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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

SEP 14 1994

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Ethalfluralin

FROM: Ray Landolt *8/31/94*
Review Section I
Toxicology Branch II
Health Effects Division (7509C)
and
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Manager, Carcinogenicity Peer Review Committee
Science Analysis Branch
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TO: Joanne Miller
Product Manager #23
Fungicide-Herbicide Branch
Registration Division (7505C)
and
Jay Ellenberger, Chief
Reregistration Branch
Special Review and Reregistration Division (7508W)

THROUGH: Stephanie R. Irene, Ph.D. *Stephanie R. Irene*
Acting Deputy Director, Health Effects Division (7509C)

The Health Effects Division Carcinogenicity Peer Review Committee (CPRC) met on June 08, 1994 to discuss and evaluate the weight-of-the-evidence on ethalfluralin with particular reference to its carcinogenic potential. The CPRC concluded that ethalfluralin should be classified as Group C - possible human carcinogen - and recommended that for the purpose of risk characterization, a low dose extrapolation model be applied to the animal data for the quantification of human risk (Q_1). This was based on a statistically significant increase in adenomas/fibroadenomas of the mammary gland in female rats at more than one dose, which exceeded the historical control range. Positive genotoxicity data supported the carcinogenicity concern.

SUMMARY

Administration of ethalfluralin in the diet to Fisher 344 rats resulted in statistically significant increases in mammary gland fibroadenomas and adenomas/fibroadenomas combined in the female at both the mid and high doses. The incidences of these tumors were well in excess of the upper range of historical controls. The highest dose in this study did not elicit clinical signs of toxicity or weight gain changes in either sex. The CPRC determined that the highest dose in the male rats was inadequate. Since there were tumors in the female rat, the dosing was not considered to be inadequate; however, it was agreed that the females could have tolerated a higher dose. A single adenoma of the kidney and of the urinary bladder in male rats at the mid dose, and a single carcinoma of the kidney at the mid- and high doses in female rats, were also noted. These are sites which were the targets for the structural analog trifluralin. Another structurally related analog, flumetralin, also increased mammary tumors (adenocarcinomas and combined adenomas/adenocarcinomas) in female rats. It was suggested that at higher doses, the adenomas in the mammary gland might progress to carcinomas.

Administration of ethalfluralin in the diet to B6C3F₁ mice did not result in increases of any tumors; however, dosing in this study was considered to be inadequate in both sexes. This was based on absence of significant toxicity, other than focal hyperplasia (which was not considered much of an effect). The differences in weight gain between controls and the treated mice was not considered to be significant.

[Details are provided in Section G. "The Weight of Evidence".]

The Committee nevertheless did not recommend repeating any of the studies, as there was sufficient information to perform a risk characterization, based on the mammary gland tumors in the female rat alone.

The classification of Group C was based on increases in mammary tumors in the female rat, by both pair-wise and trend analysis (at two doses, and well in excess of historical controls, even though dosing was not as high as the animals could have tolerated). There were also positive data for genotoxicity and a suggestion of two other tumor types in the rat: bladder (in males) and kidney (in both sexes), similar to that observed with other aromatic amine chemicals of this class, which at higher doses of ethalfluralin might have been more pronounced.

The CPRC agreed that these data were sufficient for the purpose of risk characterization, and that a low dose extrapolation model be applied to the animal data for the quantification of human risk (Q₁), based on the mammary gland tumors in the female rat.

A. Individuals in Attendance at the meetings:

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

Penny Fenner-Crisp

William Burnam

Karl Baetcke

Marcia Van Gemert

Hugh Pettigrew

Esther Rinde

Yin Tak Woo

[Handwritten signatures: Penny Fenner-Crisp, William Burnam, Karl Baetcke, Marcia Van Gemert, Hugh Pettigrew, Esther Rinde, Yin Tak Woo]

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

Ray Landolt¹

Michael Ioannou

Lori Brunsman

Michael Stedham²
(PAI/Clement)

[Handwritten signatures: Ray Landolt, Michael Ioannou, Lori Brunsman, Michael Stedham]

3. Other Attendees:

Bernice Fisher (HED)

¹Also a member of the PRC for this chemical; signature indicates concurrence with the peer review unless otherwise stated.

²Signature indicates concurrence with pathology report.

B. Material Reviewed

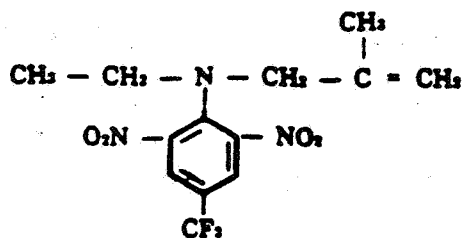
The material available for review consisted of DERs and other data summaries prepared by Roland A. Gessert, Irving Mauer, Tim McMahon and Ray Landolt from studies submitted by Eli Lilly (Dow Elanco). Statistical analysis was provided by Lori Brunsman. The material reviewed is attached to the file copy of this report.

C. Background Information

Ethalfluralin (EL-161) with the trade name Sonalin^R is registered for terrestrial food uses by Dow Elanco. Tolerances are established for residues of [N-ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl) benzenamine in or on a wide range of raw agricultural commodities including meat and milk listed in 40 CFR 180.416 at 0.05 ppm. This dinitroaniline herbicide is registered for preemergence control of annual grasses and broad leaf weeds.


A Notice of Determination Not to Indicate a Rebuttal Presumption Against Registration (RPAR) was published in Federal Register January 4, 1984. Ethalfluralin was under consideration because the RPAR criteria for carcinogenicity and teratogenicity had been met. In evaluating the risks and benefits associated with the proposed uses of ethalfluralin, the Agency determined that the benefits exceeded the risk posed. A conditional registration was issued to allow time for the registrant to conduct a second species teratogenicity, a non-rodent chronic feeding and a dermal penetration study. The data on file in support of tolerances on ethalfluralin are complete.

The TOX Chem No. is 453B. The P.C. Code of ethalfluralin is 113101. The Chemical Abstract Registry Number (CAS No.) of ethalfluralin is 55283-68-6. The structure of ethalfluralin is presented below:



Ethalfluralin

The impurity nitrosamine is present in the technical (94.5%) ethalfluralin used in the various toxicological studies.



MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

D. Evaluation of Carcinogenicity Data

1. Rat 2-Year Carcinogenicity Study

Reference: Two-Year Dietary Evaluation of Ethalfluralin in the Fischer 344 Rat, Lilly Research Laboratories, Reports R-267 and R-277, June 1981, MRID 00094776 and 92062013.

a. Experimental Design

In replicate studies ethalfluralin was fed to 30 Fischer 344 rats/sex/group at levels of 100, 250 or 750 ppm, equivalent to 4.2, 10.7 or 32.2 mg/kg/day, respectively, with 60 rats/sex in the controls for 24 months.

b. Discussion of Tumor Data

There were no significant compound-related tumors observed in male rats. However, a single adenoma of the kidney and of the urinary bladder in male rats at the mid-dose, and a single carcinoma of the kidney at the mid- and high doses in female rats, were noted. Female rats had significant increasing trends, and significant differences in pair-wise comparisons of the 32.3 mg/kg/day dose group with the controls, for mammary gland fibroadenomas and combined mammary gland adenomas and/or fibroadenomas, all at $p < 0.01$. Female rats also had significant differences in the pair-wise comparisons of the 10.7 mg/kg/day dose group with the controls for mammary gland fibroadenomas ($p < 0.01$) and combined mammary gland adenomas and/or fibroadenomas ($p < 0.05$).

Since there was no significant statistical evidence of differential mortality with increasing doses of ethalfluralin, these statistical analyses were based upon the Cochran-Armitage trend test and the Fisher's Exact test for pair-wise comparisons of the dosed groups with the controls. See Table 1 for female rat tumor analysis results.

Table 1. Ethalfluralin - Harlan Industries Fischer 344 Combined Rat Studies

Female Mammary Gland Tumor Rates[†] and Cochran-Armitage Trend Test and Fisher's Exact Test Results (p values)

	<u>Dose (mg/kg/day)</u>			
	0	4.2	10.7	32.3
Adenomas (%)	4/60 (7)	1 ^a /60 (2)	5/59 (8)	1/59 (2)
p =	0.168	0.182 ⁿ	0.489	0.187 ⁿ
Fibroadenomas (%)	9/60 (15)	10/60 (17)	21 ^b /59 (36)	28/59 (47)
p =	0.000 ^{**}	0.500	0.008 ^{**}	0.000 ^{**}
Combined (%)	13/60 (22)	11/60 (18)	25 ^c /59 (42)	29/59 (49)
p =	0.000 ^{**}	0.410 ⁿ	0.013 [*]	0.002 ^{**}

[†]Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

ⁿNegative change from control.

^aFirst adenoma observed at week 80, dose 4.2 mg/kg/day.

^bFirst fibroadenoma observed at week 70, dose 10.7 mg/kg/day.

^cOne animal in the 10.7 mg/kg/day dose group had both an adenoma and a fibroadenoma of the mammary gland.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If ^{*}, then $p < 0.05$. If ^{**}, then $p < 0.01$.

Table 2. Historical Control Incidence of Fibroadenomas in Female Fischer 344 Rats (Data obtained at Lilly Research Laboratories from May 1977 to February 1979).

Study Number	Termination of study	Number of Untreated females	Number of Females with Fibroadenomas	Percent Incidence
R-695	May 1977	120	16	13.3%
R-1136*	November 1978	30	8	26.7%
R-1146*	November 1978	30	5	16.7%
R-1246*	December 1978	30	5	16.7%
R-1256*	December 1978	29	4	13.8%
R-87*	February 1979	30	0	0.0%
R-97*	February 1979	30	7	23.3%
R-167*	February 1979	30	6	20.0%
R-177*	February 1979	<u>30</u>	<u>4</u>	<u>13.3%</u>
Total		359	55	Overall Incidence 15.3%

*Replicate Studies

The historical control incidence (Table 2) ranges from 0 to 26.7% with an average incidence of 15.3% for this tumor. This compares with an incidence in the ethalfluralin study of 22% in the controls, 18% in the low dose, 42% in the mid dose and 49% in the high dose. The incidences of combined mammary gland adenomas and/or fibroadenomas in the mid (250 ppm) and high (750 ppm) dose exceed the historical control values for this tumor.

c. Non-Neoplastic Lesions and other findings

There were no changes in body weight gain related to the dose levels administered as compared to the controls in male or female rats.

The statistical evaluation of mortality indicates no significant incremental changes with increasing doses of ethalfluralin in male or female rats.

No clinical signs of toxicity were observed related to the dose levels administered, except for a dose related incidence of dark yellow urine indicative of the presence of ethalfluralin metabolites.

There were no changes in hematology or clinical chemistry values attributed to the administration of ethalfluralin as compared to the controls at the termination of the study.

There were no significant differences in organ weights related to the administration of ethalfluralin with the exception of a slight increase in relative liver weight in males (8.9%) and females (4.1 and 4.7%) at the highest dose levels as compared to controls

Histopathological non-neoplastic findings were limited to yellow coloration of fat at the high dose level.

d. Adequacy of Dosing for Assessment of Carcinogenicity

The dose selections for the 2-year rat feeding study were based on levels of 250, 500, 1100, 2500 or 5000 ppm fed to Fischer 344 rats for 90 days (Study No. R-936). The NOEL and LOEL in the 90-day study are 500 and 1100 ppm (29 and 63 mg/kg/day) respectively, with an increase in absolute and relative liver weights (20%) at the 1100 ppm level. The 750 ppm level was selected as the high dose for the 2 year feeding study. Based on the parameters investigated in the 2-year feeding study the systemic NOEL is greater than 750 ppm. The highest dose tested (750 ppm) was considered inadequate in males. Since there were tumors in the female rat, the dosing was not considered inadequate; however it was agreed that female rats could have tolerated a higher dose. No new studies are required.

2. Mouse 2-Year Carcinogenicity Study

Reference: Two-Year Dietary Evaluation of Ethalfluralin in the B6C3F₁ Mouse, Lilly Research Laboratories Reports M-9167 and M-9177, July 1981, MRID 00094777 and 92062016.

a. Experimental Design

In replicate studies ethalfluralin was fed to 40 B6C3F₁ mice/sex/group at levels of 100, 400 or 1500 ppm, equivalent to 10.3, 41.9 or 163.3 mg/kg/day, respectively, with 60 mice/sex in the controls.

b. Discussion of Tumor Data

No increased incidences of neoplasms were observed in these replicate studies at the dose levels administered for 24 months.

c. Non-neoplastic lesions and other findings

Evaluation of mortality incidence indicates no change with increasing doses of ethalfluralin were observed as compared to the controls.

No clinical signs of toxicity were observed related to the dose levels administered.

Mean body weight gain of the control and test levels were comparable during the experimental period until the 24th month when a significant ($p < 0.05$) decrease in body weight gain of 18-30% as compared to the control was reported for males and females at the high dose level.

A significant ($p < 0.05$) decrease in hematocrit, hemoglobin and erythrocyte count, accompanied by a significant ($p < 0.05$) increase in mean corpuscular hemoglobin concentration was reported in the high dose females at the termination of the study.

A significant ($p < 0.05$) increase in alkaline phosphatase values was reported at the termination of the study in male and female mice at the high dose level.

Relative liver weights of females of the high dose level were significantly ($p < 0.05$) increased as compared to the controls. Relative kidney weights of females of the mid and high dose levels were significantly ($p < 0.05$) increased. Relative heart weight of females of the mid and high dose levels were significantly ($p < 0.05$) increased as compared to the controls.

both replicate studies for the low, mid and high dose levels.

Table 3 - Incidence of Hepatocellular Hyperplasia in Male and Female Mice of Studies M-9167 and M-9177 Combined

	<u>CONTROL</u>	<u>100 ppm</u>	<u>400 ppm</u>	<u>1500ppm</u>
Males	5.9% (7/119)	11.3% (9/80)	15.0% (12/80)	17.7% (14/79)
Females	1.7% (2/120)	5.0% (4/80)	7.5% (6/80)	26.3% (21/80)

Table 4 Historical Control Incidence Of Hepatocellular Hyperplasia in B6C3F₁ Mice (Data obtained at Lilly Research Laboratories from February 1977 to April 1979)

<u>Study Number</u>	<u>Males</u>	<u>Females</u>
M-9087	15.5% (9/60)	8.3% (5/60)
M-9097	10.0% (6/60)	10.0% (6/60)
M-9067	3.3% (2/60)	8.3% (5/60)
M-9077	1.7% (1/60)	1.7% (1/60)

The studies with ethalfluralin were conducted from June 1977 to June 1979.

On inspection of the incidence of hepatocellular hyperplasia observed in the low dose with ethalfluralin and the historical control incidence, a variation in the spontaneous incidence of hepatocellular hyperplasia can occur in the mouse. On this basis the NOEL and LEL is 100 ppm (10.3 mg/kg/day) and 400 ppm (41.9 mg/kg/day), respectively.

d. Adequacy of Dosing for Assessment of Carcinogenicity

The dose selection for this 2-year study is based on levels of 560, 1100, 2250, 4000 or 8000 ppm, fed to B6C3F₁ mice for 90-days (Study No. M-9286). The NOEL and LOEL in the 90-day study are 560 and 1100 ppm, respectively, with decreases in bilirubin values and absolute kidney weights.

The increased incidence of hepatocellular hyperplasia exceeded the concurrent and historical control incidence for this lesion at the mid and high dose. The Committee considered the incidence of hepatocellular hyperplasia adaptive and not life threatening. In the absence of significant toxicity or significant weight gain depressions, the CPRC considered the highest dose not high enough and therefore inadequate in both sexes of mice. A new carcinogenicity study is not required.

E. **Additional Toxicological Data on Ethalfluralin**

1. Metabolism

Reference: Absorption, Distribution and Elimination of ¹⁴C Ethalfluralin in Rats. Study No. DR-0233-3655-001, June 22, 1993, MRID 428229-01, Submitted by Dow Chemical Company.

Disposition of ¹⁴C ethalfluralin was investigated in male and female Fischer 344 rats at single low (10 mg/kg) and high (100 mg/kg) oral doses, and repeated low oral doses of 10 mg/kg followed by a single radiolabeled dose. Absorption of ethalfluralin was estimated at between 79-87%, based on data supplied by the registrant. Feces represented the major route for excretion of ethalfluralin derived radioactivity, with 50.9-63.2% excreted in 72 hours by this route for male and female rats. Tissue levels at study termination (72 hours post-dose) were negligible (less than 0.3% of the administered dose in any one tissue):

The major urinary metabolite identified in male and female rats was the acid metabolite of ethalfluralin, or (2-methyl, 2-hydroxy-3-[2,6-dinitro-4-trifluoro-methyl] phenylamine] propanoic acid). This metabolite comprised between 7.2-9.0% of the administered dose in urine of female rats 0-24 hour post-dose, and between 13-17.3% of the administered dose in male rat urine. All other urinary metabolites comprised less than 6.2% of the dose in urine of males and females.

In feces, the major metabolite identified was an amino product of ethalfluralin metabolism, N-ethyl-N-(2-methyl-2-propenyl)-2-nitro-6-amino-4-(trifluoromethyl)benzenamine. This metabolite comprised between 2.7-7.1% of the administered dose in male and female rat feces across all dose groups, and appeared in slightly higher percentage in feces from high dose rats. A second metabolite identified in feces comprised between 1.6-4.6% of the dose and co-eluted with a standard of ethalfluralin in both male and female rats. Although a specific metabolic scheme was not presented for ethalfluralin in rats, major metabolites were identified in this study.

