deferral to : _____ Biological Analysis Branch/BEAD

_____ Science Coordination and Analysis Branch

_____ TB - Insecticide/Rodenticide Support Branch

_____ TB - Herbicide/Fungicide/Antimicrobial Support Section

21

Elanco Products has submitted a rebuttal to an estimate of the exposure of children to their fungicide isoxaben, used on residential turf. The original submission did not contain any data specific to isoxaben but rather provided an exposure and risk assessment using surrogate dislodgeable residue data for another chemical used on turf. In response to this submission, NDEB provided an estimate for exposures of children on residential turf treated with isoxaben in a memorandum dated 14 March 1989 (1). Two methods, one estimating exposure from a correlation found in the scientific literature between dislodgeable residues and dermal exposure of fruit harvesters and the other derived using certain arbitrary assumptions were used for the assessment. Neither of these techniques has been substantiated. These two methods yielded similar dermal exposures, differing by only about 20 percent. The fruit harvester correlation, while adequate for some situations, was judged by the registrant not to be representative of a scenario where children might be playing on treated lawns. Estimates based on other assumptions were considered to be more appropriate for this scenario.

Most of the assumptions used by NDEB and the registrant were similar. The few assumptions that were different resulted in unequal estimates of exposure. Detailed explanations of the effects of changes for each of the assumptions are presented in the following sections.

EFFECT OF INITIAL DISLODGEABLE RESIDUE LEVELS OF ISOXABEN ON TREATED TURF

The registrant estimated the initial amount of dislodgeable isoxaben residue on a treated lawn to be 1.69 ug/cm^2 using a ratio of total to dislodgeable residues of 4.4. This factor was derived from data obtained in a field dissipation study measuring dislodgeable residues of the growth regulator flurprimidol (Cutless 50W) on turf. No data were submitted to support the use of this compound as a surrogate for isoxaben. The registrant cited similarities in usage pattern, application rate,and formulation type in defense of the use of this information to estimate isoxaben residues. Flurprimidol is not structurally similar to isoxaben. It is not certain whether isoxaben and flurprimidol exhibit the same properties with regard to dislodgeable residues and environmental fate. NDEB used the conservative assumption that the surface residue level was 11.2 ug per cm^2, approximately 6.6 times that estimated by the registrant using surrogate data from a different compound, and that all of this material was available for transfer to the child. It is NDEB's opinion that surrogate data, while useful for worker exposure estimation in some situations, are not applicable for any exposure estimate that can be appreciably affected by the nature of residues, environmental fate, other specific properties of the compound in question.

EFFECT OF DISSIPATION PATTERN OF ISOXABEN ON TURF

The proposed label requires that the treated turf be watered within 21 days of application to activate the fungicide. The assessment conducted by NDEB assumed that, since the effects of watering the treated area on residue levels are not known, the quantity of isoxaben residue on treated turf remained constant over a 21 day period. It was further assumed that all of the material deposited on a treated surface was available for transport to the skin. After this time all material was assumed to be dissipated and no further exposure would occur. The registrant assumed that residues dissipation followed first order kinetics and that this decay began immediately after application. The registrant referenced a soil dissipation study which included measurements of isoxaben in turf and in the first 6 inches of soil. The half life of isoxaben residues on treated turf was estimated to be 67 days. A soil dissipation study was reviewed in the Isoxaben Addendum to the Registration Standard. This study measured soil residues after application of isoxaben at 1.0 lb ai per acre. The initial total residues on the turf or in first six inches of soil were 0.96 lb per acre. The relative contribution of residues on the turf foliage to this total was not reported (2). The registrant then reduced this half life estimate to 21 days based on the behavior of other lawn treatment chemicals and on the assumption that the watering of the turf would decrease the surface residues within a 21 day period. After 21 days the residues were assumed to remain constant at 10 percent of those estimated for day 21. No supporting data for either of these assumptions are available. The two dissipation patterns assumed by NDEB and the registrant are presented in Figure 1. The soil dissipation study cited above found that, after 211 days and after receipt of 28 inches of water by the treated plot, the residues in the surface/six inch samples were still at a level of 0.17 lb ai per acre (1.9 ug per cm^2 if all material is assumed to be located on the surface). If the unsubstantiated assumption that dislodgeable residues are decreased by a factor of 4.4 is accepted, the dislodgeable residues would then be 0.43 ug/cm^2. The effect of dissipation pattern on exposure varies with the spacing of the exposure events. The overall exposure is more dependent on other assumptions rather than any specific dissipation pattern. If other assumptions are kept constant, the dissipation pattern changes the exposure by a factor of about 1.1-1.4

EFFECT OF CLOTHING

Both NDEB and the registrant assumed that the entire surface area of the body would be uniformly exposed to the surface residues of isoxaben and that transfer from the treated surface to the skin was 100 percent efficient. The surface areas of a 2-6 year old and a child at ages 7-12 were assumed to be 0.7 and 0.9 m^2, respectively. No adjustments were made for the wearing of clothing in NDEB's previous assessment. The trunk of the body of a child has been estimated to contribute approximately 34 percent to the

total surface area (3). If it is assumed that the trunk is covered by clothing (ie, wearing a tee shirt and shorts) and that the clothing completely protects covered areas, the dermal component of exposure would be reduced by that percentage. NDEB notes that clothing is unlikely to provide complete protection, particularly if it is wet or torn. Uniform exposure of each area of the body is not likely to occur and this factor contributes to the uncertainty associated with these estimates.

