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

MEMORANDUM OCT - 9

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

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

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

SUBJECT: EPA Reg.#1471-REE. 1471-EUP-63. PP#8G2118; 2F2645; SONALAN E.C. Herbicide. Ethalfluralin

Petitioner: Elanco. Division of Eli Lilly and Company
Greenfield, Indiana

Action Requested:

Applicant requests registration of SONALAN E.C., an emulsifiable concentrate containing 35.1% of the herbicide ethalfluralin.

Applicant also requests a tolerance of 0.05 ppm for ethalfluralin in or on the raw agricultural commodity grouping seed and pod vegetables, forage legumes, and cucurbits.

Recommendations:

Toxicology Branch cannot recommend favorably. The following points are noted:

1. The 2-year rat feeding study indicates that ethalfluralin causes an increased incidence of benign mammary gland fibroadenomas in females at the high doses (250 and 750 ppm). The NOEL for this effect is 100 ppm (0.01%) in the diet. Historical data provided by the registrant indicate an incidence of fibroadenomas in untreated Fischer 344 rats to be from 0% to 26.7%, with an overall incidence of 15.3%. In comparison, incidences of 35% and 46.7% for the mid-dose and high-dose females in this study would categorize ethalfluralin as an oncogen for benign mammary gland adenomas. Due to the above positive findings a risk assessment is required. (See review page 21)

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2. In the 2-year mouse chronic feeding and oncogenicity study, results were negative for oncogenicity at 100, 400, or 1500 ppm in the diet. However, a NOEL has not been determined due to a dose related biologically significant increase in focal hepatocellular hyperplasia at 100 ppm (LDT). (See review page 41)

3. In the second rabbit teratology study (#B7160) an apparent teratogenic response is evident as manifested by an increased incidence of cleft palate and open eyelids at the 250 mg/kg dose level. However, due to the high mortality, abortion rate, and numbers of non-pregnant animals in the study and the resultant reduced number of litters available for examination, a meaningful assessment was not possible. Furthermore, the stress apparently induced by the dosing procedure may have further complicated the results of the study. A final determination concerning the teratogenic potential of ethalfluralin should be based on a repeat study. (See review page 80)

4. In the rat teratology study there was an increased incidence of hydronephrosis (without hydroureter in the treated groups and this is considered a potentially positive teratogenic response. This effect is noted at increased levels over the controls in this study and also as compared to the historical data provided. (No hydronephrosis was detected in the 3-generation reproduction study utilizing the same strain of animals as in the teratology study although historical data indicates an expected incidence of 12.1%).

The top dose for this rat study was based on reduced food consumption in a rabbit study, and no maternal toxicity was seen in this rat study; dosage therefore was inadequate. Also various performance values for control rats were very poor and comparison to treated groups was not considered meaningful. Therefore, it will be necessary to repeat the study. (See review page 89)

5. In the 3-generation reproduction study, no reproductive effects were seen. The NOEL is 250 ppm based on slightly depressed mean body weight gains in males at 750 ppm in all 3-generations. However, no findings of hydronephrosis were reported, which is a discrepancy between the rat teratology study and historical data for the laboratory. Data are Core Supplementary pending an explanation of this discrepancy. (See review page 95)

6. The Ames Mutagenicity Test is reported to be negative. However, the report is unacceptable in the absence of detailed data and the failure to use maximal concentrations up to the level of toxicity or crystallization. References also should be cited. (See review page 103)

7. No signs of a dominant lethal effect were seen with ethalfluralin. However, the study is unacceptable in the absence of evidence of absorption of the chemical, too few males and females being used, inadequate dosing schedule, and lack of a positive control. (See review page 107)

8. The Three-Week Dermal Toxicity Study in Rabbits conducted with the EC ethalfluralin formulation, is considered Core Supplementary because there were only 5 rabbits per sex group and because dermal irritation scores were not provided. (See review page 66)

9. The following studies have been classified as Core Supplementary and should be repeated unless the firm is able to provide additional information or data which will allow upgrading of the studies to Core-Minimum or Satisfactory.

Acute Oral LD_{50}, Rat, on technical ethalfluralin and formulation
Acute Dermal Toxicity on technical ethalfluralin and formulation
Primary Dermal Irritation on formulation
Primary Eye Irritation on technical ethalfluralin
Acute Inhalation Toxicity on technical ethalfluralin and formulati
Three-Week Dermal Toxicity on formulation
Teratology Study in the Rabbit
Teratology Study in the Rat
Reproduction Study in the Rat
Mutagenicity: Ames Test
Mutagenicity: Dominant Lethal Test

10. Toxicology Branch needs to know the identity of the plant metabolites so it may be determined if the plant metabolites are the same as, or comparable to, the animal metabolites already studied. Registrant has submitted additional information, now undergoing review.