2. Genotoxicity

Reference: The Effect of Ethalfluralin on the Induction of Bacterial Mutation, Using a Modification of the Ames Test, MRID 00128693; The Effect of Ethalfluralin on the Induction of Reverse Mutations in Salmonella Typhimurium using the Ames Test, MRID 00128694; The Effect of Ethalfluralin in the Induction of Forward Mutation at the Thymidine Kinase Locus of L5178Y Mouse Lymphoma Cells, MRID 00128696; Chromosome Aberrations in Chinese Hamster Ovary Cells (CHO) In Vitro, MRID 00152219; The Effect of Ethalfluralin on the Induction of DNA Repair Synthesis in Primary Cultures of Adult Rat Hepatocytes, MRID 00128695.

Ethalfluralin was positive in two Salmonella assays for increased revertants with and without metabolic activation and negative at the TK Locus in L5178Y cells of the mouse lymphoma assay. Induction of chromosomal aberrations in CHO cells was positive with S-9 activation and negative in the absence of S-9 metabolic activation. Ethalfluralin was not mutagenic in the unscheduled DNA synthesis assay.

3. One-Year Chronic Toxicity

Reference: The Toxicity of Ethalfluralin (EL-161) Administered Orally to Beagle Dogs for One Year, Lilly Research Laboratories, Report No.D01684, November 1985, MRID 00153371 and 92062014.

Thirty-Two 7-to-8-month old Beagle dogs weighing between 7.3 and 8.3 Kg were divided into four groups of 4 dogs/sex/ dose and received ethalfluralin orally by capsule at 0, 4, 20 and 80 mg/kg/day. The NOEL and LEL are 4 and 20 mg/kg/day, respectively, with variations in erythrocyte morphology, increased thrombocyte count, increased erythroid series of the bone marrow and bilirubin in the urine. Amber urine indicative of

ethalfluralin metabolites and elevated alkaline phosphatase values were observed at the mid and high dose groups. Siderosis of the liver was observed in both sexes at the high dose level.

Reference: One-Year Dietary Toxicity Study with Ethalfluralin in the Fischer 344 Rat. Lilly Research Laboratories, Report No. R-257, August 1979, MRID 00094775.

Ethalfluralin was fed to 15 Fischer 344 rats/sex/dose of 0, 100, 250 and 750 ppm, (equivalent to 3.9, 9.7 and 28.4 mg/kg/day for males and 4.9, 11.9 and 34.4 mg/kg/day for females). The NOEL and LEL are 100 and 250 ppm, respectively, with significant increased blood creatinine and BUN values determined at the termination of the study.

Reference: One-Year Dietary Toxicity Study with Ethalfluralin in the B6C3F₁ Mouse. Lilly Research Laboratories, Report No. M-9157, April 1981; MRID 00094778.

Ethalfluralin was fed to 15 B6C3F₁ mice/sex/dose at 0, 100, 400 and 1500 ppm (equivalent to 12, 47.0 and 173 mg/kg/day for males and 12, 49 and 184 mg/kg/day for females). The NOEL and LEL are 100 ppm and 400 ppm, respectively. Alkaline phosphatase values were elevated at the mid and high dose levels. At the high dose level, SGPT values and relative liver weights were increased and BUN and creatinine values were decreased at the termination of the study.

4. 90-Day Subchronic Toxicity

Reference: Subchronic Toxicity of EL-161 Technical Grade in Dogs; Lilly Research Laboratories Report No. D-3733, August 1974, MRID 00135193.

Thirty-two 10 to 14 month old Beagle dogs weighing between 6.4 and 13.8 kg were divided into four groups of 4 dogs/sex/dose administered orally by capsule at 6.25, 27.5 or 125 mg/kg/day. On day 15 the high dose was discontinued due to intolerance (after a 16-day recovery period) then reduced to 80 mg/kg/day for 90 days. The NOEL and LEL are 27.5 and 80 mg/kg/day, respectively, with elevated alkaline phosphatase, cholesterol and BUN values. Slight fatty metamorphosis of the liver was reported at the high dose level.

Reference: Three-Month Oral Toxicity Study of Ethalfluralin (EL-161) in Rats. Lilly Research Laboratories, Report No. R-936, February 1978, MRID 00135191.

Ethalfluralin was fed to 10 Fischer 344 rats/sex/dose of 0, 250, 500, 1100, 2500 or 5000 ppm equivalent to 14, 29, 63, 146 or 313 mg/kg/day, respectively, for 90 days.

This study is referenced for the dose selection used in the one (R-257) and two (R-267 and R-277) year studies. The NOEL and LEL are 500 and 1100 ppm, respectively, with a significant ($p < 0.05$) increase in absolute and relative liver weights (20%), decrease in RBC, hematocrit and hemoglobin values and increased relative kidney weights were reported at this dose. Bright yellow urine and stained fur (indicative of ethalfluralin metabolites) were observed at the 500 ppm level and above. Hematology and clinical chemistry values were determined at the termination of the study.

Reference: Three Month Oral Toxicity Study of Ethalfluralin (EL-161) in Mice, Lilly Research Laboratories Report No. M-9286, May 1978, MRID 0094774.

Ethalfluralin was fed to 15 B6C3F₁ mice/sex/dose at 0, 560, 1100, 2250, 4000 or 8000 ppm equivalent to 68, 136, 285, 538 or 1205 mg/kg/day, respectively. The NOEL and LEL are 560 and 1100 ppm, respectively, with decrease in bilirubin values and absolute kidney weights at this level. A dose related incidence of yellow-orange urine was observed, indicative of ethalfluralin metabolites.

5. Structure-Activity Relationships

Ethalfluralin is structurally related to four other dinitro-trifluoro herbicides (Figure 1) and is expected to behave in a biologically similar manner. The limited information provided by the toxicology one-liners indicate the following carcinogenic concerns:

Benefin - Significant ($p < 0.05$) increasing trend in liver cell adenomas and carcinomas combined in B6C3F₁ mice. The mouse and rat studies are not adequate for carcinogenic evaluation. Not mutagenic in the test systems assayed.

Flumetralin (Prime^R) - Significant increase in mammary adenocarcinomas and combined adenomas/adenocarcinomas in female rats at the 30 ppm level. Significant increase in adrenal pheochromocytomas in male rats at the 30 ppm level. No carcinogenic potential in mice. Positive

with and without activation in the gene mutation assay in Salmonella typhimurium (Ames test). Negative in the mouse micronucleus assay.

Profluralin - Significant ($p < 0.05$) increase in hepatoma B in male mice. No carcinogenic potential in rats. No genotoxicity studies on file. No registered uses in the U.S.

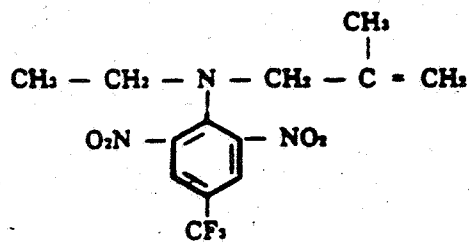
Trifluralin - Classified as a Group C Carcinogen based on tumors of the renal pelvis and urinary bladder in male and female Fischer 344 rats. No carcinogenic potential in mice. Not mutagenic in the test systems assayed.

6. Carcinogenicity in animals -- Ethalfluralin

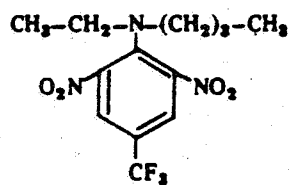
After a full evaluation of all of the data and supporting information regarding animal carcinogenicity, the Committee concludes that exposure to ethalfluralin resulted in an increased incidence of fibroadenomas and combined adenomas/fibroadenomas of the mammary gland in female rats. The induction of mammary gland tumors occurred at more than one dose and the incidence of tumors at both the mid- and high doses exceeded the historical control range (even though the dosing was not as high as the animals could have tolerated). A structurally related analog, flumetralin, also increased mammary tumors (adenocarcinomas and combined adenomas/adenocarcinomas) in female rats. With higher dosing, it may have been possible for the mammary tumors induced by ethalfluralin to progress to carcinomas, as was seen with flumetralin. There was also a suggestion of other tumor types, at the same sites (kidney and urinary bladder) which are the targets for another analog, trifluralin. Ethalfluralin induced mutations in the Salmonella assay \pm activation and was clastogenic in cultured mammalian cells with activation; this genotoxic activity supports a carcinogenicity concern for ethalfluralin. The relevance of the tumor data to an evaluation of ethalfluralin's potential for human carcinogenicity is discussed elsewhere in this report.

Figure 1

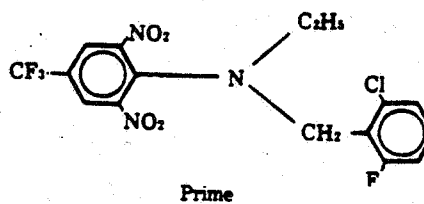
Structural Analogues of Ethalfluralin



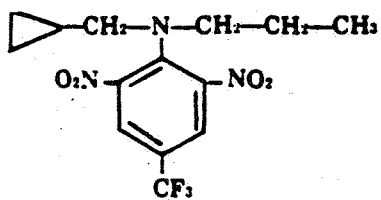
Ethalfluralin



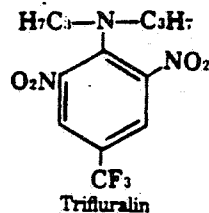
Benefin



Prime



Profluralin



Trifluralin

F. Weight of the Evidence Consideration

The Committee considered the following observations regarding the toxicology of ethalfluralin for a weight-of-the-evidence determination on its carcinogenic potential.

1. Male and female rats were fed 0, 4.2, 10.7 or 32.3 mg/kg/day in replicate studies for two years. The highest dose tested in male rats was considered inadequate. The highest dose in females was not inadequate; however, females could have tolerated a higher dose. There were no significant compound-related tumors observed in male rats.

Female rats had significant dose-related increasing trends, and significant differences in the pair-wise comparisons of both the 10.7 and the 32.3 mg/kg/day dose groups with the controls, for mammary gland fibroadenomas and combined mammary gland adenomas and/or fibroadenomas. The incidence of combined mammary gland adenomas and/or fibroadenomas in the mid and high dose group exceeds the historical control values for this tumor.

2. Male and female mice were fed 0, 10.3, 41.9 or 163.3 mg/kg/day in replicate studies for two years. The highest dose tested in mice was considered inadequate in both sexes.
3. From the studies submitted ethalfluralin is positive in two Salmonella assays, negative in the mouse lymphoma assay and unscheduled DNA synthesis assay. Ethalfluralin is positive for chromosomal aberrations in the CHO assay with activation and negative without activation. The genotoxic activity by ethalfluralin supports a carcinogenicity concern.
4. Ethalfluralin is structurally related to four other dinitrotrifluoro compounds: benefin, flumetralin, profluralin and trifluralin with demonstrated carcinogenic potential in mice and/or rats.

G. Classification of Carcinogenic Potential:

The Peer Review Committee considered the criteria contained in the EPA's "Guidelines for Carcinogen Risk Assessment" [FR51: 33992-34003, 1986] for classifying the weight of evidence for carcinogenicity.

The Peer Review Committee agreed that ethalfluralin should be classified as a Group C - possible human carcinogen and that a low-dose extrapolation methodology (Q^*) be applied to the animal data. This decision was based on evidence of increased incidences of mammary gland tumors in the female rat at two doses, and the incidences at these doses were well in excess of historical controls, even though dosing was not as high as the animals could have tolerated. A structurally related analog, flumetralin, also increased mammary tumors (adenocarcinomas and combined adenomas/adenocarcinomas) in female rats. With higher dosing, it may have been possible for the mammary tumors induced by ethalfluralin to progress to carcinomas, as was seen with flumetralin. There was also a suggestion of other tumor types in the rat, at the same sites (kidney and urinary bladder) which are the targets for another analog, trifluralin along with positive data for genotoxicity.

Administration of ethalfluralin in the diet to B6C3F₁ mice did not result in increases of any tumors; however, dosing in this study was considered to be inadequate in both sexes. The highest dose administered to male rats was also considered to be inadequate. The Committee nevertheless did not recommend repeating any of the studies, as there was sufficient information to perform a risk characterization, based on the mammary tumors in the female rat alone.

The Group C classification was based on mammary tumors in the female rat with a suggestion of bladder tumors in females and kidney tumors in both sexes, genotoxicity and SAR to other aromatic amine chemicals. A low dose extrapolation model will be applied to the animal data for the quantification of human risk (Q_1), based on the mammary tumors in the female rat.



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

Subject: Ethalfluralin, Quantitative Risk Assessment, Comparison of 2 Q_1 's (old, 1981 vs new, 1994), from Combined Data from Two Chronic/Oncogenicity Studies in Fischer 344 Rats, 1981

From: Bernice Fisher, Biostatistician
Statistics Section
Science Analysis Branch
Health Effects Division (7509C)

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

Caswell No. 453B

To: Ray Landolt, Pharmacologist
Review Section I
Toxicology Branch II
Health Effects Division (7509C)

Thru: Hugh Pettigrew, Ph.D., Section Head
Statistics Section
Science Analysis Branch
Health Effects Division (7509C)

Hugh Pettigrew 8/3/94

The unit risk, Q_1 (mg/kg/day)⁻¹ of Ethalfluralin, based upon female rat mammary gland (adenomas and/or fibroadenomas) tumor rates is 8.90×10^{-2} in human equivalents (converted from animals to humans by use of the 3/4's scaling factor-1994, Tox Risk, 3.5-K.Crump)*. The dose levels used in the combined rat studies were 0, 4.2 10.7 and 32.3 mg/kg/day of Ethalfluralin. The corresponding combined tumor rates were 13/60, 11/60, 25/59, and 29/59. (Using the previous method of converting to human equivalents by means of the 2/3's surface area adjustment in the above data set, the unit risk, Q_1 is 1.38×10^{-1} (mg/kg/day)⁻¹ in human equivalents.)

The previous (1981) unit risk, Q_1 (mg/kg/day)⁻¹ of Ethalfluralin, based upon only female rat mammary gland fibroadenoma tumor rates, was 1.3×10^{-1} in human equivalents (converted from animals to humans by use of the 2/3's surface area adjustment and the Global79 computer program of K.Crump). The dose levels used were the same as for the current estimation of unit risk. However the corresponding tumor rates used, fibroadenomas only, were 9/60, 10/60, 21/60 28/60.

* See Memo - Deriving Q_1 's Using the Unified Interspecies Scaling Factors, P.A. Fenner-Crisp, Director-HED, 7/1/94.

cc: Debra Edwards, CCB
Larry Dorsey, OREB

James Koriya, SAB
Caswell File





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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review of Ethalfluralin

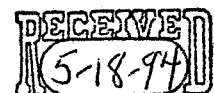
FROM: Ray Landolt *RL 5/17/94*
Review Section I
Toxicology Branch II
Health Effects Division (7509C)

TO: Esther Rinde, Ph.D.
Manager, Carcinogenicity Peer Review Committee
Science Analysis Branch
Health Effects Division (7509C)

THRU: Mike Ioannou, Ph.D., Section Head *J.M. Ioannou 5/17/94*
Review Section I
Toxicology Branch II
Health Effects Division (7509C)

and
Marcia van Gemert, Ph.D., Branch Chief
Toxicology Branch II
Health Effects Division (7509C) *M. van Gemert 5/18/94*

Attached is an overview of the carcinogenic potential of ethalfluralin prepared for the weight-of-the-evidence consideration by the Health Effects Division Carcinogenicity Peer Review Committee.



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I. Scientific Issues Considered by Toxicology Branch II, Health Effects Division, in Connection with the Classification of Ethalfluralin as a Carcinogen

- A. Material Reviewed
- B. Background Information
- C. Evaluation of Carcinogenicity Evidence
- D. Additional Toxicology Data on Ethalfluralin
- E. Structure-Activity Considerations
- F. Weight of evidence Considerations

II. Attachments

DER - Combined Chronic Toxicity and Carcinogenicity
Study with Ethalfluralin Technical in
Fischer 344 Rats
Historical control tumor data from Lilly
Research Laboratories
Tumor tables for Report Nos. R-267 and R-277

DER - Carcinogenicity Study with Ethalfluralin
Technical in B₆C₃F₁ Mice
Historical control tumor data from Lilly
Research Laboratories
Statistical Analysis by Lori L. Brunzman.
Tumor tables for Report Nos. M-9167 and
M-9177

Selected One-Liners

A. Material Reviewed

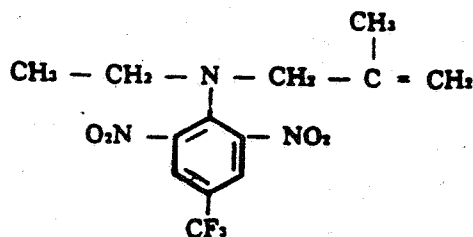
The material available for review consisted of DERs and other data summaries prepared by Roland A. Gessert, Irving Mauer, Tim McMahon and Ray Landolt from studies submitted by Eli Lilly (Dow Elanco). Statistical analysis was provided by Lori Brunsman. The material reviewed is attached to the file copy of this report.