EFFECT OF ACTIVITY PATTERN

Both NDEB and the registrant assumed a total of 42 exposure days per year for 10 years (ages 2-12). NDEB assumed daily exposure for 21 consecutive days immediately after treatment with 2 applications per year. The registrant assumed the 42 exposure days were spread out over a 183 day period between two treatments. Children may be exposed to treated turf for more than 42 days, depending on climate. The average residues, assuming the registrant's dissipation pattern, were 0.22 ug per cm^2 as opposed to the 11.2 ug per cm2 under NDEB's scenario. Both parties assumed 100 percent transfer between turf residues and the skin and that the entire surface of a child is exposed only one time per day. The differences in the spacing of the exposure periods result in a 1.7-9 fold difference between the exposure estimates provided by NDEB and the registrant, depending on the other assumptions for a given scenario.

CONCLUSIONS

NDEB has calculated exposures for various combinations of initial residue level, dissipation order, and activity pattern. The calculations are explained in detail in Appendix A. The dermal component greatly exceeded exposure by the oral route. The registrant and NDEB used some different assumptions to estimate exposures. Data to support the assumptions used in any of these calculations are currently lacking. It is reasonable to assume that the watering of a treated lawn would affect the available residues in some way. However, since the soil dissipation study neglected to determine the fraction that remained in or on the treated turf, it is not possible to determine the magnitude of this effect. Appreciable residues were present in the turf/six inch fraction after considerable time and after extensive watering of the treated area. NDEB believes that, in lieu of additional information, any assumption of a decay pattern, such as the 21-day half life with 90 percent reduction after day 21, is unsupported and that the conservative assumption of no decay for 21 days is both prudent and appropriate. The registrant's assumption of a dislodgeable residue level of 1.69 ug per cm^2 is based on surrogate data for chemicals whose environmental fate and dislodgeable fraction may, or may not, adequately represent those of isoxaben. The assumption of complete dissipation after 21 days, which was used by NDEB, is not so conservative and may underestimate actual

dislodgeable residues. The differing assumptions and their approximate effects on the total exposures estimated are summarized in Table 1. The actual exposure estimates for each scenario are presented in Table 2 and also depicted in Figure 2.

NDEB notes that a number of other factors could influence the exposures of individuals to materials on residential turf. The number of exposure days could be quite different than the 42 assumed in this assessment. The clothing worn could range from minimal, such as a diaper or shorts to long sleeve shirt and trousers. The activity pattern will change as the individual grows older. Exposure is also likely to continue to some extent through adulthood. The effects of rainfall and the presence of moisture on the turf could affect both the dissipation of the material and the rate of transfer from the treated surface to the skin. The effects of these factors is not known. It is clear that the exposures of individuals to compounds used on residential turf presents a complex matrix of possibilities. Should Toxicology Branch decide that the risks justify a more refined exposure assessment additional data, both compound specific and addressing activity patterns, will be necessary.

POSSIBLE OPTIONS TO MITIGATE EXPOSURE

There are some options available for mitigating exposures of persons contacting treated residential turf that should be considered. Typical actions such as protective clothing, engineering controls and extended reentry intervals are not suitable for a residential turf scenario. One reasonable change that could be incorporated into the isoxaben label would be to limit the number of applications to one time per year. A second possibility would be to require watering the treated lawn before the 21 day interval currently recommended by the label. NDEB notes that the effect of watering on dislodgeable residues is currently unknown and that data addressing these residues would still be necessary in order to conduct a more reliable exposure assessment.

cc: Correspondence file
Isoxaben file
Circulation
TB-HFAS
SACB

Table 1. Comparison of Assumptions Used by Elanco Products and NDEB to Estimate the Exposures of Children Playing on Lawns Treated with Isoxaben (Gallery.

NDEB ASSUMPTIONS:	**Difference Factor**	**ELANCO ASSUMPTIONS:**
The surface residue on treated turf is 11.2 ug/cm^2. All of this is considered to be available for transfer to the skin. Isoxaben is applied at the maximum label rate of 1.33 lb product per acre (1.0 lb ai/A). Isoxaben is applied 2 times per year at 183 day intervals.	6.2-6.8	**Initial dislodgeable residue on treated turf is 1.69 ug/cm^2.** This estimate is based ratio of total to dislodgeable residues of 4.4 for a surrogate compound, flurprimidol, which is applied in the same manner. Flurprimidol is **NOT** structurally similar to isoxaben. Isoxaben is applied at rate of 0.75 lb product per acre, less than the maximum label rate. Isoxaben is applied 2 times per year at 183 day intervals.
Residues on the treated surface remain constant for 21 days after application. Following this interval all residues are assumed to have dissipated.	1.1-1.4	**Residues on the treated surface dissipate with a half life of 21 days. After 21 days the surface residues drop to 0.085 ug/cm^2 and remain constant at this level until the next treatment.** The average dislodgeable residue level over this interval is 0.22 ug/cm^2.

Table 1 (Continued). Comparison of Assumptions Used by Elanco Products and NDEB to Estimate the Exposures of Children Playing on Lawns Treated with Isoxaben (Gallery).

Exposure occurs once per day on the 21 days immediately following treatment. Dermal exposure occurs over the entire surface of the child (0.7 and 0.9 m^2 for a 2-6 year old and a 7-12 year old child, respectively). Repeated contact is assumed not to occur. Body weights are assumed to be 17 kg for children 2-6 and 31 kg for ages 7-12.	1.7-9	**Children are exposed to isoxaben for 42 days per year, spread over 183 day intervals after each treatment.** Such exposure occurs once per day. Repeated contact is assumed not to occur. Dermal exposure occurs over the entire surface of the child (0.7 and 0.9 m^2 for a 2-6 year old and a 7-12 year old child, respectively). Body weights are assumed to be 17 kg for children 2-6 and 31 kg for ages 7-12.
Oral exposure occurs from licking a surface equal to that of one hand **AND** from licking the surface of a 3 inch diameter ball. Transfer of isoxaben from the treated surface to the skin is assumed to be 100 percent efficient. Skin surface residues are equal to those on the treated area. Lifetime exposure of a child will encompass 10 years (ages 2-12). A child is exposed for 42 days per year.	1.03	Oral exposure occurs from licking a surface equal to that of one hand **OR** from licking the surface of a 3 inch diameter ball. Transfer of isoxaben from the treated surface to the skin is assumed to be 100 percent efficient. Skin surface residues are equal to those on the treated area. Lifetime exposure of a child will encompass 10 years (ages 2-12). A child is exposed for 42 days per year.

