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

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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 — Ric 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
Diane Beal
Jack Quest — Jack E. Quest

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

K. Clark Swentzel 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.

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TABLE 2

ISOXABEN - $B_6C_3F_1$ MOUSE STUDY

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

| Females<br>Dose (ppm) | 0 | 100 | 1000 | 12500 |
|---|---|---|---|---|
| Hepatocellular carcinomas | 0/52<br>(0) | 1/52<br>(2) | 0/46<br>(0) | 2/52<br>(4) |
| Hepatocellular adenomas | 0/52**<br>(0) | 3/52<br>(6) | 2/46<br>(4) | 7/52**<br>(13) |
| Combined carcinomas and/or adenomas | 0/52**<br>(0) | 4/52<br>(8) | 2/46<br>(4) | 9/52**<br>(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<br>(2) | 0/30-2/30<br>(0-7) |
| Hepatocellular adenomas | 7/637<br>(1) | 0/30-2/30<br>(0-7) |
| Combined carcinomas and/or adenomas | 20/637<br>(3) | |

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

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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

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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).

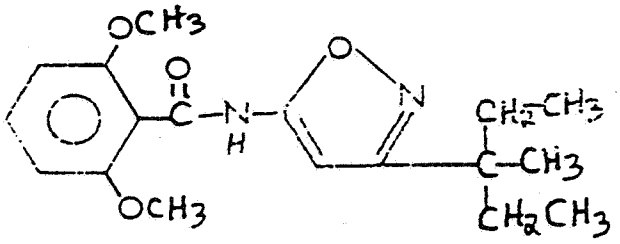
B. Material Reviewed:

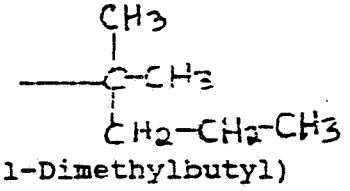
The material available for review consisted of DER's, one-liners, and other data summaries prepared by Ms. Jones. Tables and statistical data analyses for the mouse and rat studies were provided by C.J. Nelson [Memo, 5/1/87]. The material reviewed and the above memo are attached to the file copy of this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

C. Background Information:

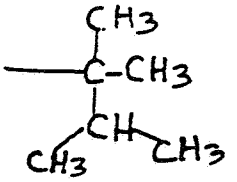
Isoxaben, a new chemical, is a pre-emergent herbicide which has been proposed for experimental use on wheat and barley, and for permanent use on ornamentals to control broadleaf weeds. Technical Isoxaben is 95.5% pure and consists of three isomers, differing only in the isoxazolyl side-chain.

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

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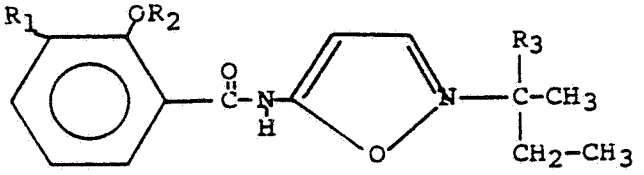
## FIGURE 1

### 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.