B. Background Information

Ethalffluralin (EL-161) with the trade name Sonalin^R is registered for terrestrial food uses by Dow Elanco. Tolerances are established for residues of [N-ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4 (trifluoromethyl) benzenamine in or on a wide range of raw agricultural commodities including meat and milk listed in 40 CFR 180.416 at 0.05 ppm. This dinitroaniline herbicide is registered for preemergence control of annual grasses and broad leaf weeds.

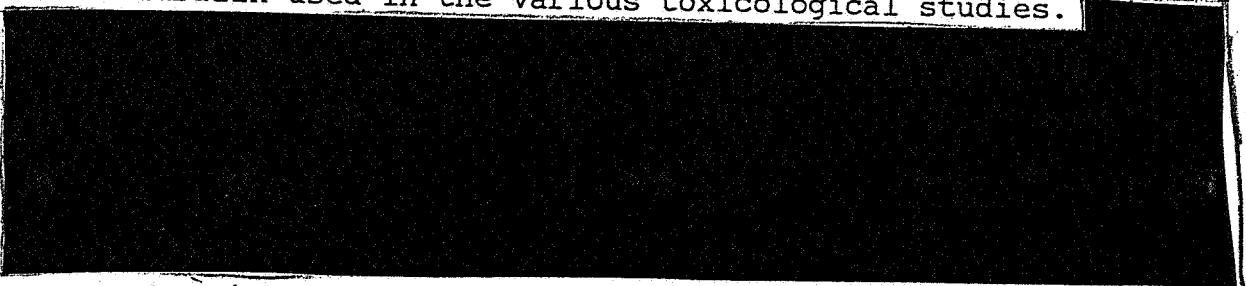
A Notice of Determination Not to Indicate a Rebuttal Presumption Against Registration (RPAR) was published in Federal Register January 4, 1984. Ethalffluralin was under consideration because the RPAR criteria for carcinogenicity and teratogenicity had been met. In evaluating the risks and benefits associated with the proposed uses of ethalffluralin, the Agency determined that the benefits exceeded the risk posed. A conditional registration was issued to allow time for the registrant to conduct a second species teratogenicity, a non-rodent chronic feeding and a dermal penetration study. The data on file in support of tolerances on ethalffluralin are complete.

The TOX Chem No. is 453B. The P.C. Code of ethalffluralin is 113101. The Chemical Abstract Registry Number (CAS No.) of ethalffluralin is 55283-68-6. The structure of ethalffluralin is presented below:



Ethalffluralin

The impurity nitrosamine is present in the technical (94.5%) ethalfluralin used in the various toxicological studies.



C. Evaluation of Carcinogenicity Data

1. Rat 2-Year Carcinogenicity Study

Reference: Two-Year Dietary Evaluation of Ethalfluralin in the Fischer 344 Rat, Lilly Research Laboratories, Reports R-267 and R-277, June 1981, MRID 070678.

a. Experimental Design

In replicate studies ethalfluralin was fed to 30 Fischer 344 rats/sex/group at levels of 100, 250 or 750 ppm, equivalent to 4.2, 10.7 or 32.2 mg/kg/day, respectively, with 60 rats/sex in the controls for 24 months.

b. Discussion of tumor Data

There were no significant compound-related tumors observed in male rats. Female rats had significant increasing trends, and significant differences in pair-wise comparisons of the 32.3 mg/kg/day dose group with the controls, for mammary gland fibroadenomas and combined mammary gland adenomas and/or fibroadenomas, all at $p < 0.01$. Female rats also had significant differences in the pair-wise comparisons of the 10.7 mg/kg/day dose group with the controls for mammary gland fibroadenomas ($p < 0.01$) and combined mammary gland adenomas and/or fibroadenomas ($p < 0.05$).

Since there was no significant statistical evidence of differential mortality with increasing doses of ethalfluralin, these statistical analyses were based upon the Cochran-Armitage trend test and the Fisher's Exact test for pair-wise comparisons of the dosed groups with the controls. See Table 1 for female rat tumor analysis results.

Table 1. Ethalfluralin - Harlan Industries Fischer 344 Combined Rat Studies

Female Mammary Gland Tumor Rates⁺ and Cochran-Armitage Trend Test and Fisher's Exact Test Results (p values)

	<u>Dose (mg/kg/day)</u>			
	0	4.2	10.7	32.3
Adenomas (%)	4/60 (7)	1 ^a /60 (2)	5/59 (8)	1/59 (2)
p =	0.168	0.182 ^a	0.489	0.187 ^a
Fibroadenomas (%)	9/60 (15)	10/60 (17)	21 ^b /59 (36)	28/59 (47)
p =	0.000 ^{**}	0.500	0.008 ^{**}	0.000 ^{**}
Combined (%)	13/60 (22)	11/60 (18)	25 ^c /59 (42)	29/59 (49)
p =	0.000 ^{**}	0.410 ^a	0.013 [*]	0.002 ^{**}

⁺Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

^aNegative change from control.

^aFirst adenoma observed at week 80, dose 4.2 mg/kg/day.

^bFirst fibroadenoma observed at week 70, dose 10.7 mg/kg/day.

^cOne animal in the 10.7 mg/kg/day dose group had both an adenoma and a fibroadenoma of the mammary gland.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If ^{*}, then $p < 0.05$. If ^{**}, then $p < 0.01$.

Table 2. Historical Control Incidence of Fibroadenomas in Female Fischer 344 Rats (Data obtained at Lilly Research Laboratories from May 1977 to February 1979).

Study Number	Termination of study	Number of Untreated females	Number of Females with Fibroadenomas	Percent Incidence
R-695	May 1977	120	16	13.3%
R-1136*	November 1978	30	8	26.7%
R-1146*	November 1978	30	5	16.7%
R-1246*	December 1978	30	5	16.7%
R-1256*	December 1978	29	4	13.8%
R-87*	February 1979	30	0	0.0%
R-97*	February 1979	30	7	23.3%
R-167*	February 1979	30	6	20.0%
R-177*	February 1979	<u>30</u>	<u>4</u>	<u>13.3%</u>
Total		359	55	Overall Incidence 15.3%

***Replicate Studies**

The historical control incidence (Table 2) ranges from 0 to 26.7% with an average incidence of 15.3% for this tumor. This compares with an incidence in the ethalfluralin study of 22% in the controls, 18% in the low dose, 42% in the mid dose and 49% in the high dose. The incidences of combined mammary gland adenomas and/or fibroadenomas in the mid (250 ppm) and high (750 ppm) dose exceed the historical control values for this tumor.

c. Non-Neoplastic Lesions and other findings

There were no changes in body weight gain related to the dose levels administered as compared to the controls in male or female rats (Table 3).

The statistical evaluation of mortality indicates no significant incremental changes with increasing doses of ethalfluralin in male or female rats. See Tables 4 and 5 for mortality test results.

No clinical signs of toxicity were observed related to the dose levels administered, except for a dose related incidence of dark yellow urine indicative of the presence of ethalfluralin metabolites.

The following table summarizes the mean body weight gain in male and female rats at the 3 and 24 month interval for studies R-267 and R-277.

Table 3. Mean Body Weight(g) with (%) Gain as Compared to Controls

Month	Controls		4.2 mg/kg/day		10.7 mg/kg/day		32.2 mg/kg/day	
	<u>R-267</u>	<u>R-277</u>	<u>R-267</u>	<u>R-277</u>	<u>R-267</u>	<u>R-277</u>	<u>R-267</u>	<u>R-277</u>
<u>Males</u>								
0	134	120	136	123	137	120	136	126
3	337	350	338(0.4%)	344(4%)	341(0.4%)	346(1%)	343(2%)	345(4%)
24	419	406	488(6%)	433(8%)	468(2%)	413(2%)	425(1%)	419(2%)
<u>Females</u>								
0	106	96	107	96	108	95	107	96
3	200	205	199(2%)	200(5%)	194(9%)	197(6%)	194(7%)	199(6%)
24	307	298	315(3%)	310(6%)	303(3%)	292(2%)	313(2%)	296(1%)

Table 4. Ethalfluralin - Harlan Industries Fischer 344 Combined Rat Studies

Female Mortality Rates⁺ and Cox or Generalized K/W Test Results

Dose (mg/kg/day)	<u>Weeks</u>				Total
	1-26	27-52	53-78	79-105 ^f	
0	0/60	0/60	3/60	24/57	27/60 (45)
4.2	0/60	0/60	2/60	18/58	20/60 (33)
10.7	0/59 ^a	0/59	4/59	18/55	22/59 (37)
32.3	1/60	0/59	4/59	17/55	22/60 (37)

⁺Number of animals that died during interval/Number of animals alive at the beginning of the interval.

^aOne accidental death at week 22, dose 10.7 mg/kg/day.

^fFinal sacrifice at week 105.

() Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 5. Ethalfluralin - Harlan Industries Fischer 344 Combined Rat Studies

Male Mortality Rates⁺ and Cox or Generalized K/W Test Results

Dose (mg/kg/day)	<u>Weeks</u>				Total
	1-26	27-52	53-78	79-105 ^f	
0	0/60	0/60	3/60	15/57	18/60 (30)
4.2	0/60	1/60	1/59	16/58	18/60 (30)
10.7	0/60	0/60	2/60	18/58	20/60 (33)
32.3	0/60	0/60	3/60	14/57	17/60 (28)

⁺Number of animals that died during interval/Number of animals alive at the beginning of the interval.

^fFinal sacrifice at week 104.

() Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

There were no changes in hematology or clinical chemistry values attributed to the administration of ethalfluralin as compared to the controls at the termination of the study.

There were no significant differences in organ weights related to the administration of ethalfluralin with the exception of a slight increase in relative liver weight in males (8.9%) and females (4.1 and 4.7%) at the highest dose levels as compared to controls

Histopathological non-neoplastic findings were limited to yellow coloration of fat at the high dose level.

d. Adequacy of Dosing for Assessment of Carcinogenicity

The dose selections for the 2-year rat feeding study were based on levels of 250, 500, 1100, 2500 or 5000 ppm fed to Fischer 344 rats for 90 days (Study No. R-936). The NOEL and LOEL in the 90-day study are 500 and 1100 ppm (29 and 63 mg/kg/day) respectively, with an increase in absolute and relative liver weights (20%) at the 1100 ppm level. The 750 ppm level was selected as the high dose for the 2 year feeding study. Based on the parameters investigated in the 2-year feeding study the systemic NOEL is greater than 750 ppm. The 750 ppm level was not adequate for carcinogenic testing.

2. Mouse 2-Year Carcinogenicity Study

Reference: Two-Year Dietary Evaluation of Ethalfluralin in the B₆C₃F₁ Mouse, Lilly Research Laboratories Reports M-9167 and M-9177, July 1981, MRID 070680.

a. Experimental Design

In replicate studies ethalfluralin was fed to 40 B₆C₃F₁ mice/sex/group at levels of 0, 100, 400 or 1500 ppm, equivalent to 10.3, 41.9 or 163.3 mg/kg/day, respectively, with 60 mice/sex in the controls.

b. Discussion of Tumor Data

No increased incidences of neoplasms were observed in these replicate studies at the dose levels administered for 24 months.

c. Non-neoplastic lesions and other findings

Evaluation of mortality incidence indicates no change with increasing doses of ethalfluralin were observed as compared to the controls.

No clinical signs of toxicity were observed related to the dose levels administered.

Mean body weight gain of the control and test levels were comparable during the experimental period until the 24th month when a significant ($p < 0.05$) decrease in body weight gain of 18-30% as compared to the control was reported for males and females at the high dose level.

A significant ($p < 0.05$) decrease in hematocrit, hemoglobin and erythrocyte count, accompanied by a significant ($p < 0.05$) increase in mean corpuscular hemoglobin concentration was reported in the high dose females at the termination of the study.

A significant ($p < 0.05$) increase in alkaline phosphatase values was reported at the termination of the study in male and female mice at the high dose level.

Relative liver weights of females of the high dose level were significantly ($p < 0.05$) increased as compared to the controls. Relative kidney weights of females of the mid and high dose levels were significantly ($p < 0.05$) increased. Relative heart weight of females of the mid and high dose levels were significantly ($p < 0.05$) increased as compared to the controls.

A dose-related increased incidence of focal hepatocellular hyperplasia (Table 6) was reported in males and females of both replicate studies for the low, mid and high dose levels.

Table 6 - Incidence of Hepatocellular Hyperplasia in Male and Female Mice of Studies M-9167 and M-9177 Combined

	<u>CONTROL</u>	<u>100 ppm</u>	<u>400 ppm</u>	<u>1500ppm</u>
Males	5.9% (7/119)	11.3% (9/80)	15.0% (12/80)	17.7% (14/79)
Females	1.7% (2/120)	5.0% (4/80)	7.5% (6/80)	26.3% (21/80)

Table 7 Historical Control Incidence Of Hepatocellular
Hyperplasia in B₆C₃F₁ Mice (Data obtained at Lilly
Research Laboratories from February 1977
to April 1979)

<u>Study Number</u>	<u>Males</u>	<u>Females</u>
M-9087	15.5% (9/60)	8.3% (5/60)
M-9097	10.0% (6/60)	10.0% (6/60)
M-9067	3.3% (2/60)	8.3% (5/60)
M-9077	1.7% (1/60)	1.7% (1/60)

The studies with ethalfluralin were conducted from June 1977 to June 1979.

On inspection of the incidence of hepatocellular hyperplasia observed in the low dose with ethalfluralin and the historical control incidence, a variation in the spontaneous incidence of hepatocellular hyperplasia can occur in the mouse. On this basis the NOEL and LEL is 100 ppm (10.3 mg/kg/day) and 400 ppm (41.9 mg/kg/day), respectively.

d. Adequacy of Dosing for Assessment of Carcinogenicity

The dose selection for this 2-year study is based on levels of 560, 1100, 2250, 4000 or 8000 ppm, fed to B₆C₃F₁ mice for 90-days (Study No. M-9286). The NOEL and LOEL in the 90-day study are 560 and 1100 ppm, respectively, with decreases in bilirubin values and absolute kidney weights.

Based on an increased incidence of hepatocellular hyperplasia as compared to the concurrent and historical control incidence for this lesion, the mid dose (400 ppm) is considered adequate for carcinogenicity testing in the two-year study.

D. Additional Toxicological Data on Ethalfluralin

1. Metabolism

Reference: Absorption, Distribution and Elimination of ¹⁴C Ethalfluralin in Rats. Study No. DR-0233-3655-001, June 22, 1993, MRID 428229-01, Submitted by Dow Chemical Company.

Disposition of ¹⁴C-ethalfluralin was investigated in male and female Fischer 344 rats at single low (10 mg/kg) and high (100 mg/kg) oral doses, and repeated low oral doses of 10 mg/kg followed by a single radiolabeled dose. Absorption of ethalfluralin was estimated at between 79-87%, based on data supplied by the registrant. Feces represented the major route for excretion of ethalfluralin derived radioactivity, with 50.9-63.2% excreted in 72 hours by this route for male and female rats. Tissue levels at study termination (72 hours post-dose) were negligible (less than 0.3% of the administered dose in any one tissue).

The major urinary metabolite identified in male and female rats was the acid metabolite of ethalfluralin, or (2-methyl, 2-hydroxy-3-[2,6-dinitro-4-trifluoro-methyl] phenylamine] propanoic acid). This metabolite comprised between 7.2-9.0% of the administered dose in urine of female rats 0-24 hour post-dose, and between 13-17.3% of the administered dose in male rat urine. All other urinary metabolites comprised less than 6.2% of the dose in urine of males and females.

In feces, the major metabolite identified was an amino product of ethalfluralin metabolism, N-ethyl-N-(2-propenyl)-2-nitro-6-amino-4-(trifluoromethyl) benzenamine. This metabolite comprised between 2.7-7.1% of the administered dose in male and female rat feces across all dose groups, and appeared in slightly higher percentage in feces from high dose rats. A second metabolite identified in feces comprised between 1.6-4.6% of the dose and co-eluted with a standard of ethalfluralin in both male and female rats. Although a specific metabolic scheme was not presented for ethalfluralin in rats, major metabolites were identified in this study.

2. Genotoxicity

Reference: The Effect of Ethalfluralin on the Induction of Bacterial Mutation, Using a Modification of the Ames Test, MRID 250475; The Effect of Ethalfluralin on the Induction of Reverse Mutations in Salmonella Typhimurium using the Ames Test, MRID 254075; The Effect of Ethalfluralin in the Induction of Forward Mutation at the Thymidine Kinase Locus of L5178Y Mouse Lymphoma Cells, MRID 250475; Chromosome Aberrations in Chinese Hamster Ovary Cells (CHO) In Vitro, MRID 259342; The Effect of Ethalfluralin on the Induction of DNA Repair Synthesis in Primary Cultures of Adult Rat Hepatocytes, MRID 250475.

Ethalfluralin was positive in two salmonella assays for increased revertants with and without metabolic activation and negative at the TK Locus in L5178Y cells of the mouse lymphoma assay. Induction of chromosomal aberrations in CHO cells was positive with S-9 activation and negative in the absence of S-9 metabolic activation. Ethalfluralin was not mutagenic in the unscheduled DNA synthesis assay.

3. One-Year Chronic Toxicity

Reference: The Toxicity of Ethalfluralin (EL-161) Administered Orally to Beagle Dogs for One Year, Lilly Research Laboratories, Report No.D01684, November 1985, MRID 260434 and 262711.