FIGURE 1.

ESTIMATED ISOXABEN RESIDUES ON RESIDENTIAL TURF

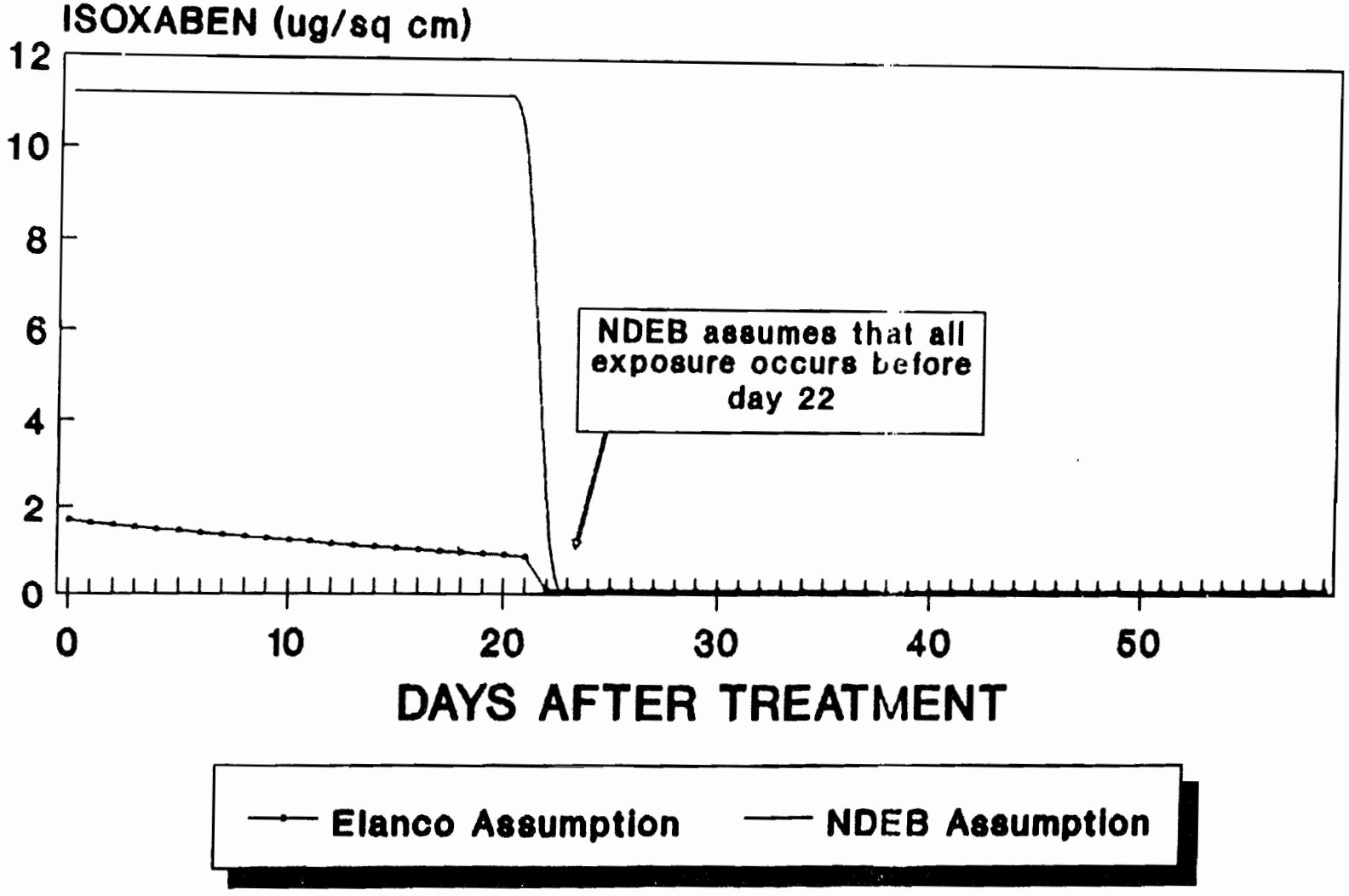
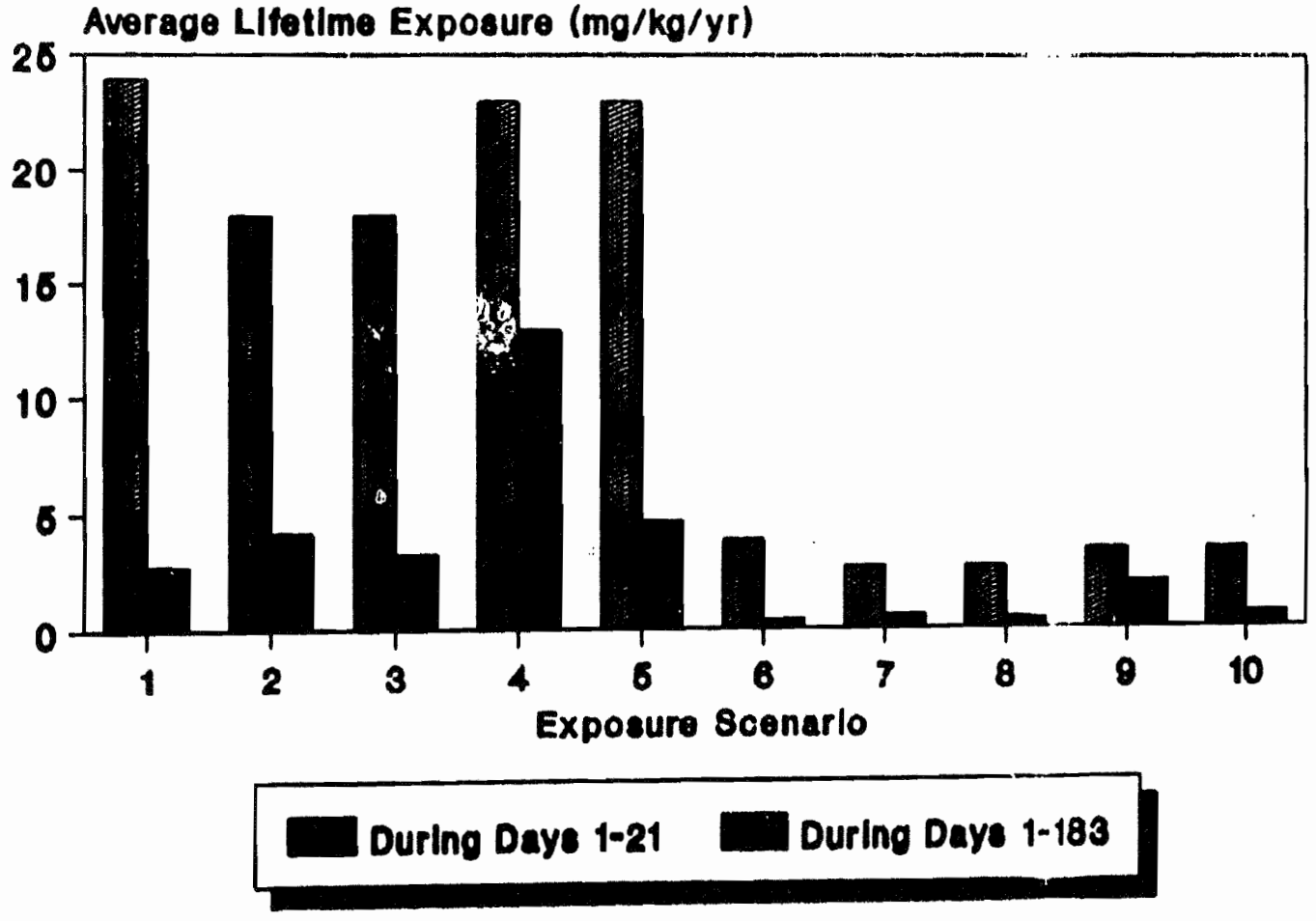