Thirty-Two 7-to-8-month old Beagle dogs weighing between 7.3 and 8.3 Kg were divided into four groups of 4 dogs/sex/ dose were administered ethalfluralin orally by capsule at 0, 4, 20 and 80 mg/kg/day. The NOEL and LEL are 4 and 20 mg/kg/day, respectively, with variations in erythrocyte morphology, increased thrombocyte count, increased erythroid series of the bone marrow and bilirubin in the urine. Amber urine indicative of ethalfluralin metabolites and elevated alkaline phosphatase values were observed at the mid and high dose groups. Siderosis of the liver was observed in both sexes at the high dose level.

Reference: One-Year Dietary Toxicity Study with Ethalfluralin in the Fischer 344 Rat. Lilly Research Laboratories, Report No. R-257, August 1979, MRID 070678.

Ethalfluralin was fed to 15 Fischer 344 rats/sex/dose of 0, 100, 250 and 750 ppm, (equivalent to 3.9, 9.7 and 28.4 mg/kg/day for males and 4.9, 11.9 and 34.4 mg/kg/day for females). NOEL and LEL are 100 and 250 ppm, respectively, with significant increased blood creatinine and BUN values determined at the termination of the study.

Reference: One-Year Dietary Toxicity Study with Ethalfluralin in the $B_6C_3F_1$ Mouse. Lilly Research Laboratories, Report No. M-9157, April 1981; MRID 070679.

Ethalfluralin was fed to 15 $B_6C_3F_1$ mice/sex/dose at 0, 100, 400 and 1500 ppm (equivalent to 12, 47.0 and 173 mg/kg/day for males and 12, 49 and 184 mg/kg/day for females. NOEL and LEL are 100 ppm and 400 ppm, respectively. Alkaline phosphatase values were elevated at the mid and high dose levels. At the high dose level, SGPT values and relative liver weights were increased and BUN and creatinine values were decreased at the termination of the study.

4. 90-Day Subchronic Toxicity

Reference: Subchronic Toxicity of EL-161 Technical Grade in Dogs; Lilly Research Laboratories Report No. D-3733, August 1974, MRID 097327.

Thirty-two, 10 to 14 month old Beagle dogs weighing between 6.4 and 13.8 Kg were divided into four groups of 4 dogs/sex/dose administered orally by capsule at 6.25, 27.5 or 125 mg/kg/day. On day 15 the high dose was discontinued due to intolerance (after a 16-day recovery period) then reduced to 80 mg/kg/day for 90 days. NOEL and LEL are 27.5 and 80 mg/kg/day, respectively, with elevated alkaline phosphatase, cholesterol and BUN values. Slight fatty metamorphosis of the liver was reported at the high dose level.

Reference: Three-Month Oral Toxicity Study of Ethalfluralin (EL-161) in Rats. Lilly Research Laboratories, Report No. R-936, February 1978, MRID 097327.

Ethalfluralin was fed to 10 Fischer 344 rats/sex/dose of 0, 250, 500, 1100, 2500 or 5000 ppm equivalent to 14, 29, 63, 146 or 313 mg/kg/day, respectively, for 90 days.

This study is referenced for the dose selection used in the one (R-257) and two (R-267 and R-277) year studies. The NOEL and LEL are 500 and 1100 ppm, respectively, with a significant ($p < 0.05$) increase in absolute and relative liver weights (20%), decrease in RBC, hematocrit and hemoglobin values and increased relative kidney weights were reported at this dose. Bright yellow urine and stained fur (indicative of ethalfluralin metabolites) were observed at the 500 ppm level and above. Hematology and clinical chemistry values were determined at the termination of the study.

Reference: Three Month Oral Toxicity Study of Ethalfluralin (EL-161) in Mice, Lilly Research Laboratories Report No. M-9286, May 1978, MRID 070678.

Ethalfluralin was fed to 15 B₆C₃F₁, mice/sex/dose at 0, 560, 1100, 2250, 4000 or 8000 ppm equivalent to 68, 136, 285, 538 or 1205 mg/kg/day, respectively. The NOEL and LEL are 560 and 1100 ppm, respectively, with decrease in bilirubin values and absolute kidney weights at this level. A dose related incidence of yellow-orange urine was observed, indicative of ethalfluralin metabolites.

5. Developmental and Reproduction Studies

Reference: A Teratology Study of Orally Administered Ethalfluralin in the Rat, Bio Research Laboratories Report No. 81182, November, 1985, MRID 260434

Sprague-Dawley rats were dosed orally by gavage at 0, 50, 250 or 1000 mg/kg/day from day 6 to 15 of gestation, inclusive. Maternal NOEL and LEL are 50 and 250 mg/kg/day, respectively, with decrease in body weight gain and elimination of dark urine during days 7 through 16 of the study. Developmental NOEL is greater than 1000 mg/kg/day

Reference: A Teratology Study of Orally Administered Ethalfluralin in the Rabbit, Lilly Research Laboratories Report No. B01383, June 1983, MRID 250596

Dutch Belted rabbits were dosed orally by gavaged at 0, 25, 75, 150 or 300 mg/kg/day from day 6 to 18, inclusive. Maternal NOEL and LEL are 75 and 150 mg/kg/day, respectively, with decreased food consumption and abortions (2/15) reported at this level. Developmental NOEL and LEL are 75 and 150 mg/kg/day with increased resorptions, increased sternal and cranial variants reported at this level.

Reference: A Multi-Generation Reproduction study with Ethalfluralin in the Fischer 344 Rat. Lilly Research Laboratories Report No. R-68, R-738 and R-1248, March 1981 MRID 070682.

In a 3 generation reproduction study, male and female Fischer 344 rats were fed 0, 100, 250 or 750 ppm, equivalent to 0, 5, 12.5 and 37.5 mg/kg/day, respectively. The systemic (parental) NOEL and LEL are 250 and 750 ppm, respectively, with a decrease in body weight gain in males of all 3 generations.

Reproductive NOEL is greater than 750 ppm. The quality of this study was questioned by California Department of Pesticide Regulation based on the lack of histopathology on reproductive organs of F₀ and F₁ adults with a request for 7 months multigeneration bridging study.

Reference: A 7-Month Multigeneration Bridging Study of Ethalfluralin (EL-161) Administered in the Diet to Fischer 344 Rat, Lilly Research Laboratories Report No. R16890 and R16990 April 1992, MRID 42300301.

This study is supplementary to the preceding multigeneration reproduction study. In a 7-month multigeneration reproduction bridging study, Fischer 344 rats were fed 0, 100, 250 or 750 ppm, equivalent to 0, 8, 20 and 61 mg/kg/day, respectively. The NOEL and LEL for parental toxicity are 250 and 750 ppm, respectively, with increase in absolute and relative liver weights. No treatment related histopathological effects were noted. Reproductive NOEL is equal to or greater than 750 ppm and the LEL is greater than 750 ppm.

E. Structure-Activity Relationships

Ethalfluralin is structurally related to four other dinitro-trifluro herbicides (Figure 1) and is expected to behave in a biologically similar manner. The limited information provided by the toxicology one-liners indicate the following carcinogenic concerns.

Benefin - Significant ($p < 0.05$) increasing trend in liver cell adenomas and carcinomas combined in B₆C₃F₁ mice. The mouse and rat studies are not adequate for carcinogenic evaluation. Not mutagenic in the test systems assayed.

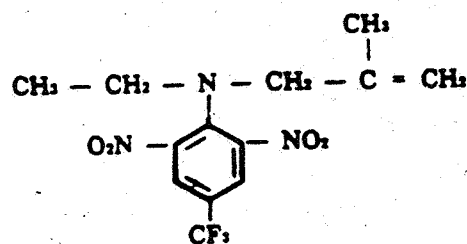
Prime^R - Significant increase in mammary adenocarcinomas and combined adenomas/adenocarcinomas in female rats. Significant increase in adrenal pheochromocytomas in male rats. No carcinogenic potential in mice. Positive with and without activation in the gene mutation assay in Salmonella typhimurium (Ames test). Negative in the mouse micronucleus assay.

Profluralin - Significant ($p < 0.05$) increase in hepatoma B in male mice. No carcinogenic potential in rats. No genotoxicity studies on file. No registered uses in the U.S.

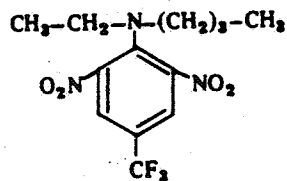
Trifluralin - Classified as a Group C Carcinogen based on tumors of the renal pelvis and urinary bladder in male and female Fischer 344 rats. No carcinogenic potential in mice. Not mutagenic in the test systems assayed.

Figure 1.

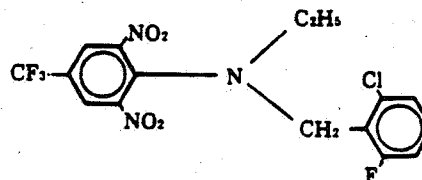
Structural Analogues of Ethalfluralin



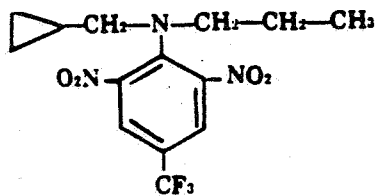
Ethalfluralin



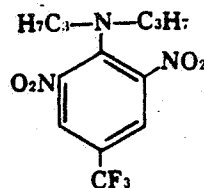
Benefin



Prime



Profuralin



Trifluralin

F. Weight of the Evidence Consideration

The Committee considered the following observations regarding the toxicology of ethalfluralin for a weight-of-the-evidence determination on its carcinogenic potential.

1. Male and female rats were fed 0, 4.2, 10.7 or 32.3 mg/kg/day in replicate studies for two years. The highest dose tested was not adequate for carcinogenic testing.

There were no significant compound-related tumors observed in male rats.

Female rats had significant dose-related increasing trends, and significant differences in the pair-wise comparisons of both the 10.7 and the 32.3 mg/kg/day dose groups with the controls, for mammary gland fibroadenomas and combined mammary gland adenomas and/or fibroadenomas. The incidence of combined mammary gland adenomas and/or fibroadenomas in the mid and high dose group exceeds the historical control values for this tumor.

2. Male and female mice were fed 0, 10.3, 41.9 or 163.3 mg/kg/day in replicate studies for two years. The dietary levels fed were adequate for carcinogenic testing based on an increased incidence of focal hepatocellular hyperplasia in the mid and high dose male and female mice.
3. From the studies submitted ethalfluralin is positive in the Salmonella assay, negative in the mouse lymphoma and unscheduled DNA synthesis assay.
4. Ethalfluralin is structurally related to four other dinitrotrifluoro compounds: benefin, Prime^R, profluralin and trifluralin with demonstrated carcinogenic potential in mice and/or rats.



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

FILE COPY

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

May 19, 1994

MEMORANDUM

SUBJECT: Carcinogenicity Peer Review Meeting on ETHALFLURALIN

FROM: Esther Rinde, Ph.D. *E.R.*,
Manager, Carcinogenicity Peer Review
Health Effects Division (7509c)

TO: Addressees

Attached for your review is a package on ETHALFLURALIN prepared by Ray Landolt.

A meeting to reconsider the carcinogenicity classification of this chemical is scheduled for Wednesday June 08, 1994, at 10:00 am in Room 817, CM2.

Addressees

P. Fenner-Crisp
R. Engler
W. Burnam
K. Baetcke
M. Van Gemert
K. Dearfield
H. Pettigrew
B. Fisher
L. Brunsman
E. Doyle
R. Landolt
M. Ioannou
R. Hill
Y. Woo
R. DiLavore/L. Brennecke



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

APR 13 1994

MEMORANDUM

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

SUBJECT: Ethalfluralin Qualitative Risk Assessment Based On
Harlan Industries Fischer 344 Rat Dietary Studies

Caswell No. 453B

TO: Ray Landolt, Pharmacologist
Review Section I
Toxicology Branch II
Health Effects Division (7509C)

FROM: Lori L. Brunsman, Statistician
Statistics Section
Science Analysis Branch
Health Effects Division (7509C)

Lori L. Brunsman
4/13/94

THROUGH: Hugh M. Pettigrew, Section Head
Statistics Section
Science Analysis Branch
Health Effects Division (7509C)

Hugh M. Pettigrew
4/13/94

Summary

This qualitative risk assessment of Ethalfluralin was based upon two chronic oncogenicity feeding studies conducted in Harlan Industries Fischer 344 rats, both initiated in March, 1977. The animals received doses of 0.0, 4.2, 10.7, or 32.3 mg/kg/day of Ethalfluralin for 104 weeks. These studies have been combined for this qualitative risk assessment.

The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of Ethalfluralin in male or female rats.

There were no significant compound-related tumors observed in male rats.

Female rats had significant dose-related increasing trends, and significant differences in the pair-wise comparisons of both the 10.7 and the 32.3 mg/kg/day dose groups with the controls, for mammary gland fibroadenomas and combined mammary gland adenomas and/or fibroadenomas.



Background

Two replicate chronic oncogenicity feeding studies in Fischer 344 rats supplied by Harlan Industries, Inc., Cumberland, Indiana, were conducted simultaneously by Lilly Research Laboratories, Greenfield, Indiana, for Elanco Products Company, Indianapolis, Indiana, and issued June 29, 1981 (Study Nos. R-267 and R-277; MRID Nos. 00094776 and 92062013; Accession No. 070678).

The study design allocated groups of 30 rats per sex per study replicate to dietary concentrations of 0.0, 0.01, 0.025, or 0.075 percent of Ethalfluralin for 104 weeks. The animals received time-weighted average doses (sexes and replicate studies combined) of 0.0, 4.2, 10.7, or 32.3 mg/kg/day.

Survival Analysis

The statistical evaluation of mortality indicated no significant incremental changes with increasing doses of Ethalfluralin in male or female rats. See Tables 1 and 2 for mortality test results.

The statistical evaluation of mortality was based upon the Thomas, Breslow and Gart computer program.

Tumor Analysis

There were no significant compound-related tumors observed in male rats.

Female rats had significant increasing trends, and significant differences in the pair-wise comparisons of the 32.3 mg/kg/day dose group with the controls, for mammary gland fibroadenomas and combined mammary gland adenomas and/or fibroadenomas, all at $p < 0.01$. Female rats also had significant differences in the pair-wise comparisons of the 10.7 mg/kg/day dose group with the controls for mammary gland fibroadenomas ($p < 0.01$) and combined mammary gland adenomas and/or fibroadenomas ($p < 0.05$).

Since there was no significant statistical evidence of differential mortality with increasing doses of Ethalfluralin, these statistical analyses were based upon the Cochran-Armitage trend test and the Fisher's Exact test for pair-wise comparisons of the dosed groups with the controls. See Table 3 for female rat tumor analysis results.

Table 1. Ethalfluralin - Harlan Industries Fischer 344 Combined Rat Studies

Male Mortality Rates⁺ and Cox or Generalized K/W Test Results

Dose (mg/kg/day)	<u>Weeks</u>				Total
	1-26	27-52	53-78	79-105 ^f	
0	0/60	0/60	3/60	15/57	18/60 (30)
4.2	0/60	1/60	1/59	16/58	18/60 (30)
10.7	0/60	0/60	2/60	18/58	20/60 (33)
32.3	0/60	0/60	3/60	14/57	17/60 (28)

⁺Number of animals that died during interval/Number of animals alive at the beginning of the interval.

^fFinal sacrifice at week 104.

() Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 2. Ethalfluralin - Harlan Industries Fischer 344 Combined Rat Studies

Female Mortality Rates⁺ and Cox or Generalized K/W Test Results

Dose (mg/kg/day)	<u>Weeks</u>				Total
	1-26	27-52	53-78	79-105 ^f	
0	0/60	0/60	3/60	24/57	27/60 (45)
4.2	0/60	0/60	2/60	18/58	20/60 (33)
10.7	0/59 ^a	0/59	4/59	18/55	22/59 (37)
32.3	1/60	0/59	4/59	17/55	22/60 (37)

⁺Number of animals that died during interval/Number of animals alive at the beginning of the interval.

^aOne accidental death at week 22, dose 10.7 mg/kg/day.

^fFinal sacrifice at week 105.

() Percent.

Note: Time intervals were selected for display purposes only.

Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

Table 3. Ethalfluralin - Harlan Industries Fischer 344 Combined Rat Studies

Female Mammary Gland Tumor Rates⁺ and Cochran-Armitage Trend Test and Fisher's Exact Test Results (p values)

	<u>Dose (mg/kg/day)</u>			
	0	4.2	10.7	32.3
Adenomas (%)	4/60 (7)	1 ^a /60 (2)	5/59 (8)	1/59 (2)
p =	0.168	0.182 ^a	0.489	0.187 ^a
Fibroadenomas (%)	9/60 (15)	10/60 (17)	21 ^b /59 (36)	28/59 (47)
p =	0.000 ^{**}	0.500	0.008 ^{**}	0.000 ^{**}
Combined (%)	13/60 (22)	11/60 (18)	25 ^c /59 (42)	29/59 (49)
p =	0.000 ^{**}	0.410 ^a	0.013 [*]	0.002 ^{**}

⁺Number of tumor bearing animals/Number of animals examined, excluding those that died or were sacrificed before week 53.

^aNegative change from control.

^aFirst adenoma observed at week 80, dose 4.2 mg/kg/day.

^bFirst fibroadenoma observed at week 70, dose 10.7 mg/kg/day.

^cOne animal in the 10.7 mg/kg/day dose group had both an adenoma and a fibroadenoma of the mammary gland.

Note: Significance of trend denoted at control.

Significance of pair-wise comparison with control denoted at dose level.

If *, then $p < 0.05$. If **, then $p < 0.01$.

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Two-Year Dietary Evaluation of Ethalfluralin in the Fisher 344 Rat. Studies R-267 and R-277 conducted by Elizabeth R. Adams, M.S. (Project Leader) et al. Lilly Research Laboratories, Greenfield, Indiana. June, 1981. Accession#070678.

Ethalfluralin (EL-161; Lilly Compound 94961) was administered to Fisher 344 rats in the diet for 2-years in replicate studies, to evaluate the chronic toxicity and oncogenic potential of the chemical. Dietary concentrations of the chemical were 0.0, 0.01, 0.025 and 0.075 percent, and were based on a 3-month study in rats. In the 3-month test 500 ppm was the NOEL; a statistically significant increase in liver weight (about 20%) occurred at 0.11 percent (1100 ppm). An increased liver weight of 20% in a 3-month study was considered excessive for a longterm 2-year study, so the high dose chosen of 0.075% was a dose interpolated to cause a 10% increase in liver weight. The low dose, 0.01%, was selected to provide a 100x multiple if residue levels were as great as 1 ppm. The middle dose, of 0.025%, was chosen as an acceptable intermediate.

Four hundred-eighty rats were used in the study; 360 receiving ethalfluralin, and 120 controls. These 480 rats were divided into two replicates, with 30 rats/sex/group in each replicate. All the rats were housed in one room. Replicate studies were conducted primarily to increase validity of judgements concerning possible treatment related effects based on reproducibility of these effects in the replicate studies. Replication was also done with different data collection days, so that one technician could better perform the study measurements at the proper times. Since the replicate studies were similar, the data were combined where appropriate.

Test Material:

Ethalfluralin, chemically is N-ethyl-N(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl)-benzenamide. The lot used was B30-Y64-35B, [REDACTED]

[REDACTED] the purity of the lot was 94.5%.

The concentrations of ethalfluralin in the diet were not adjusted for purity. The nitrosamine content of the ethalfluralin was [REDACTED]

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

Animals:

Fisher 344 rats, 4-5 weeks of age, were supplied as litters by Harlan Industries, Inc., Cumberland, Indiana in 2 shipments, one week apart. Extra rats were ordered to allow for culling before starting the study, and to replace during the first month any rat not adjusting to the laboratory conditions. Eight rats were replaced: 2 controls, one low dose, 3 middle dose, and 2 high dose. Replacements were due to respiratory problems, or weight loss, and one death (high dose).

Rats were held 2 weeks by litter and sex to adapt to caging, feeding, and watering systems of the laboratory. Rats differing greatly in weight from the general population, or which were in poor physical condition were not used.

Test rats were caged individually in stainless steel wire mesh cages (18 cm x 20 cm x 36 cm) suspended over absorbant cage board. The cage board was changed weekly. Racks and cages were washed and autoclaved monthly, and feeders every 2 weeks. The rats were kept in one room at $24 \pm 3^{\circ}\text{C}$, with relative humidity of 45%. Twelve hours of artificial light and 12 hours darkness were provided by automatic timers.

Identification:

Each rat had an ID number consisting of study number and animal number that specified sex and treatment. The number was attached to the cage and was used for all records, samples, and reports. Also, the rats were ear punched to denote dose level.

A standard mash ration was prepared every 2 to 4 weeks by the Lilly Feed Mill, and a fresh dietary mixture was provided at least every 2 weeks, the quantity for each diet level being prepared in a single batch.

The lot of ethalfluralin used in the study was analysed for nitrosamine contaminant at 4, 6, 9, 12, 15, 19, and 28 months after the start of the tests.

Samples of each diet level were assayed for ethalfluralin content at the beginning of the test and also after aging for 1 and 2 weeks under animal room conditions, to confirm presence and stability of ethalfluralin in the test diets. Thereafter, samples of a fresh dietary mixture were assayed every 4 months.

All animals were examined daily to determine if any were dead, dying, or moribund. At least once weekly each rat was examined closely noting muscle tone, teeth, eyes, pelage, secretions, and excretions. Any external masses or lesions were measured when first observed and as changes in size became apparent.

All rats were weighed once weekly using an automatic self taring balance connected to a computer tape recorder. Food consumption was measured at the same time and in the same manner; the efficiency of food utilization was calculated at the same time.

Hematology and Clinical Chemistry were conducted at the end of the 2-year treatment period, the rats being fasted overnight and bled before being killed for necropsy.

Hematology parameters evaluated on all animals included hematocrit, hemoglobin, erythrocyte count, total and differential leucocyte counts, erythrocyte morphology, mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration.

Clinical chemistry determinations performed on rats at 2-years included serum concentrations of glucose, urea nitrogen, creatinine, bilirubin (T.B.), alkaline phosphatase, and glutamic pyruvic transaminase.

Organ weights were recorded at necropsy for rats surviving to termination on the following trimmed organs: liver, kidneys, heart, spleen, thyroids, adrenals, prostate and testes, or uterus and ovaries. Organ weight: body weight ratios were calculated.

Dead, moribund killed, and rats killed at the end of the study were necropsied. Besides the usual careful systematic examination, particular attention was given to chronic, neoplastic, treatment-related and metastatic lesions. Necropsies were performed by qualified pathologists, and their findings were recorded.

The following organ and tissues were collected and fixed in 10% buffered formalin: gross lesions, skin, mammary gland, salivary gland, lung, heart, thyroid (with parathyroid), stomach, duodenum, jejunum, ileum, colon, liver, skeletal muscle, thymus, pancreas, spleen, kidney, adrenal, urinary bladder, prostate, testis, ovary, uterus, lymph node, cerebrum, cerebellum, brain stem, pituitary, eye, bone, and bone marrow.

Preparations of organs and tissues collected at necropsy were evaluated by a certified veterinary pathologist. The findings were recorded and tabulated and a summary of important pathologic alterations was prepared.

Dunnett's (two-tailed) statistical method was used to analyze differences between control and treated group means for parameters which are generally distributed normally: body weight gain, hematology, clinical chemistry, and organ weights.

Tumor data were examined separately using an "Analysis of Tumor Incidence in Chronic Toxicity Tests", by S. Stanley Young, Ph.D., presented at ASA Meeting, Boston, August 23-27, 1976.

Results:

Nitrosamine Content of Ethalfluralin Lot Used in Study.



Ethalfluralin Assay of the feed gives values of 80 to 96% of theory, with an overall value of 86% of theory. Part of the 'loss' apparently is due to the chemical having a 'potency' or purity of 94.5%. The additional loss apparently is due to losses in recovery in the assay procedure. The registrant states ethalfluralin appeared stable in the diet for two weeks.

Survival of the rats does not appear to be affected by ethalfluralin at levels as administered in these studies. Survival at 24 months in males was 70% in controls and at 0.01% in the diet; 67% at 0.025%, and 72% at 0.075% in the diet. In the females, survival was lowest in controls (55%), and 67%, 62%, and 63% in the low, mid, and high dose groups.

Mean body weights also did not appear to be affected by ethalfluralin in the diet, as determined by terminal body weights. The growth curves of the mid and high dose females tended to be a little lower than the controls and low dose females from 75 or 100 days until about 610 or 700 days, but then all dose levels meeting prior to termination of the study. There were no dose-related differences between the middle and high dose groups. The weight gain charts show the mean weight gains to be statistically less ($p < 0.05$) in the mid and high dose females at 9 and 12 months and also at 18 months at the mid dose in replicate 267. In replicate 277, statistically significant lower mean body weight gains appear in the mid and high dose females at 9 months and in the high dose females only at 12 months.

Feed Consumption and Feed Efficiency were unaffected by treatment.

Toxic Signs:

There were no physical signs of toxicity which could be attributed to ethalfluralin administration. Rats receiving ethalfluralin had urine of a darker yellow color than controls, the intensity being dose related. However, ethalfluralin is not detectable in the urine, so color must be derived from metabolites. Physical conditions noted in low incidence but not related to treatment or dose level included chromadacryorrhea, head tilt (probable middle ear infection), eye opacities, respiration problems, vaginal bleeding, chromorhinorrhea, body sores and dermatitis, and palpable masses in the abdomen. The palpable masses appeared to be enlarged spleens, verified at necropsy. Toward the end of the study cachexia and weakness were noted in rats which subsequently died or were killed.

Hematology:

There were no differences between groups related to chemical administration in HCT, HGB, RBC, and MCHC. However, 1 female control rat in replicate 267, 1 male control rat in replicate 277, and two female mid-dose rats in replicate 277 had mononuclear cell leukemia which resulted in unmeaningful group mean values for WBC, MCV, and MCH. Excluding the data from these rats resulted in no hematology which could be attributed to administration of the chemical.

Urinalyses were not conducted in these replicate 2-year feeding studies, or in the 1-year feeding study. However, gross and microscopic examination of the kidneys and urinary bladders revealed no lesions which could be attributed to administration of the chemical.

Blood Chemistry:

There were isolated aberrant values in individual rats in BUN in control and low dose groups. Also, blood glucose values exceeded 150 mg% in two female rats in the high dose group in replicate 267; and an AP value of 1038 mu/ml was seen in a single female rat in the high dose group in replicate 277. These rats were diseased with leukemia, glomerulonephritis, and emaciation, so the values probably were not treatment or dose related. The individual AP values range from 39-570 mu/ml for the controls and 36-438 mu/ml for the high dose rats (excluding the isolated 1038 value).

There were apparent significantly high mean blood glucose values in all treated males in replicate 277 and in high dose females in replicate 267. In examining individual glucose values of rats in all groups, one sees many values in "normal" groups which far exceed the "high" mean values of the groups in question. Also, the mean blood glucose value for the male controls in replicate 277 (103.1 mg%) is low compared with all the other control groups in the study (113-116 mg%).

For comparison of these values see tables on pages 27, 28 and 29.

Mean Organ/Body Weight Ratios:

There are a few statistically significant differences in mean organ/body weight ratios between controls and treated rats. However, they are scattered, and do not appear to be dose related. For example, the mean relative heart weights are slightly but statistically less than controls in female rats in replicate 267 at the low and mid dose levels. The mean thyroid/body weight ratio in high dose females in replicate 267 is more than twice that of controls. In examining individual rat pathology reports it is noted that 2 rats have thyroid follicular cell adenomas and one has a C-cell carcinoma. The thyroids of rats in replicate 277 have "normal" thyroid/body weight ratios.

Blood Glucose mg %

Dose Level	MALES			FEMALES		
	R257 1-Year	R267 2-Year	R277 2-Year	R-257 1-Year	R267 2-Year	R277 2-Year
Control	148.3	113.4	103.1	130.3	115.9	113.4
100 ppm	153.3	116.3	120.6*	133.5	127.9	123.5
250 ppm	160.3	118.4	121.5*	133.6	124.4	127.1
750 ppm	174.0*	122.9	128.3*	148.9	131.8*	119.9

*Statistically different from controls.
 $p \leq 0.05$, two-tailed Dunnett's "t"

BUN mg %

Dose Level	MALES			FEMALES		
	R257 1-Year	R267 2-Year	R277 2-Year	R257 1-Year	R267 2-Year	R-277 2-Year
Control	15.08	17.40	21.16	14.60	24.65 ^a	14.48
100 ppm	15.35	24.55 ^a	17.44	15.27	15.15	14.55
250 ppm	15.35	18.64	18.84	16.47*	19.69	17.71
750 ppm	15.84	17.63	19.60	17.41*	18.94	17.18

^aMean value includes aberrant value from one rat.

*Statistically different from controls.
 $p \leq 0.05$, two-tailed Dunnett's "t"

Creatinine mg %

Dose Level	MALES			FEMALES		
	R257 1-Year	R267 2-Year	R277 2-Year	R-257 1-Year	R267 2-Year	R277 2-Year
Control	.49	.67	.73	.46	.54	.55
100 ppm	.43	.69	.63	.51	.48	.48
250 ppm	.39	.62	.60*	.57*	.50	.46*
750 ppm	.43	.63	.64	.57*	.46	.49

*Statistically different from controls.
 $p \leq 0.05$, two-tailed Dunnett's "t"

Alkaline Phosphatase mU/ml

Dose Level	MALES			FEMALES		
	R257 1-Year	R267 2-Year	R277 2-Year	R257 1-Year	R267 2-Year	R-277 2-Year
Control	113.6	108.4	128.3	91.2	145.8	102.4
100 ppm	100.5	103.7	145.1	83.2	84.6*	113.4
250 ppm	106.5	178.1	111.7	67.6	93.4	133.4
750 ppm	102.9	91.1	96.6	60.6*	89.6	142.7

*Statistically different from controls.
 $p \leq 0.05$, two-tailed Dunnett's "t"

Total Bilirubin mg %

Dose Level	MALES			FEMALES		
	R257 1-Year	R267 2-Year	R277 2-Year	R-257 1-Year	R267 2-Year	R277 2-Year
Control	.342	.263	.403	.426	.527	.357
100 ppm	.322	.297	.304*	.406	.274	.337
250 ppm	.318	.315	.338	.437	.322	.359
750 ppm	.307*	.277	.313*	.433	.325	.704

*Statistically different from controls.
 $p \leq 0.05$, two-tailed Dunnett's "t"

The spleen/body weight ratios of females in the low and mid dose groups of replicate 267 are statistically less than control females. However, in looking at the other replicate it is apparent the spleen weights of 5 control females of this replicate are abnormally high, while those of the treated rats are "normal". Four of these abnormally heavy spleens have mononuclear cell leukemia, while the 5th spleen is enlarged due to increased hematopoiesis.

Table 35 shows treatment groups to be significantly different from the controls in heart/body weight ratios. Again, it appears the heart/body weight ratios of the treated animals are normal, while the controls appear to be abnormal. In the controls we have four heart ratios exceeding .4 g (.40, .42, .45, and .48; also .35 and .39). All six of these rats have tumors. In addition, one heart showed thrombosis of one atrium. The heart ratios of rats in the treated groups were much more closely grouped.

Table 36 shows the high dose males of replicate 277 to have a significantly higher testes weight than controls. In this group there are 5 testes weights which exceed 7 g. (7.29, 7.74, 8.58, 8.73 and 10.1). In the control and other treatment groups there are no testes weights of 7 g or higher. Both testes in these 5 rats contain Leydig Cell tumors.

In these studies there were no statistically significant effects of ethalfluralin on liver weight, although slight increases in relative liver weights can be observed in high dose females of both replicates and high dose males of replicate 267. Increased liver weight in the 90-day studies had been the basis for dose selection in these studies.

In summary, there were no differences in organ weights which could be considered related to administration of ethalfluralin with the possible exception of slight increases in liver weight at the high dose level.

Neoplasms

Tissues of all animals were examined histologically. The only neoplasms occurring which appeared to be related to administration of the chemical were benign mammary gland fibroadenomas. A summary of their incidence is listed below:

<u>Control</u>		<u>Low Dose</u>		<u>Mid Dose</u>		<u>High Dose</u>		<u>Replicate Number</u>
<u>Male</u>	<u>Female</u>	<u>Male</u>	<u>Female</u>	<u>Male</u>	<u>Female</u>	<u>Male</u>	<u>Female</u>	
0/30	6/30	0/30	4/30	0/30	12/30	1/30	15/30	267
0/30	<u>3/30</u>	1/30	<u>6/30</u>	0/30	<u>9/30</u>	0/30	<u>13/30</u>	277
TOTAL	9/60 15%		10/60 16.7%		21/60 35%		28/60 46.7%	

The NOEL for oncogenicity therefore is 0.01% (100 ppm) in the diet.

In the registrant's report narrative, the pathologist states:

"The total numbers of benign and malignant neoplasms among the control and ethalfluralin treated groups were comparable. Furthermore, there was no evidence of a decreased latency period for any type of neoplasm in the ethalfluralin treated groups.

"A perusal of the incidence of individual neoplasms revealed an increase in the number of benign mammary neoplasms in females of the 0.025 and 0.075 percent dose groups (the Z-statistic was significant at the high dose), but the control and 0.01 percent groups had comparable numbers. Since adenomas and fibroadenomas are similar and may in fact be anatomic variants of a common neoplasm, they were combined (hereafter referred to as fibroadenoma) to give the total incidence of benign mammary neoplasms. The apparently increased incidence of this very benign neoplasm was probably of no biological significance because: (1) there was no difference in the morphologic appearance between the control and treated groups, and the latency period was not affected; (2) the incidence of malignant mammary neoplasms was not increased; (3) there was no effect on the well-being or survival of the rats. Fibroadenomas are very common in the Fischer 344 female, but there is wide variation in incidence as report in the literature. Perusal of reports of Bioassays for Carcinogenicity of the National Cancer Institute revealed an incidence range of 0 to 48 percent among numerous individual studies for control Fischer 344 females."

Dr. Louis Kasza, Toxicology Branch pathologist, comments as follows: To establish oncogenicity, both benign and malignant tumors are considered. It is known that fibroadenomas frequently are present in the Fischer 344 female rat, but the average incidence is considered to be about 14%. (Pathology of Laboratory Animals. By K. Benirschke, F.M. Garner, and T.C. Jones. Pg. 1057. Springer-Verlag, New York. 1978).