Table 2. Estimated Average Lifetime Exposures of Individuals to Isoxaben Applied to Residential Turf. Exposure is assumed to occur for 42 days per year during ages 2-12 only. Margins of Safety (MOS) are based on a 2 year rat study and were provided by Toxicology Branch.

Initial Residue (ug/cm^2)	Dissipation Pattern	Exposed on First 21 Days After Treatment Only			Exposure Spread Over 183 Days After Treatment		
		Average Residues (ug/sq cm)	Lifetime Exposure mg/kg/yr	(MOS)	Average Residues (ug/sq cm)	Lifetime Exposure mg/kg/yr	(MOS)
(1) 11.2	None for 21 days, 0 thereafter	11.2	24	(76)	1.29	2.82	(647)
(2) 11.2	First order with 21 day half life	8.1	18	(100)	1.87	4.07	(448)
(3) 11.2	First order with 21 day half life, constant at 0.56 after 21 days.	8.1	18	(100)	1.46	3.23	(565)
(4) 11.2	First order with 87 day half life	10.3	23	(79)	5.9	12.54	(146)
(5) 11.2	First order with 87 day half life, constant at 0.56 after 21 days.	10.3	23	(79)	2.07	4.59	(398)
(6) 1.69	None for 21 days, 0 thereafter	1.69	3.8	(480)	0.19	0.42	(4345)
(7) 1.69	First order with 21 day half life	1.22	2.7	(676)	0.28	0.63	(2897)
(8) 1.69	First order with 21 day half life, constant at 0.085 after 21 days.	1.22	2.7	(676)	0.22	0.52	(3510)
(9) 1.69	First order with 87 day half life	1.56	3.4	(537)	0.89	1.98	(922)
(10) 1.69	First order with 87 day half life, constant at 0.085 after 21 days.	1.56	3.4	(537)	0.31	0.73	(2500)

FIGURE 2.

LIFETIME EXPOSURES TO ISOXABEN ON TURF
(42 Days of Exposure Per Year)



See Table 2 for Scenario Description

Appendix A.
Calculation of Lifetime Exposures of Individuals to Isoxaben Applied to Residential Turf

Assumptions:

1) Children are exposed to isoxaben 42 days per year for 10 years (ages 2-12). No further exposure occurs after that time.

2) The body weights for a 2-6 and 7-12 year old child are 17 and 31 kg, respectively. The corresponding surface areas are 7000 and 9000 cm^2.

3) The entire surface area of the child is exposed to the treated surface. Exposure occurs only one time per day.

4) Transfer of isoxaben from the treated surface is 100 percent efficient. Residues on the skin are equal to those on the turf.

5) Dermal penetration is assumed to be 11 percent as provided by the registrant.