Alfredo Nunziata and Alberta Storino, CRF Centro Ricerca Farmaceutica, Rome, Italy in their review of Spontaneous Neoplastic Pathology in Control Rats (VETERINARY AND HUMAN TOXICOLOGY. Vol. 24, No. 4, pg. 243. August, 1982) lists the following incidence of mammary gland tumors in Fischer 344 rats:

Life Science Laboratories	females - 41%; males, 23%
Alderly Park	females - 25.5%; males - 0
Alderly Park SPF weanlings	females - 2.0%; males - 0
Fischer Inbred weanlings	females - 19.5%; males - 6.3%

The authors attribute the variation to various parameters (and laboratory conditions), citing diet as one of the most important factors.

INCIDENCE OF FIBROADENOMAS IN UNTREATED FEMALE FISHER 344 RATS
FROM FIVE TWO-YEAR TOXICOLOGY STUDIES

Study Number	Termination of Study	Number of Untreated Females	Number of Females with Fibroadenomas	Percent Incidence
R-695	May 1977	120	16	13.3%
R-1136*	November 1978	30	8	26.7%
R-1146*	November 1978	30	5	16.7%
R-1246*	December 1978	30	5	16.7%
R-1256*	December 1978	29	4	13.8%
R-87*	February 1979	30	0	0.0%
R-97*	February 1979	30	7	23.3%
R-167*	February 1979	30	6	20.0%
R-177*	February 1979	<u>30</u>	<u>4</u>	<u>13.3%</u>
Total		359	55	Overall Incidence 15.3%

*Replicate studies

The registrant subsequently (October 7, 1982) submitted historical data on the incidence of fibroadenomas in untreated female Fischer 344 rats in their laboratories. They cite an overall incidence of 15.3%, with an incidence of 26.7% in control replicate R-136, November, 1978, the highest control incidence cited. It therefore appears ethalfluralin causes mammary gland fibroadenomas at levels of 250 and 750 ppm in the diet of Fischer 344 rats.

Nonneoplastic Findings

The only nonneoplastic findings which can be related to treatment was yellow coloration of the fat in 15/30 high dose females in replicate 267 and in 1/30 high dose males and 4/30 high dose females in replicate 277. The coloration appears to be due to metabolites of ethalfluralin and is not expected to have any toxicologic significance.

Conclusions:

- 1) Nitrosamines of ethalfluralin were absent, or were present near the limits of detection in the chemical used in these tests. Nitrosamines were not considered to be a factor in these studies.
- 2) Feeding ethalfluralin had no adverse effect on survival.
- 3) While ethalfluralin had no significant effect on terminal mean body weights, weight gains were statistically lower in the mid dose and high dose females from three to 18 or 22 months, and recovering prior to termination of the study.
- 4) There were no physical signs of toxicity which could be attributed to the chemical.
- 5) No hematological or blood chemistry effects attributable to the chemical were seen.
- 6) Apparent statistical differences in organ weights and organ/body weight ratios were not actually related to chemical administration. However, while not statistically significant, high dose females in both replicates and high dose males in one replicate showed slight increases in relative liver weights.

7) Administration of ethalfluralin results in an increased incidence of benign mammary gland fibroadenomas in females at the mid and high dose levels. The registrants provided data on the incidence of mammary fibroadenomas in untreated control females in other studies in their laboratories which demonstrate an incidence ranging to 26.7% (average or overall incidence of 15.3%). This compares with an incidence in the current studies of 15% in the controls, 16.7% in the low dose (100 ppm), 35% in the mid dose (250 ppm), and 46.7% in the high dose (750 ppm) females. Therefore, no increase in fibroadenomas was noted in the low dose level (100 ppm). (Statistical evaluation is now underway.) *L. K.*

8) Urinalyses were not performed. However, no gross or microscopic pathology was found in the urinary tract which could be attributed to administration of the chemical.

9) Blood was taken only at the termination of the study; interim hematology and blood chemistry values were not determined. Therefore, blood chemistry values from the one-year rat feeding study are charted with the values from these 2-year feeding studies.

The NOEL for the study is 100 ppm based on increased incidence of mammary gland fibroadenomas in the females at 250 ppm and 750 ppm.

The data are considered to be equivalent to Core-Minimum.

ETHAL FLURALIN

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Pages 61 through 65 are not included.

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 - ☐ Identity of product impurities.
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A TWO-YEAR DIETARY EVALUATION OF ETHALFLURALIN (COMPOUND 94961)

IN THE FISCHER 344 RAT

STUDIES R-267 AND R-277

Project Leader: E. R. Adams

Pathologist: N. V. Owen

Report Prepared By: E. R. Adams

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

June 1981

TRIFLURALIN

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

FILE

Caswell

453B

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DEC 23 1982

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

THRU: Orville E. Paynter, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769) *W. Burin 12.16.82*

SUBJECT: EPA Reg.#1471-REE; 1471-EUP-63; PP#8G2118; SONALAN E.C.
Herbicide. Registrant's submission of November 4, 1982;
Ethalfluralin.

The following is the Toxicology Branch analysis and comments on the registrant's rebuttals received November 4, 1982 to our evaluation of their toxicology submission for ethalfluralin dated October 29, 1982.

The rabbit and rat teratology studies were re-examined by Gary Burin and discussed in his memos of November 24 and November 29, 1982.

Chronic Feeding/Oncogenicity:

Two-Year Rat Study (R-267, R-277). The registrant and EPA agreed that no increase in benign mammary gland fibroadenomas were noted at the lowest dose level (100 ppm or 4.2 mg/kg/day on a time weighted basis) and that the incidence of these tumors is significant at 250 and 750 ppm (10.7 and 32.3 mg/kg, respectively).

The registrant also submitted with this study (2/4/82) a risk analysis which reflected the following findings:

<u>Individual Exposed</u>	<u>Chemical Exposed to</u>	<u>PDLE (mg/kg/day)</u>	<u>Slope (mg/kg/day)⁻¹</u>	<u>Risk</u>
Applicator/Mixer/Loader/Field Worker	Ethalfifluralin	1.58 x 10 ⁻⁵	0.0871	1.4 x 10 ⁻⁶
Consumer	Ethalfifluralin	<9.98 x 10 ⁻⁷	0.0871	<8.7 x 10 ⁻⁸
Bystander (during application)	Ethalfifluralin	7.32 x 10 ⁻⁸	0.0871	6.4 x 10 ⁻⁹
Bystander (after application)	Ethalfifluralin	2.54 x 10 ⁻⁷	0.0871	2.2 x 10 ⁻⁸
Applicator/Mixer/Loader/Field Worker	[REDACTED] (nitrosamine)	<1.54 x 10 ⁻⁸	33.5	<5.2 x 10 ⁻⁷
Consumer	[REDACTED]	<2.50 x 10 ⁻⁹	33.5	<8.4 x 10 ⁻⁸
Bystander (during application)	[REDACTED]	6.26 x 10 ⁻¹⁴	33.5	2.1 x 10 ⁻¹²
Bystander (after application)	[REDACTED]	1.10 x 10 ⁻¹⁰	33.5	3.7 x 10 ⁻⁹

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

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The registrant's risk analysis was forwarded to the Toxicology Branch Statistician, Mr. B. Litt for review; any question concerning the status of this risk assessment should be addressed to the Toxicology Deputy Branch Chief.

The Environmental Fate Branch concurred with the human exposure calculated by the registrant; a copy of this review is attached.

Two-Year Mouse Study (M-9167, M-9177). Hepatocellular Hyperplasia. The registrant and EPA agree that hepatocellular hyperplasia was found, but disagreed on the LEL (or NOEL). The Toxicology Branch position was originally based on Dr. L. Kasza's (the Branch pathologist) examination of the data reported in the two replicates in this study alone and not in consideration of the additional historical control data recently submitted (November, 1982). Of the two replicates listed in the table below only one animal group (the low-dose female group in replicate M-9167) fails to show an increased incidence at the 100 ppm (10.3 mg/kg on a time-weighted basis) dose level. Both replicates show an increased incidence over the control group at the 400 ppm and 1500 ppm dose levels (41.9 and 163.3 mg/kg, respectively). See tables below:

		<u>Control</u>	<u>100 ppm</u>	<u>400 ppm</u>	<u>1500 ppm</u>
Males	M-9167	8.3% (5/60)	10.0% (4/40)	17.5% (7/40)	22.5% (9/40)
Males	M-9177	3.4% (2/59)	12.5% (5/40)	12.5% (5/40)	12.5% (5/39)
Females	M-9167	1.7% (1/60)	0.0% (0/40)	10.0% (4/40)	20.0% (8/40)
Females	M-9177	1.7% (1/60)	10.0% (4/40)	5.0% (2/40)	32.5% (13/40)

Replicates combined

	<u>Control</u>	<u>100 ppm</u>	<u>400 ppm</u>	<u>1500 ppm</u>
Males	5.9% (7/119)	11.3% (9/80)	15.0% (12/80)	17.7% (14/79)
Females	1.7% (2/120)	5.0% (4/80)	7.5% (6/80)	26.3% (21/80)

Therefore, it was originally concluded that there was an effect at all treatment levels.

The following historical control data were recently submitted (November, 1982) by the registrant:

<u>Study Number</u>	<u>Males</u>	<u>Females</u>
M-9087	15.0% (9/60)	8.3% (5/60)
M-9097	10.0% (6/60)	10.0% (6/60)
M-9067	3.3% (2/60)	8.3% (5/60)
M-9077	1.7% (1/60)	1.7% (1/60)

The above table reflects a high incidence of hepatocellular hyperplasia in both males and females of studies #M-9087 and #M-9097; the incidence in the remaining two studies is relatively low, except for one female group (8.3% in replicate M-9067). It is not clear on what basis these control data were selected. However, 1) if these data were randomly selected from a pool of controls available during the same period of the study and 2) assuming that these controls were kept under similar experimental conditions, one may note that a variation can occur in the spontaneous incidence of hepatocellular hyperplasia in the mouse. Dr. L. Kasza stated that both hepatocellular hyperplasia and tumors are proliferative lesions (or changes), but he also

- indicated that hepatocellular hyperplasia is considered to be a less significant lesion than neoplastic changes and does not necessarily interfere with the normal function of the liver.

The biological significance of the hepatocellular hyperplasia noted in this study in the low dose group (10.3 mg/kg on a time-weighted basis) may be considered debatable due to the fact that the lesions were not graded in the study and that the selection method for the submitted historical data was not identified. At any event, the level of hepatocellular hyperplasia at the low dose level may at present be considered as marginal because the lesion occurred in a similar fashion in the control and low dose groups (i.e., examination of the description of the lesion in both the control and low dose groups did not reflect significant differences in size or color). Bert Litt, Toxicology Branch statistician, conducted a Log Rank Test of Trend on hepatocellular hypertrophy and liver tumors in the mouse study. As suggested by the name of the test, it is designed to determine the significance of any trend. This analysis did not reflect a NOEL for hepatocellular hypertrophy.

However, according to Dr. L. Kasza, in considering the historical control data submitted from the two other studies conducted by Lilly, the mean incidence of hepatocellular hyperplasia in the males is 7.5% overall (12.5% and 2.5%), and in the females is 7.1% overall (9.2% and 5%). So when comparing the incidence of this lesion in the low dose group of the 2-year mouse study (100 ppm) (average combined replicate incidence is 11.3% for males and 5% for females) with the average incidence values in the historical control, we note that the hepatocellular hyperplasia in both males and females of the low dose level are within the range of expected variation. This indicates that 100 ppm (10.3 mg/kg) may be considered a NOEL in the mouse.

In conclusion, it appears that the NOEL in the mouse study may be 10.3 mg/kg. However, for Toxicology Branch to reach a final conclusion on this issue, we need the actual dates and method of selection of the recently submitted historical data.

If the above requested data are supplied and demonstrate the proper utility of the historical data, the ADI will then be based on the 2-year rat study since at present the NOEL of 4.2 mg/kg in this study is apparently the lowest value obtained in these chronic feeding studies in both rats and mice. As noted previously, it is also a positive study and the risk assessment report will be reviewed separately.

Three-Generation Rat Reproduction Study: EPA acknowledges that the rat teratology study and the 3-generation rat study were conducted in different strains. The Fischer 344 rat was used in the reproduction study and the Wistar rat was used in the teratology study. The registrant indicated in submissions of October, 1982 and November, 1982 that the Fischer 344 rat strain has a very low incidence of hydronephrosis (1/466 gestation-day-20 control fetus was affected in the Lilly Laboratory). Consequently, the apparent discrepancy in incidence of hydronephrosis between the rat teratology study and the Three-Generation Study is explained

Toxicology Branch has some historical data on the Fischer 344 rat strain (from other testing facilities) which are inconsistent with the level of other normally occurring gross abnormalities noted in the submitted study. We therefore request that a complete set of historical data be submitted by the registrant before a final recommendation can be made on the adequacy of the reproduction study.

Furthermore, Toxicology Branch notes that if it were to attempt to resolve issues relative to hydronephrosis (observed in the teratology study) by utilizing this study, the reproduction study would need to be conducted in the same strain of rats as the teratology study. Elanco responded to this question raised in our recent meeting on November 10, 1982 and in the subsequent report in November 1982, that it was basically Elanco's policy to perform the reproduction study and the 2-year chronic feeding study in the same strain of rats. However, it is noted that this statement is inconsistent with Elanco's recent submission on EL-919 where both the teratology and reproduction studies were performed on the same strain of animals, the Wistar rat, while the subchronic toxicity studies (assumed to be the basis for the chronic toxicity studies) were performed on the Fischer 344 strain.

Mutagenicity:

The registrant agreed to repeat the Quantitative Ames Test.

The registrant also agreed to repeat the Dominant Lethal Test. However, we find that the registrant's resources will be best utilized if the following tests are first performed:

1. Mouse micronucleus test;
2. Rat or Mouse sister chromatid exchange;
3. In vitro mammalian cell gene mutation test.

Guinea Pig Sensitization:

Since the registrant conducted both, the Buhler topical patch test and the Magnusson-Kligman sensitization test, we cannot ignore the positive response seen in the Magnusson-Kligman test. On their own initiative and in accordance with their own judgement the registrant chose to conduct the Magnusson-Kligman test. It should also be noted (in response to the registrant's comments in their recent submission) that we do not always know the extent to which individual animal tests adequately and accurately reflect potential effects to humans. On the otherhand, we cannot ignore the positive response obtained in the Magnusson-Kligman test, and labeling should appropriately reflect the positive findings.

Roland A. Gessert LBC
12/17/82
Roland A. Gessert, D.V.M.
Toxicology Branch
Hazard Evaluation Division (TS-769)

I concur with the pathology findings/
conclusions presented.

Louis Kasza
Louis Kasza, D.V.M., Ph.D.
Toxicology Branch
Hazard Evaluation Division (TS-769)

TS-769:th:TOX/HED:RAGessert:12-14-82:card 3

A Two-Year Dietary Evaluation of Ethalfluralin in the B₆C₃F₁ Mouse. Studies M-9167 and M-9177. Conducted by Elizabeth R. Adams, M.S. (Project Leader) et. al., Lilly Research Laboratories; Greenfield, Indiana. July, 1981. Accession#070680.

Ethalfluralin (Compound 94961, EL-161) was administered to B₆C₃F₁ mice in the diet for 2-years in replicate studies, to evaluate its chronic toxicity and oncogenic potential. Each replicate contained 60 control mice of each sex and 40 mice per sex in each treated group for a total of 720 mice on test. The dietary concentrations of ethalfluralin and the resultant estimated time-weighted average exposures (average of males and females), were as follows:

<u>Ethalfluralin, % in diet</u>	<u>Ethalfluralin; mg/kg/day (time-weighted average)</u>
0.01 = 100 ppm	10.3
0.04 = 400 ppm	41.9
0.15 = 1500 ppm	163.3

These dietary concentrations of 0, 0.01, 0.04 and 0.15 percent (0, 100, 400, and 1500 ppm) were based on results of a 3-month study in mice, M-9286.

Ethalfluralin is N-ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl)-benzenamine. The lot used was B30-Y64-35B,

Mash diet containing the 3 ethalfluralin concentrations was pelleted every 2-months.

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

The mice, 5 to 7 weeks of age, were supplied by Charles River Breeding Laboratories, Inc.; Wilmington, Massachusetts. Two shipments of mice, one for each replicate, were received one week apart.

Mice were held for 2-weeks housed 3 or 4/sex/cage to permit adaptation to the caging, housing, and watering system of the laboratory. Mice which were significantly different in weight from the general population, or which were in poor physical condition were not used. Mice of the first shipment were assigned to replicate M-9167, and mice of the second shipment to replicate M-9177. At the time of distribution, each mouse was examined by the technician. At the start of the tests, the mice were 7 to 9 weeks old and the males of M-9167 weighed 24.2 ± 0.1 g and the females 20.3 ± 0.1 g; the males of M-9177 weighed 23.6 ± 0.1 g and the females 19.7 ± 0.1 g.

The test mice were housed 3 or 4 per stainless-steel hanging cage, 18 x 18 x 25 cm, on dry racks. Racks, cages, water bottles, and feed cups were washed and autoclaved every two weeks.

All test mice were maintained at $24 \pm 3^\circ\text{C}$ with a minimum relative humidity of 45 percent in one room. A light-dark cycle of 12 hours light was maintained by automatic timers.

Each mouse was assigned an identification number indicating study number and animal number that specified sex and treatment. This number was attached to the cage and was used for all records samples, and reports. Additionally, each mouse was ear punched in the left ear to identify dose group and in the right ear to identify mice within a cage.