6) Oral exposure occurs from licking an area equal to that of one hand and the surface of a 3 inch diameter ball. The hand surface area is assumed to be 140 cm^2 for a 2-6 year old and 180 cm^2 for ages 7-12.

Calculation of Exposure:

The daily dermal exposure of a child to isoxaben is calculated by the following equation:

$$\text{Daily Dermal Exposure (mg/kg)} = \frac{\text{Surface Residues (ug/cm}^2\text{)} \times \text{Surface Area (cm}^2\text{)}}{\text{Body weight (kg)} \times 1000 \text{ ug/mg}}$$

The annual exposure, adjusted for 11 percent dermal absorption would be:

$$\text{Annual Dermal Exposure (mg/kg/yr)} = \text{Daily Dermal Exposure} \times 42 \text{ days/yr} \times 0.11$$

Appendix A.

Calculation of Lifetime Exposures of Individuals to Isoxaben Applied to Residential Turf

Combining equations and substituting the appropriate constants yields the following:

$$\text{Mean Lifetime Exposure (mg/kg/yr)} = \frac{\left(\frac{\text{Residue} \times SA_1}{BW_1 \times 1000}\right) + \left(\frac{\text{Residue} \times SA_2}{BW_2 \times 1000}\right) \times \frac{42\ \text{day}}{\text{year}} \times 5\ \text{yr} \times PF}{70\ \text{years}}$$

where:

Residue = Average residue (ug/cm^2) during the exposure period
SA_1 = Surface area of a 2-6 year old child = 7000 cm^2
SA_2 = Surface area of a 7-12 year old child = 9000 2m^2
BW_1 = Body Weight of a 2-6 year old child = 17 kg
BW_2 = Body Weight of a 2-6 year old child = 31 kg
PF = Penetration factor = 11 % = 0.11

Substituting the constant values:

$$\text{Exposure} = \frac{\left(\frac{7000\ \text{cm}^2}{17\ \text{kg}}\right) + \left(\frac{9000\ \text{cm}^2}{31\ \text{kg}}\right) \times \frac{42\ \text{days/yr} \times 5\ \text{yr} \times 0.11}{1000\ \text{ug/mg}}}{70\ \text{years}}$$

or, after combining constant values:

Exposure = Residue x 2.11

For an average residue level of 11.2 ug/cm^2, this lifetime exposure becomes:

Average lifetime exposure (mg/kg/yr) = 11.2 ug/cm^2 x 2.11

= 24 mg/kg/yr

Each of the scenarios considered yields a different average residue value. The oral component of exposure can be calculated in the same manner by substituting the appropriate hand surface areas and the surface area of a 3 inch diameter ball into the above equations. The corresponding multiplication factor for estimation of exposure from surface residues reduces to 0.091.

REFERENCES

1) Memorandum from M. Firestone (NDEB) to R. Mounfort (RD) titled "Isoxaben Exposure Estimate for Children Playing on Treated Lawns", dated 14 March 1989.

2) Rutherford, B.S. and O.D. Decker (1988) Isoxaben Turf Field Dissipation Study - Illinois Site. MRID No. 40532102.

3) U.S. Dept of Commerce (1985) Development of Statistical Distributions of Standard Factors Used in Exposure Assessments. NTIS No. PB85-24_667

3/14/89

007755

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, DC 20460

OFFICE OF
PESTICIDES AND
TOXIC SUBSTANCES

14 MAR 1989

MEMORANDUM

SUBJECT: **ISOXABEN** EXPOSURE ESTIMATE FOR CHILDREN PLAYING ON TREATED LAWNS (NO HED PROJECT NUMBER)

TO: Richard F. Mountfort
Product Manager 23
Registration Division (H7509C)

FROM: Michael P. Firestone, Ph.D., Chief
Review Section 1
Non-Dietary Exposure Branch/HED (H7509C)

THRU: Charles L. Trichilo, Ph.D., Chief
Non-Dietary Exposure Branch/HED (H7509C)

DEFERRAL TO: TB-HFAS X

I. INTRODUCTION

NDEB has been requested by TB-HFAS to provide exposure estimates for children who may be exposed to isoxaben postapplication via contact with treated home lawns, despite the lack of any chemical specific data. It should be noted that NDEB previously concluded that using foliar dislodgeable residue (FDR) data for a surrogate chemical is not acceptable assessing exposure to isoxaben; also, data should be generated to delineate the relationship between home lawn FDR and human exposure (see M. Firestone memorandum of February 28, 1989).

Despite the lack of any actual data, this assessment will provide a very rough estimate of dermal and ingestion exposure utilizing modified versions of two unsubstantiated methodologies used previously by the Agency. The specific use in question involves the application of a dry flowable formulation of isoxaben (GALLERY 75 DF; 75% ai) to home lawns. Both daily and annual exposures have been estimated.

II. DETAILED CONSIDERATIONS

The degree of conservativeness of the assumptions utilized in this exposure estimation can not be determined without further research. Previous exposure estimates provided for children playing on home lawns treated with other pesticides have been estimated using data showing the pattern of decline of dislodgeable residues on turf grass. Upon receipt of acceptable chemical specific turf foliar dissipation data (reflecting different grass varieties at several geographically diverse sites), NDEB can refine the exposure estimates provided in this report to more closely represent the actual exposure level (note: a protocol for such a study should be submitted for Agency approval prior to its conduct).

The following assumptions were used in this exposure assessment:

1. Respiratory exposure is insignificant compared to dermal or ingestion exposure.

2. Isoxaben is applied to home lawns twice per year at the maximum application rate of 1 lb ai/A.

3. Exposure is assumed to occur daily for 21 days after each application. In the absence of foliar residue data, NDEB will assume both that uniform coverage of the home lawn will occur and that foliar residues will not dissipate during this exposure period; after 21 days, residue levels will dissipate to a level approaching zero due to watering-in (note: according to the registrant, a worst-case half-life of 87 days has been determined, thus, residue levels would not be expected to significantly decline during the first 21 days after application; however, according to the label, "Gallery must be activated within 21-days of application to be fully effective").

4. Lifetime exposure will encompass 10 years (ages 2 through 12) as assumed by the registrant.

5. Dermal exposure to the child will occur as a result of contact with the treated grass through such activities as crawling and rolling.

   Dermal exposure can be estimated by utilizing either of two unsubstantiated methodologies used previously by the Agency.

   a) In the first method, contact will occur over the entire body surface area. Any dermal contact will result in a quantitative transfer of residues from the foliage to the surface of the skin. This method may not be conservative since a child could be exposed to much higher levels if dermal absorption is rapid as he/she contacts different areas of the treated lawn.

b) The second method of estimating dermal exposure is to use a modification (corrected for the relative child:adult body surface area ratio) of the relationship between dermal exposure by fruit harvesters and FDR developed by Zweig, et al. (Journal of Environmental Science and Health, Volume B20, pp. 27-59, 1985), where:

dermal exposure mg/hr = antilog [(log FDR ug/cm^2) + 0.603]

A child is assumed to play outdoors 4 hours per day.

6. As referenced by the registrant (ICRP 1984), for ages 2 to 6, assumptions include: total body surface area of 7,000 cm^2, hand surface area of 140 cm^2,and body weight of 17 kg; for ages 6 to 12, assumptions include total body surface area of 9,000 cm^2, hand surface area of 180 cm^2, and body weight of 31 kg.

7. All dermal exposure values correspond to the amount of chemical impinging on the skin surface corrected for a dermal penetration factor of 11% (see below).

8. We assume that during the course of an exposure episode, the child will lick an area of his body equal to the surface area of both hands and will lick the surface area of a 3-inch diameter ball. Licking is assumed to quantitatively remove residues from each respective surface. The surface residues on the ball are assumed to be equal to the surface residues on the grass. Possible oral exposure resulting from the ingestion of contaminated soil has not been considered.

II. EXPOSURE CALCULATIONS

A. Dermal Exposure - Method 1: Quantitative Transfer

1. If isoxaben is applied at 1 lb ai/A, the surface residues, assuming uniform coverage, is:

$$\frac{1\text{ lb ai}}{\text{acre}} \times \frac{1\text{ acre}}{4047\text{ m}^2} \times \frac{454\text{ g}}{1\text{ lb}} \times \frac{10^3\text{ mg}}{\text{g}} = 112\text{ mg/m}^2$$

The daily dermal exposure to a 2 to 6 year old child is:

$$\frac{112\text{ mg}}{\text{m}^2} \times \frac{0.70\text{ m}^2}{\text{child}} \times \frac{1}{17\text{ kg}} = 4.6\text{ mg/kg/day}$$

The annual dermal exposure to a 2 to 6 year old child is:

194 mg/kg/year (4.6 mg/kg/day x 21 days/ treatment x 2 treatments/year)

The daily dermal exposure to a 7 to 12 year old child is:

$$\frac{112 \text{ mg}}{\text{m}^2} \times \frac{0.90 \text{ m}^2}{\text{child}} \times \frac{1}{31 \text{ kg}} = 3.3 \text{ mg/kg/day}$$

The annual dermal exposure to a 7 to 12 year old child is:

137 mg/kg/year (3.3 mg/kg/day x 21 days/ treatment x 2 treatments/year)

2. Average lifetime dermal exposure:

[(194 mg/kg/year x 6 years) + (137 mg/kg/year x 6 years)] / 70 years = 28 mg/kg/yr or 7.8×10^{-2} mg/kg/day

Assuming (as per the registrant's January 25, 1989 submission) a dermal penetration factor of 11% (note: NDEB defers the adequacy of this value to TB-HFAS), lifetime average daily exposure would be 8.6×10^{-3} mg/kg/day.

B. Dermal Exposure - Method 2:
Zweig-Leffingwell-Popendorf Correlation

1. As in II-A(1) above, uniform coverage results in a FDR level of 112 mg/m^2 (11.2 ug/cm^2).

The daily dermal exposure to a 2 to 6 year old child is:

4 hours x 1/17 kg x antilog [(log 11.2) + 0.603] mg/hr

x 0.7 m^2/2.1 m^2 child:adult surface area ratio

= 3.5 mg/kg/day

The annual dermal exposure to a 2 to 6 year old child is:

147 mg/kg/yr (3.5 mg/kg/day x 21 days/ treatment x 2 treatments/year)

The daily dermal exposure to a 7 to 12 year old child is:

4 hours x 1/31 kg x antilog [(log 11.2) + 0.603] mg/hr

x 0.9 m^2/2.1 m^2 child:adult surface area ratio

= 2.5 mg/kg/day

The annual dermal exposure to a 7 to 12 year old child is:

105 mg/kg/yr (2.5 mg/kg/day x 21 days/ treatment x 2 treatments/year)

2. Average lifetime dermal exposure:

[(147 mg/kg/year x 6 years) + (105 mg/kg/year x 6 years)] / 70 years = 22 mg/kg/yr

Since methods 1 (28 mg/kg/yr) and 2 (22 mg/kg/yr) give similar results, the results from method 1 will be used throughout the rest of this exposure estimation.