Prepelleted mash diets and pellets containing ethalfluralin were assayed every other time that pellets were prepared, and also after aging two months. Two months was the maximum age of the pellets that mice were fed.

All mice were examined daily to determine if any were dead or moribund. At least weekly each mouse was closely observed, noting muscle tone, pelage, eyes, teeth, secretions, and excretions. Any external masses or lesions were measured when first observed and when any changes in size became apparent.

All mice were weighed weekly for the first three months and every 2 weeks thereafter. Weight data were collected with a self-taring balance connected with a computer tape recorder.

Hematology & Blood Chemistry

At the end of the 2-year treatment period the mice were fasted overnight and bled before being killed for necropsy.

Hematology tests included hematocrit (HCT), hemoglobin (HGB), erythrocyte count (RBC), leucocyte count (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

Blood chemistry determinations included glucose, urea nitrogen (BUN), creatinine, bilirubin, alkaline phosphatase, and glutamic pyruvic transaminase (SGPT).

Organ Weights

Were determined for mice surviving to termination. The following organs were trimmed and weighed at necropsy: liver, kidneys with adrenals attached, heart, spleen, testes, and uterus with ovaries attached.

Pathology

Dead, moribund killed, and mice killed at the end of the study were necropsied, being given a complete, careful, systematic examination. The following organs and tissues were collected and fixed in 10% buffered formalin: gross lesions, mammary gland, salivary gland, lung, heart, thyroid (with parathyroid), stomach, duodenum, ileum, jejunum, colon, liver, thymus, skin, pituitary, bone, pancreas, spleen, kidney, adrenal, urinary bladder, prostate, seminal vesicle, testis, ovary, uterus, lymph node, skeletal muscle, cerebrum, cerebellum, brain stem, eye, and bone marrow.

These histologic preparations was examined by a certified veterinary pathologist. Consultations with other pathologists were used, and the criteria for diagnosis were those of classical pathology.

Statistical analysis of differences between control and treated group means for parameters which were generally distributed normally was by Dunnett's method. Another method (which was described) was used to analyze tumor data.

*Yellow House?*Results:Diet Assays

The mean assay percents of theory of all the pellets assayed were: low level, 103.2%; mid dose level, 99.9%, and high dose level, 79.6%. Since these are mean values, naturally some of the individual assays fall above those values and some fall below. The lowest assay for the high dose level pellets was 68.7%, occurring in the second batch pelleted, one month following the initial batch. The lowest assays for pellets in the low and mid dose levels occurred nearly midway in the study and gave values of 77% and 75% of theory, respectively. Assays for other pelleting batches near 100% for the low and mid dose levels. Since a single pelleting batch would not be fed for a period exceeding two months, the effect of feeding pellets of lower ethalfluralin content would be minimized. However, from the available data it appears that the high dose group received approximately 80% of the intended dose.

Toxic Signs

No physical signs of toxicity were observed.

Survival

Feeding ethalfluralin had no apparent effect on numbers of mice surviving for two years. Two-year survival was 70.0% for controls and 69.4%-78.1% for treatment groups.

Weight Gain

Mean weight gains were statistically reduced in females of both replicates at 24-months, and also in males of replicate M-9167 at the high (0.15%) dietary level. In replicate M-9167 there was a tendency of reduced weight gain in high dose levels, although not statistically significant, in males and females beginning at 9 months. However, at the low and mid dose levels there were actual increased mean weight gains from 1 month through 18 months in the females of replicate M-9167. In replicate M-9177, treatment at the low and mid dose levels appeared to have no detectable adverse effect on mean weight gains in either males or females at any time of the study; mean weight gains frequently were greater than controls for these groups. It is only at 24-months that noticeable adverse weight effects were seen in this replicate at the high dose level. 10-30%

Hematology

Mean hematology values show few statistically significant differences between treated and control mice. In replicate M-9167 hematocrit, hemoglobin, and red blood cell count were statistically less than controls at the high dose level only in females. ^{10%}32.9% vs 36.35% for HCT; 12.44 g vs 13.84 g for HGB; ^{10%} and 7.022 million vs 7.754 million for RBC)%(significant at 0.05 level). And the mean corpuscular hemoglobin concentration was statistically greater than control in females (39% vs 38.1%). ^{3%}

MCHC

Other statistically significant differences from controls at the 0.05 level are (1) the mean corpuscular hemoglobin concentration ↑ in females on the low dose level in both replicates (39% vs 38.1%) in replicate M-9167; 38.03% vs 37.06% in replicate (M-9177).

(2) Also the MCHC in males at the mid-dose level in replicate M-9177 (37.88% vs 36.77%).

(3) And the mean corpuscular volume in the mid-dose males in replicate M-9177 (45.4 vs 46.8/cu microns).

White blood cell counts of all groups including controls, are normal, although there are extremely wide variations in individual animal values (also including controls). There are no statistically significant differences in mean white blood cell differential counts between treated and control mice.

Blood Chemistry (See attached tables).

Alkaline phosphatase was significantly increased over controls at the 0.05 level at the high dose level in males of both replicates and in females of replicate M-9177. Some trend was also noted, though not significant at the .04 level. Other parameters including glucose, BUN, creatinine, and total bilirubin showed no statistically significant differences between treated and control mice. Minor consistent but not statistically significant increases were seen in SGPT values in males and females of both replicates.

See discussion at the end of this review.

Body weights of females in both replicates were statistically less than control females at the high dose level.

002251

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Mouse Alkaline Phosphatase mU/ml

Dose Level	MALES				FEMALES			
	90-Day	1-Year	2-Year	2-Year	90-Day	1-Year	2-Year	2-Year
Control	50.9	58.6	82.5	85.3	88.6	120.1	234.1	185.1
0.01%		64.3	84.9	83.6		119.4	244.5	220.5
0.04%		76.3*	106.7	91.7		116.7	240.2	186.6
0.056%	64.8				95.5			
0.110%	67.9				96.8			
0.15%		84.0*	117.8* 43%	116.9* 37%		140.7	242.8	321.4* 74%
0.225%	77.7				97.9*			
0.400%	104.3*				95.6			
0.800%	148.5*				108.1*			

*Statistically significant from controls.
 $p \leq 0.05$.

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Mouse SGPT mU/ml

Dose Level	MALES				FEMALES			
	90-Day	1-Year	2-Year	2-Year	90-Day	1-Year	2-Year	2-Year
Control	65.5	30.1	57.8	44.3	21.8	35.7	63.3	47.6
0.01%		32.6	61.9	55.7		42.3	80.5	63.9
0.04%		36.3	78.8	51.5		36.6	61.6	69.1
0.056%	61.4				19.8			
0.110%	47.3				19.2			
0.15%		56.3*	90.9	81.4		31.5	78.8	84.2
0.225%	47.1				23.2			
0.400%	79.6				20.9			
0.800%	250.0*				100.9*			

*Statistically different from controls.
 $p \leq 0.05$.

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Mouse Creatinine mg/%

Dose Level	MALES				FEMALES			
	90-Day	1-Year	2-Year	2-Year	90-Day	1-Year	2-Year	2-Year
Control	1.17	0.24	0.37	0.29	1.11	0.44	0.31	0.29
0.01%		0.26	0.38	0.26		0.47	0.34	0.31
0.04%		0.28	0.31	0.29		0.34	0.33	0.31
0.056%	1.27				1.13			
0.110%	1.22				1.05			
0.15%		0.33	0.37	0.29		0.29*	0.33	0.31
0.225%	1.26				0.93			
0.400%	1.29				0.93			
0.800%	1.31				0.90*			

*Statistically different from controls.
 $p \leq 0.05$.

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Mouse BUN mg/%

Dose Level	MALES				FEMALES			
	90-Day	1-Year	2-Year	2-Year	90-Day	1-Year	2-Year	2-Year
Control	32.17	20.21	28.06	25.57	16.92	15.56	16.98	15.41
0.01%		20.18	24.34	25.28		13.89	18.49	19.06
0.04%		19.88	23.76	23.98		14.07	16.68	15.28
0.056%	28.11				15.18			
0.110%	33.89				15.93			
0.15%		18.08	27.31	22.51		13.29*	22.01	17.82
0.225%	35.35				17.14			
0.400%	29.81				18.23			
0.800%	20.98*				21.19*			

*Statistically different from controls.
 $p \leq 0.05$.

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Mean relative liver weights were statistically greater in females on the high dose level (6.03 g) than control (4.1 g) in replicate M-9177. Tumors complicated mean liver weight picture in other groups, but by deleting livers with tumors there still appeared to be increased liver weight in the high dose groups and possibly in the mid dose groups. Although not statistically significant, in Replicate 9167 there was a 11.1% increase in liver weights between the 0.04% group and the 0.01% and control groups, (tumor removed). 47% ↑

Mean relative kidney weights were statistically greater at the 0.05 level in females on the high (0.15%) dose levels (1.55 g and 1.49 g, kidneys attached) than controls (1.24 g and 1.22 g) in both replicates. This also was true at the mid-dose level (1.50 g vs 1.22 g) for females in replicate M-9177. 25% ↑

Mean relative heart weights were statistically reduced (0.48 g vs 0.52 g for controls) at the 0.05 level in the low dose (0.01%) males of replicate M-9167; and were statistically increased at the mid- and high-dose levels (0.48 g and 0.50 g vs 0.42 g for controls) in females in replicate M-9177. 14% ↑

Mean relative testes weights for the high dose level in replicate M-9167 were statistically higher than controls (0.76 g vs 0.59 g), but because this effect was not seen in the other treatment levels of this replicate, and was not seen in the other replicate it is not considered to be treatment related.

Histopathology - Neoplasms

Tissues from 718 of 720 test mice were evaluated histologically. Numerous neoplasms were found in all groups, including controls, but no increased incidence of neoplasms were found which could be related to treatment.

The only non-neoplastic condition which appeared to be related to treatment was an apparent dose-related increase in the incidence of focal hepatocellular hyperplasia in males and females of both replicates. This is considered to be biologically significant at all dose levels (100 ppm, 400 ppm, 1500 ppm). See table on following page.

002251

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Incidence of Hepatocellular Hyperplasia in 2-Year Mouse Feeding Studies

Study Number	Control		100 ppm		400 ppm		1500 ppm	
	Male	Female	Male	Female	Male	Female	Male	Female
M-9167	5/60 8.3%	1/60 1.7%	4/40 10%	0/40	7/40 17.5%	4/40 10%	9/40 22.5%	8/40 20%
M-9177	2/60 3.3%	1/60 1.7%	5/40 12.5%	4/40 10%	5/40 12.5%	2/40 5%	5/40 12.5%	13/40 32.5%
Total 9167 & 9177	7/120 5.8%	2/120 1.7%	9/80 11.25%	4/80 5%	12/80 15%	6/80 7.5%	14/80 17.5%	21/80 26.25%

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Discussion:

Testing guidelines specify that blood chemistry determinations and urinalysis be performed at least at two interim points during administration of the test diets, and at the termination of the study. The mouse studies were conducted for durations of 90 days, 1 year, and 2 years, with blood chemistry determinations being made only at the termination of the studies. No urinalyses were performed.

To help moderate the deficiencies, tables were prepared listing side by side blood chemistry values for alkaline phosphatase, SGPT, BUN, and creatinine for the 90 day, 1 year, and 2 year studies (see tables). Asterisks denote values which are statistically different from controls at the 0.05 level.

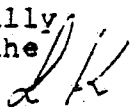
The greatest blood chemistry effect was an increase in alkaline phosphatase values, particularly in males, on a dose-related basis. There also was a trend, although not consistent, toward increased SGPT values. With the values for the various studies recorded side by side, it is interesting to note the influence of aging on the blood chemistry values, alkaline phosphatase and SGPT values tending to increase with age, and creatinine and BUN tending to decrease somewhat with age (although not consistently so).

Although urinalyses were not conducted, in these studies there was no pathology found in the kidneys, bladder, or elsewhere in the urinary tract which could be attributed to administration of the chemical.

Therefore, by combining data from the 90 day, 1 year, and 2 year studies, the studies can be considered Core Minimum, even though no urinalyses or interim blood chemistry analyses were conducted.

Conclusions:

1) According to the data evaluated, ethalfluralin is not oncogenic at 100 ppm, 400 ppm, and 1500 ppm in the diet under the conditions of these studies.

2) A NOEL has not been determined, based on a biologically significant increase in focal hepatocellular hyperplasia at the lowest dose tested (100 ppm). 

The study was submitted and reviewed as an oncogenicity and chronic feeding study. A comparison between this study and chronic feeding studies in the rat indicates the mouse is more sensitive to the chemical than the rat.

3) The study meets the requirements for Core-Minimum Data.

TRIFLURALIN

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A TWO-YEAR DIETARY EVALUATION OF ETHALFLURALIN (COMPOUND 94961)
IN THE B6C3F₁ MOUSE

STUDIES M-9167 AND M-9177

Project Leader: E. R. Adams

Pathologist: N. V. Owen

Report Prepared By: E. R. Adams

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

July 1981

ETHALFLURALIN

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

002405

MEMORANDUM

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

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

THRU: Orville E. Paynter, Ph.D.
Chief, Toxicology Branch
Hazard Evaluation Division (TS-769)

SUBJECT: SONALAN E.C. Herbicide Formulation; EPA Reg. #1471-REE;
PP#2F2645; 8G2118; Ethalfluralin. Registrant's submission
dated January 5, 1983.

In our conference with Elanco on December 22, 1982, we had requested the dates of their mouse studies M-9067, M-9077, M-9087, and M-9097, for which historical control data were submitted, and we also had asked their basis for selecting these studies for historical controls. This current submission gives the requested dates and states that these were the only 2-year studies in this strain of mouse conducted at Lilly Research Laboratories for which pathology data are currently available. The dates are:

M-9067 - February 23, 1977 - March 2, 1979
M-9077 - March 1, 1977 - March 9, 1979
M-9087 - March 23, 1977 - March 23, 1979
M-9097 - April 7, 1977 - April 11, 1979

The studies with ethalfluralin were conducted from June 16, 1977 to June 8, 1979 (M-9167) and June 22, 1977 to June 29, 1979 (M-9177). The registrant states that experimental conditions were similar for all studies. Therefore, in accordance with the data analysis by L. Kasza and R. Gessert (memo of 12/16/82), it is noted that a variation in the spontaneous incidence of hepatocellular hyperplasia can occur in the mouse. The NOEL in this study therefore is 100 ppm (10.3 mg/kg), and the study is considered to be Core-Minimum Data.

The registrant also provided cumulative and historical control data of a teratological nature on Fischer 344 rats in their laboratory. In Lilly's 3-generation reproduction study using Fischer 344 rats, no hydronephrosis was reported. In their cumulative control data utilizing this rat strain, 0/466 bilateral hydronephrosis and 1/466 unilateral hydronephrosis was reported.

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In Hernias, the rat reproduction study revealed 1/86 hernia (type unspecified) in controls and 1/108 (inguinal hernia) at the 250 mg/kg dose level. Cumulative controls showed 7/466 hernias (type unspecified).

While the applicant has provided their cumulative control data on the Fischer 344 rat, it is noted that they did not provide the date periods and how many studies and types of studies from which these data were obtained. We also note that these cumulative control data are at variance with cumulative control data on Fischer 344 rats from other laboratories in some parameters. For example, anophthalmia is known to occur in control Fischer 344 rats in some studies in other laboratories, whereas there was no anophthalmia reported in the Lilly cumulative control data. In a series of studies in Fischer 344 rats we would have expected Lilly to see this condition in at least some of their studies.

The rat reproduction study remains Core Supplementary until the dates and types of studies for the cumulative control data are provided.