C. Ingestion Exposure

1. The daily ingestion exposure from licking a 3-inch diameter (7.6 cm) ball is:

$$4 \text{ pi } (7.6 \text{ cm}/2)^2 \times \frac{10^{-4} \text{ m}^2}{\text{cm}^2} \times \frac{112 \text{ mg}}{\text{m}^2} \times \frac{1}{17 \text{ or } 31 \text{ kg}} =$$

0.12 mg/kg/day for a 2 to 6 year old; or

0.07 mg/kg/day for a 7 to 12 year old

The annual ingestion exposure from licking the ball is:

daily exposure x 21 days/treatment x 2 treatments/year =

5.0 mg/kg/year for a 2 to 6 year old; or

2.9 mg/kg/year for a 6 to 12 year old

2. The daily ingestion exposure from licking a body surface area equivalent to that of both hands is:

$$\frac{140 \text{ or } 180 \text{ cm}^2}{10{,}000 \text{ cm}^2/\text{m}^2} \times 112 \text{ mg/m}^2 \times \frac{1}{17 \text{ or } 31 \text{ kg}} =$$

0.09 mg/kg/day for a 2 to 6 year old; or

0.07 mg/kg/day for a 7 to 12 year old

Annual ingestion exposure from licking both hands is:

3.8 mg/kg/year for a 2 to 6 year old; or

2.7 mg/kg/year for a 7 to 12 year old

3. Average lifetime ingestion exposure =

$$\frac{[(5.0 + 3.8 \text{ mg/kg/yr} \times 6 \text{ years}) + (2.9 + 2.7 \text{ mg/kg/yr} \times 6 \text{ years})]}{70 \text{ years}}$$

= 1.23 mg/kg/year or 3.4×10^{-3} mg/kg/day

C. Average Daily Total (Dermal plus Oral) Exposure

Total average daily exposure summing the values for dermal exposure (corrected for dermal penetration) and oral exposure would be:

8.6×10^{-3} mg/kg/day (dermal) + 3.4×10^{-3} mg/kg/day (oral) =

1.2×10^{-2} mg/kg/day

III. CANCER RISK ASSESSMENT

Assuming the registrant's derived value for the cancer potency of 2.1×10^{-3} $(\text{mg/kg/day})^{-1}$ (note: NDEB defers the adequacy of this value to TB-HFAS), incremental lifetime risk would be 3×10^{-5} (1.2×10^{-2} mg/kg/day exposure x 2.1×10^{-3} per mg/kg/day).

IV. CONCLUSIONS

1. The estimate derived above regarding exposure is considered conservative. As stated previously, NDEB will be able to refine these exposure estimates upon receipt of actual isoxaben residue data. Additionally, the extrapolation from foliar residue levels to estimated dermal contact cannot be revised without further research in the areas of residue transfer and child behavioral patterns.

2. NDEB defers to TB-HFAS the adequacy/accuracy of both the registrant-derived dermal penetration factor of 11% and cancer potency factor of 2.1×10^{-3}.

Note to the PM: Since DEB/HED now considers pesticides used on turf grass as a food use, RD should ensure that DEB reviews this action including any label restrictions designed to prevent use on grass grown for seed (see Attachment from DEB dated January 26, 1989).

Attachment

cc: William Burnam
Sue Rathman
Marsha Van Gemert
Isoxaben file
Correspondence file
Circulation
SACB
DEB

ATTACHMENT

January 26, 1989

Note To: Anne Lindsay (RD)
Rick Tinsworth (SRRD)

Subject: Tolerance or label Restriction for Pesticides Used on Turf Grass

The recent incident in the Pacific Northwest concerning residues of Tilt on grass seed screening has pointed out the need for residue data or additional label restrictions on products used on turf grass. In the past OPP classified turf grass uses as non-food uses, since the label restrictions were to prohibit the grower from using the grass as an animal feed. However, the grass seed processors are not necessarily aware of the pesticides (and their associated label restrictions) used by the growers. In addition, recent information from BEAD indicates seed processors in Region X routinely sell screening for cattle feed (See Attached Memo).

A better way to label turf grass uses which are already classified as non-food uses would be to add or revise the restriction as follows: "Do not use on grass grown for seed":. In this way the grower, who should be aware of the label precautions, would know not to use a pesticide on grass which was being grown for seed. In those cases where removal of the label restriction is desired, tolerances would need to be established. Labels containing directions for use on "grass grown for seed" would require tolerance data to determine pesticide residues in the seed, screening and meat, milk, poultry and eggs, or removal of the seed use from the label.

I recommend that OPP implement a program to add this label restriction to turf grass uses classified as non-food uses, so that incidents such as Tilt can be avoided in the future. A Data call-in will be needed to address the older chemicals.

In addition, uses on alfalfa, clover and small grains grown for seed should also be reevaluated. I believe these are also food uses, since seed screening and other plants parts can be used as animal feed. In addition, alfalfa sprouts are a good human food. Note that OPP approved special local need uses for alfalfa grown for seed in Washington as non-food uses only after the state agreed to take the responsibility for assuring that no

part of crop (including seed screenings) was used as food or feed. See the attached emergency rules agreed to by the State of Washington.) We should follow-up with the state on how their program is actually working.

Please contact me if you have any questions concerning this subject.

Charles L. Trichilo, Chief
Dietary Exposure Banch

Attachments

cc: W. Burnam, HED
Al Jennings
Bill Jordon
A. Rathman
K. Arne, Reg X

Attachment 2 007755

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

JAN 9 1989

OFFICE OF
PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

Subject: The use of grass seed screenings in animal feed.

To: Chuck Trichilo, Chief
Residue Chemistry Branch

From: Joseph A. Ferrante, Economist
Biological and Economic Analysis Division

Thru: Allen L. Jennings, Director
Biological and Economic Analysis Division

Introduction

We have completed a market analysis of grass seed production in the Pacific Northwest (Washington Idaho, Oregon). This analysis also includes a discussion of the market for straw and screenings/chaff which are byproducts of grass seed farming and processing

Grass seeds are harvested by shaving off the top part of mature grass (ie. grass that has been allowed to flower and produce seeds) The bottom part of the plant is mowed and collected for straw. After the straw is harvested, the remaining stubble is burned in the field or plowed under (note: grass field fires have been banned in Oregon).

Grass seeds are cleaned and processed by removing the screening/chaff from the top portion of the plant. The screenings (which are comprised of parts such as the husk hull, and awns) are pelletized to produce animal feed.

The remainder of this memo briefly presents our findings from an analysis of the market for grass seed and grass seed byproducts We will also present the extent to which grass seed screenings are used in the animal feed market. This information should be useful in deciding whether or not tolerances need to be established for pesticides used on grass seed crops.

- **Findings**

  - o There are 350 000 - 400 000 acres producing grass seed in the Pacific Northwest.
  - o This acreage yields 330 - 350 million pounds of grass seed.
  - o The Pacific Northwest accounts for 60 - 70 percent of the national grass seed production.
  - o The price of grass seed ranges from $.40 per pound for low quality grass (eg. rye grass) to $1.60 per pound for high quality grass (eg. Kentucky bluegrass).
  - o Gross revenue for grass seed in the Pacific Northwest, excluding the sale of byproducts, is between $150 - $200 million.
  - o Profits vary according to the variety of grass seed produced. The per acre profits for Kentucky bluegrass (a high quality grass) are about $500 - $600.
  - o Approximately 70 - 100 million pounds of screenings are produced during grass seed processing.
  - o Almost all of the screenings (99 percent) are pelletized for cattle feed. The remaining 1 percent is used as mulch.
  - o The price of screenings is based on the market price for barley and fluctuates accordingly. Typically screenings sell for $35 - $75 per ton.
  - o Therefore gross revenues for grass seed screenings are $1 2 - $3.8 million in the Pacific Northwest.
  - o Cattle eat approximately 13.5 billion pounds of feed annually in the Pacific Northwest The feed is mainly comprised of corn, sorghum, and oats.
  - o Most grass seed screenings are consumed by cattle in Oregon Washington, and Idaho. These screenings account for less than 1 percent of the total (annual) cattle feed market in this area.

If you need any more information regarding the production of grass seeds grass seed screenings, and the relative importance of its use in the animal feed market please do not hesitate to contact me at 557-1753.

8/31/88

007755

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: CORRIGENDUM: To the memo dated May 1, 1987---
"Isoxaben, Rat and Mouse Study - Qualitative Risk Assessment of Combined Toxicity and Oncogenicity Study. Caswell #419F

FROM: C.J. Nelson, Statistician C Nelson 8/23/88
Science Support Section
Science Analysis and Coordination Branch, HED (TS-769C)

TO: Clark Swentzei
Review Section II
Toxicology Branch-Herbicide, Fungicide and Antim-Microbial Support, HED (TS-769C)

THRU: Richard Levy, M.P.H., Leader-Biostatistics Team
Science Support Section R. Levy 8-31-88
Science Analysis and Coordination Branch, HED (TS-769C)
and

John A. Quest, Ph.D., Head JA Quest 8/31/88
Science Support Section
Science Analysis and Coordination Branch, HED (TS-769C)

The following changes were brought to my attention on August 18, 1988. Two hepatocellular carcinomas in male mice were coded as hepatocellular adenomas by the reviewer. Table 6, male mouse hepatocellular carcinoma and Table 7, male mouse hepatocellular adenoma were corrected and are shown below. There was no change to Table 8, male mouse hepatocellular adenoma and/or carcinoma. The second paragraph of the tumor analysis changed slightly. It is shown below with the changes made by bold underline.

Tumor Analysis:

Hepatocellular carcinoma, adenoma, and adenoma/carcinoma were analyzed for the mouse studies (Table 6, 7, and 8 respectively). There were no survival disparities in the mouse studies, hence the same tests were used in these analyses as those used in the rat study (**Fishers exact test was used for pair-wise comparisons and the Cochran-Armitage test was used to test for trends**). There were no significant differences found for hepatocellular carcinoma for either sex. There was a significant trend for hepatocellular adenoma for both sexes (males $p < .001$, females $p = .004$). The high dose (12500ppm)

mice had significantly more adenomas than the controls for both sexes (males p = .017, females p = .006). There was a significant trend for adenomas and carcinomas combined (males p = .01, females p = .001). The high dose (12500) female mice had significantly more combined tumors than the controls (p = .001) but the high dose males were not significant (p = .18).

Table 6. ISOXABEN - Mouse Study, Hepatocellular Carcinoma Rates+, Cochran-Armitage Trend test, and Fisher's Exact Test Results

Dose (ppm)	0	100	1000	12500
Males	9/56 (16)	5/49 (10)	5/55 (9)	5/55 (9)
Female	0/52 (0)	1/52 (2)	0/46 (0)	2/52 (4)

First male tumor observed at 82 weeks in control group.
First female tumor observed at 104 weeks in 100ppm dose group.

Table 7. ISOXABEN - Mouse Study, Hepatocellular Adenoma Rates+, Cochran-Armitage Trend test, and Fisher's Exact Test Results

Dose (ppm)	0	100	1000	12500
Males	3/44 (7)**	1/41 (2)	3/47 (6)	12/48 (25) *
Female	0/52 (0)**	3/52 (6)	2/46 (4)	7/52 (13)**

First male tumor observed at 103 weeks in 12500 ppm group.
First female tumor observed at sacrifice.

cc: Bernice Fisher
Esther Rinde
Author, Chemical, Statistics, Caswell, One liner, and Reading File.