Roland A. Gessert

Roland A. Gessert, D.V.M.
Toxicology Branch

Hazard Evaluation Division (TS-769)

DC
1/14/83

TS-769:th:TOX/HED:RAGessert:1-10-83:card 3

**U.S. ENVIRONMENTAL PROTECTION AGENCY
OFFICE OF PESTICIDES/HED/TB-1
TOX ONELINERS**

PAGE 1
CASWELL#: 453B
CAS-REG#: 55283-68-6

P.C. CODE 113101- Ethalfuralin FILE LAST PRINTED: 04/15/94

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
83-1(a) and 83-2(a) Feeding-1 year Species: rat Eli Lilly Labs R-257; 8/79	Ethalfuralin 94.5% tech. [redacted] lot B30- Y64-35B	070678	NOEL = 100 ppm. LEL = 250 ppm (incr. creatinine + BUN in females). Doses 0, 100, 250, 750 ppm in Fischer 344 rats.	Minimum 002251	
83-1(a) and 83-2(b) Feeding-1 year Species: mice Eli Lilly Labs M9157; 4/81	Ethalfuralin 94.5% tech [redacted] lot B30-Y64-35B	070679	NOEL = 100 ppm. LEL = 400 ppm (alkaline phosphatase incr. in males). Increased SGPT values in high dose males. BUN and creatinine values were decreased in high dose female. Doses: 0, 100, 400, 1500 in Charles River.	Minimum 002251	
83-1(a) and 83-2(a) Feeding/carcinogenic-2 year Species: rat Eli Lilly Labs R267 & R277; 6/81	Ethalfuralin 94.5% tech. [redacted] lot B30-Y64-35B	070678	Oncogenic LOEL = 250 ppm (mammary gland fibroadenomas (F) - 35%). Doses: 0, 100, 250, 750 in Fischer 344 rats.	Minimum 002251	
83-1(a) and 83-2(b). Feeding/carcinogenic-2 year Species: mice Eli Lilly Labs M9167 & M9177; 6/81	Ethalfuralin 94.5% [redacted] lot B30-Y64-35B	070680	Oncogenic LOEL > 1500 ppm (HDT). Systemic NOEL < 100 ppm (LDT) (increase in focal hepatocellular hyperplasia). Doses tested: 0, 100 400, 1500 ppm in B6C3F1 mice.	Minimum 002251 002405	
83-1(b) Feeding-1 year Species: dog Eli Lilly Labs DO 1684; 11/85	Ethalfuralin 95.5%	260434 262711	Systemic NOEL = 4 mg/kg. Systemic LEL = 20 mg/kg (variations in erythrocyte morphology; increased thrombocyte count, increased erythroid series of the bone marrow). Levels tested: 0, 4, 20 and 80 mg/kg/day by capsule in beagles.	Supplementary 005224 Minimum 005512	
83-3(a) Developmental Toxicity study Species: rat Eli Lilly Labs R06880; 11/1/80	Ethalfuralin 93.5% tech. [redacted] lot B30-Y64-35B	070682	Maternal NOEL > 250 mg/kg (HDT). Trend toward hydronephrosis in treated litters, but no hydronephrosis. Controls performed poorly (decreased live fetuses, increased resorptions, increased females resorbing, decreased fetal weight) therefore making comparisons is questionable. Doses tested: 0, 10, 75 and 250 mg/kg in Wistar rats.	Supplementary 002251	
83-3(a) Developmental Toxicity study Species: rat BioResearch Inc. 82182; 11/85	Ethalfuralin 95.5%	260434	Maternal NOEL = 50 mg/kg. Maternal LEL = 250 mg/kg (decrease in body wt. gain and elimination of dark urine days 7-16 of gestation). A/D ratio = 50/1000 = 0.05. Developmental NOEL > 1000 mg/kg. Levels tested: 0, 50, 250, 1000 mg/kg by gavage in Charles River strain.	Guideline 005224	

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

**U.S. ENVIRONMENTAL PROTECTION AGENCY
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CASWELL#: 453B
CAS-REG#: 55283-68-6**

P.C. CODE 113101- Ethalfluralin FILE LAST PRINTED: 04/15/94

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
83-3(b) Developmental Toxicity Study Species: rabbit Eli Lilly Labs B-7079; 10/80	Ethalfluralin Tech. 94.5% [REDACTED] Lot 830-Y64-358	070682	Teratogenic NOEL > 750 mg/kg. Maternal NOEL < 250 mg/kg (abortions and decr. body wt.). Developmental NOEL < 250 mg/kg (increased resorptions and decreased litter size). Doses tested: 0, 250, 500 & 750 mg/kg to Dutch-Belted rabbits.		Supplementary 002251
83-3(b) Developmental Toxicity Study Species: rabbit Eli Lilly Labs B7160; 10/80	Ethalfluralin 94.5% [REDACTED] Lot 830-Y64-358	070682	Appears positive for teratogenicity at 250 mg/kg dose. Inadequate litters to examine. Extreme stress from dosing procedure. Doses tested: 0, 75, 250 mg/kg in Dutch Belted rabbits.		Supplementary 002251
83-3(b) Developmental Toxicity Study Species: rabbit DOW Elanco B01383; 6/83	Ethalfluralin tech. 94%; Lot 830-Y64-358	250596	Teratogenic NOEL = 75 mg/kg/day. Teratogenic LEL = 150 mg/kg/day (sternal and cranial variants). Fetotoxic NOEL = 75 mg/kg/day. Fetotoxic LEL = 150 mg/kg/day (incr. resorptions). Maternal NOEL = 75 mg/kg/day. Maternal LEL = 150 mg/kg/d. (abortions). Levels tested: 0, 25, 75, 150 and 300 mg/kg in Dutch belted strain.		Minimum 003273
83-4 Reproduction-3 generation Species: rat Eli Lilly Labs R-68, R-78, R-1248; 3/81	Ethalfluralin 94.5% Tech. [REDACTED] Lot 830-Y-348	070682	Systemic NOEL = 250 ppm. Systemic LEL = 750 ppm (slightly depressed body weight in males of all 3 generations). Reprod. NOEL > 750 ppm (HDT). (No hydronephrosis in contrast to hydronephrosis reported in rat teratology study). Doses: 100, 250, 750 ppm in Fischer 344 rats.		Supplementary 002251
Feeding-14 day Species: chicken Eli Lilly Labs H-8513; 8/74	Ethalfluralin Tech (95%) Lot 110-395A-109	097326	NOEL > 2500 ppm (HDT) (ODT). No mortalities or toxic signs. White Leghorn chicks.		Supplementary 001328
Feeding-14 day Species: rat Eli Lilly Labs R-303; 8/74	Ethalfluralin Tech (95%)	097326	Systemic NOEL < 250 ppm (LDT) (Fatty metamorphosis of liver and kidney).		Supplementary 001328
Feeding- 15 days Species: dog Eli Lilly Labs D-3193; 8/74	Ethalfluralin Tech (95%) Lot 110-395A-109	097326	Syst. NOEL < 18.75 mg/kg (LDT) (fatty metamorphosis of liver).		Supplementary 001328

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

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**PAGE 3
CASWELL#: 453B
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P.C. CODE 113101- Ethalfluralin FILE LAST PRINTED: 04/15/94

CITATION	MATERIAL	ACCESSION/ HRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
82-1(a) Feeding-3 month Species: rat Eli Lilly Labs R-813; 8/74	Ethalfluralin Tech (95%) Lots 110-395A-109; 090- 310-292	097327	Systemic NOEL = 1,100 ppm. Systemic LEL = 5,000 ppm. (HDT; body wt. gain depression, incr. liver wt., kidney and thyroid wts., decr. hemoglobin RBC and hematocrit). Urinalysis and no microscopic examination of mammary gland, spinal cord, sciatic nerve, bone marrow or pituitary gland. Doses: 0, 250, 11000, 1500 in Harlan Wistar strain		Supplementary 001328
82-1(a) Feeding-3 month Species: rat Eli Lilly Labs R-936; 2/78	Ethalfluralin Tech 94% Lot X-27900	097327	Systemic NOEL = 500 ppm. Systemic LEL = 1,100 ppm (incr. body/liver wt. ratio, decr. RBC, hematocrit and hemoglobin values in females, incr. relative kidney wts. for males). No urinalysis, serial hematological and clinical chemistry changes. Doses: 0, 250, 500, 1100, 2500 and 5000 ppm in Harlan Fischer 344 rats.		Supplementary 001328
82-1(b) Feeding-3 month Species: dog Eli Lilly Labs D-3733; 8/74	Ethalfluralin Tech	097327	Systemic NOEL = 27.5 mg/kg. Systemic LEL = 80 mg/kg (HDT; elevated alkaline phosphatase values, slight fatty metamorphosis of the liver, incr. cholesterol, BUN). Doses: 0, 6.25, 27.5, 125/80 mg/kg/d (beagles).		Minimum 001328
82-1(a) Feeding-3 month Species: mice Eli Lilly Labs M-9286; 5/78	Ethalfluralin Tech. 994%	070678	NOEL = 560 ppm (84 mg/kg/day). LEL = 1110 ppm (low bilirubin value and low mean kidney weights; both in males). Doses: 0, 560, 1100, 2250 4000 and 8000 ppm in B6C3F mice.		Minimum 002251
82-2 Dermal-3 week Species: rabbit Eli Lilly Labs B-7400; 11/81	Ethalfluralin Tech. 95.5% in formulation contain- ing 37.74% by weight	070683	Topical irrit. NOEL = 7.2 mg/kg. Topical irrit. LEL = 36 mg/kg (slight hyperkeratosis and acanthosis). Systemic NOEL = 7.2 mg/kg Systemic LEL = 36 mg/kg (decr. body wt. gain). Doses tested: 0. 3.6, 7.2 and 36 mg/kg in NZW rabbits.		Supplementary 002251
82-2 Dermal-3 week Species: rabbit Eli Lilly Labs B01384; 4/85	Ethalfluralin Tech. 95.6% a.i.	257855	NOEL => 1000 mg/kg (limit dose). Historical control data from 3, 21 day dermal toxicity studies, submitted by Lilly Research Lab, resolved the issues related to effects stated in DER 004689. The systemic effects stated in DER 004689 are within the range of historical control values and not considered effects from the applied chemical. Slight to severe irritation, slight to moderate edema and coriaceous skin with epidermal fissures. The test area was stained yellow from the test material. No systemic toxicity was observed (DER 009713 - 9/4/92).		Supplementary 004689 Minimum 009713

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

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CASWELL#: 453B
CAS-REG#: 55283-68-6**

P.C. CODE 113101- Ethalfluralin FILE LAST PRINTED: 04/15/94

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
84-2(a) Mutagenic-Ames Species: salmonella Eli Lilly Labs LBMS 1169; 11/80	Ethalfluralin 93.5% Tech. Lot B30-Y64-358	070682	Not mutagenic at conc. ranging from 1000 to 0.1 ug/ml +/- activation in salmonella strains: 646, TA1535, TA100, C3076, TA1537, D3052, TA1538, TA98 and E. coli str: WP2 and WP2 uvr A-. Absence of detailed data report and failure to use maximum concentrations up to level of toxicity or crystallization.		Unacceptable 002251
84-4 Mutagenic-DNA repair test Species: rat hepatocytes Eli Lilly Labs 791120-263; 6/80	Ethalfluralin tech 93.5% Lot B30-Y64-358	070682 -350473	Ethalfluralin at 100, 50, 10, 5, 1 and 0.5 nmoles/ml negative for UDS Cytotoxic at 500 & 1000 nmoles/ml		Acceptable 002251 003269
84-2(b) Mutagenic-dominant lethal test Species: rat Eli Lilly Labs R-159; 12/80	Ethalfluralin 93.5% Tech. Lot B30-Y64-358	070682	No evidence of dominant lethal effect. Doses tested: 5.0 g/kg (25% w/v) suspension in 10% (w/v) aq. acacia sol.		Not acceptable 002251
84-2(a) Mutagenic-Ames Species: salmonella Eli Lilly Labs 830404GPA1169; 6/83	Ethalfluralin tech 93.5% Lot B30-Y64-358	250475	Positive for increased revertants in Salmonella & E. coli. (TA1535, TA100, TA98). Tested with and without metabolic activation Doses tested: 0.1 ml - 1000 ug/ml.		Acceptable 003269
84-2(a) Mutagenic-Ames Species: salmonella Eli Lilly Labs 83037AMS1169; 5/83	Ethalfluralin tech. 93.5% Lot B30-Y64-358	250475	Positive for increased revertants (base-pair mutations) in salmonella strains: TA1537, TA1538, TA1535, TA98 & TA100; positive in TA1535 - activated, TA-100 (dose response) +/- activation. Doses tested: 0.5, 1.0, 5, 10, 50, 100, 500, 1000 nM/ml.		Acceptable 003269
84-2(a) Mutagenic- lymphoma mutation Species: mice L5178Y cells Eli Lilly Labs 830208MLA1169; 4/83	Ethalfluralin Tech. 93.5% Lot B30-Y64-358	250475	Negative for TK locus in L5178Y cells up to cytotoxic doses. Doses tested: 0.1, 0.25, 0.5, 0.75, 1.0, 2.5, 5 & 10 ug/ml.		Acceptable 003269
84-4 Mutagenic-DNA repair test Species: rat bone marrow cell Eli Lilly Labs 8302145CE1169; 3/83	Ethalfluralin Tech. 93.5% Lot B30-Y64-358	250475	Negative for SCE induction (DNA repair test) in (F) (Male not tested) Doses tested: 200, 300, 400, 500 mg/kg. Cytotoxic at 400 & 500 mg/kg.		Unacceptable 003269

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

**U.S. ENVIRONMENTAL PROTECTION AGENCY
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TOX ONELINERS**

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CASWELL#: 453B
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P.C. CODE 113101- Ethalfluralin FILE LAST PRINTED: 04/15/94

CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
84-2(b) Mutagenic- in vitro cytogen. Species: CHO cells Rep# LSR-RTC-09-5003-M-02385 8/23/85	Ethalfluralin tech	259342	Positive for the induction of chromosomal aberrations in CHO cells with S-9 metabolic activation at 85 ug/ml. Negative for chromosomal damage in CHO cells in the absence of S-9 metabolism. Doses tested: 7.24, 22.9 and 72.4 ug/ml in the absence of S-9 metabolic activation; 5.0, 15.8, 50.0 & 85.0 ug/ml in the presence of S-9 activ.	Acceptable 005224	
84-4 Mutagenic-gene mutation Species: Schizosacc. pombe P1 Rep. LSR-RTC-095005-M-030-85 8/23/85	Ethalfluralin tech 95.5%	259342	Negative for the induction of forward mutation in S. pombe P-1 cells either in the presence or absence of S-9 metabolism. Doses tested: 12.5, 25.0, 50.0, 100, 200 and 300 ug/ml.	Unacceptable 005224	
84-4 Mutagenic-DNA-repair-test Species: rat-hepatocyte- Eli Lilly Labs- 791120-263, 6/80	Ethalfluralin 93.5%; Lot B30-Y64-358	250475	Negative for induction of unscheduled DNA-synthesis up to cytotoxic doses (500 & 1000 μ M). <i>Sample 10 p. 4</i>	Acceptable 003269	
84-2(b) Mut-Sister chromatid exchange Species: Chinese hamster Eli Lilly Labs 830214SCE1169; 3/83	Ethalfluralin 93.5%; Lot B30-Y64-358	250475	Even at cytotoxic doses of 400 & 500 mg/kg, ethalfluralin did not induce sister chromatid exchange. It is recommended that spermatogonial metaphases also be sampled for SCE.	Not acceptable 003269	
Oncogenic risk assessment Species: rat DOW Elanco	Ethalfluralin	072180	The Q* value is 0.0871 mg/kg/day exp-1.	004235	
85-1 Metabolism Species: rat Eli Lilly Labs 12/81	Ethalfluralin tech 97%; Lot 721-109A-25.1 & C14- Ethalfluralin 99.6%, lot 553-834-136	070683	Following single oral dose of 100 mg/kg C14 ethalfluralin, plasma radioactivity reached peak at 1 hr., virtually zero by 3 1/2 hrs. Peak tissue concentrations in liver, fat and kidney. Biliary excretion accounted for 14% of C14 in 48 hrs. All C14 by this route found in metabolites as conjugates of glucuronic acid. 88-95% C14 excreted via urine and feces within 7 days (1/4 in urine, 3/4 in feces) unconjugated metabolites - 31% of total urinary radioactivity. Glucuronide - conjugated metabolites - 14%. Ethalfluralin not detected in urinary radioactivity - instead 4 different metabolites.	Minimum 002251	

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CITATION	MATERIAL	ACCESSION/ MRID NO.	RESULTS	TOX CAT	COREGRADE/ DOCUMENT#
85-1 Metabolism Species: rat Bio/dynamics Inc. DR-0233-3655-001; 06/22/93	14C-Ethalfluralin > 99.0% a.i., unlabeled ethalfluralin, 95.5%	428229-01	<p>Disposition of 14C-ethalfluralin was investigated in male & female Fischer 344 rats at single low (10 mg/kg) and high (100 mg/kg) oral doses and repeated low doses of 10 mg/kg followed by a single radiolabeled dose. Absorption of Ethalfluralin was estimated at between 79-87% based on data supplied by the registrant. Feces represented the major route of excretion for ethalfluralin derived radioactivity, with 50.9-63.2% excreted in 72 hrs by this route for male & female rats. Tissue levels at study termination (72 hrs post-dose) were negligible (less than 0.3% of the administered dose in any one tissue).</p> <p>The major urinary metabolite identified in male & female rats was the acid metabolite of ethalfluralin, or (2-methyl, 2-hydroxy-3-12,6-dinitro-4-trifluoro-methyl)phenylamine) propanoic acid). This metabolite comprised between 7.2-9.0% of the administered dose in urine of female rats 0-24 hrs post-dose, and between 13-17.3% of the admin. dose in male rat urine. All other urinary metabolites comprised less than 6.2% of the dose in urine of male & females.</p> <p>In feces, the major metabolite identified was an amino product of ethalfluralin metabolism, N-ethyl-N-(2-propenyl)-2-nitro-6-amino-4-(trifluoromethyl)benzenamine. This metabolite comprised between 2.7-7.1% of the administered dose in male and female rat feces across all dose groups, & appeared in slightly higher percentage in feces from high dose rats.</p> <p>A second metabolite identified in feces comprised between 1.6-4.6% of the dose and co-eluted with a standard of ethalfluralin in both male & female rats. Although a specific metabolic scheme was not presented for ethalfluralin in rats, major metabolites were identified in this study.</p>		Minimum 010541
85-2 Metabolism - dermal absorption Species: monkey Eli Lilly Labs M-6162 & PO-3282	C14-Ethalfluralin	072180	<p>Based on plasma levels of radiocarbon, 2.8% of topically applied C14-ethalfluralin is absorbed through the skin. Total excretion data demonstrate 0.9% of topically applied C14-Ethalfluralin can be absorbed systemically.</p>		Acceptable 004090 004235

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