Formulation of SONALAN E.C. Herbicide:

Active Ingredient:

Ethalfluralin* N-ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl)-benzenamine ---------- 35.1%

Inert Ingredients: ---------- 64.9%

[redacted]

*SONALAN - the registrant trademark for Elanco Products Co. ethalfluralin.

[redacted]**Applicant should provide us with the composition of [redacted] so that we may determine whether it is cleared under CFR 180.1001.

INERT INGREDIENT INFORMATION IS NOT INCLUDED

Data Base:

Studies previously reviewed by Dr. Mary Quaife (November, 1978) are as follows:

Technical Chemical:		CORE
Oral LD_{50}, rat:	greater than 10 g/kg	Supplementary
Oral LD_{50}, mouse:	greater than 10 g/kg	Supplementary
Oral LD_{50}, dog:	greater than 200 mg/kg	Supplementary
Oral LD_{50}, cat:	greater than 200 mg/kg	Supplementary
Acute Dermal LD_{50}, rabbit:	greater than 2 g/kg	Supplementary
Primary Eye Irritation, rabbit:	conjunctivitis; no corneal irritation	Supplementary
Acute inhalation, rat -	LC_{50} greater than 0.028 mg/L/hr	Supplementary
14-Day Feeding, chicken -	NOEL greater than 2500 ppm (HDT)	Supplementary
14-Day Feeding, rat -	NOEL less than 250 ppm (LDT)	Supplementary
15-Day Feeding, dog -	NOEL 18.75 mg/kg; LEL 56.25 mg/kg	Supplementary
90-Day Feeding, rat -	NOEL 1100 ppm	Supplementary
90-Day Feeding, rat -	NOEL 500 ppm	Supplementary
90-Day Feeding, dog -	NOEL 27.5 mg/kg	Minimum

SONALAN Formulation:		
Oral LD_{50}, rat -	LD_{50} greater than 2 ml/kg	Supplementary
Acute Dermal LD_{50}, rabbit -	LD_{50} greater than 2 ml/kg	Supplementary
Primary Dermal Irritation, rabbit -	Slight to moderate irritation.	Supplementary
Acute Inhalation Toxicity, rat -	LC_{50} greater than 74.4 ul/L/hour	Supplementary
Primary Eye Irritation, rabbit -	Slight iritis & conjunctivitis; corneal dulling reversible in 21 days.	Minimum

Other Core Supplementary studies listed above and not requested in Recommendation item 9 need not be repeated because the studies are not generally required (i.e., 14-day feeding in chicken and rat; 15-day feeding in dog). Additional studies are adequately superseded by studies of longer duration (i.e., 90-day feeding, rat).

Reviewed studies from this submission are Core Classified as follows: (All are on technical chemical, except 3-Week Dermal Toxicity in Rabbits).

St...	CORE CLASSIFICATION
Three-month Oral Toxicity in Mice	Minimum
One-Year Dietary Stucy in Rat (R-257) (Subchronic)	Minimum
Two-Year Dietary Study in Rat (R-267 & 277)	Minimum
One-Year Dietary Study in Mouse (M-9157) (Subchronic)	Minimum
Two-Year Dietary Study in Mouse & Oncogenicity (M-9167 & 9177)	Minimum
Guinea Pig Sensitization (G-9530 & 00379)	Minimum
Metabolism in Rats	Minimum
Three-Week Dermal Toxicity in Rabbits (on formulation)	Supplementary
Pilot Teratology Studies in Rabbit (B-7048 & 7038)	Supplementary
Teratology Studies in Rabbit (B-7079 & 7160)	Supplementary
Teratology Study in Rat (R-06880)	Supplementary
Reproduction Study in Rat (R-68, 738, 1248)	Supplementary
Mutagenicity - Ames Test (LBMS 1169)	Unacceptable
Mutagenicity - DNA Repair Synthesis	Acceptable
Mutagenicity - Dominant Lethal Test	Unacceptable

Discussion:

NITROSAMINE CONTENT OF TECHNICAL CHEMICAL USED IN STUDIES:

The ethalfluralin used in the various toxicological studies was washed to remove or reduce contamination with the nitrosamine N-ethyl-2-methyl-N-nitroso-2-propene-1-amine (ethyl-methylallyl-nitrosamine, EMANA). After the washing procedure, the nitrosamine content of ethalfluralin was about 0.05 ppm. Studies utilizing the washed technical chemical included the 2-year mouse feeding study, the one- and two-year rat feeding studies, the rabbit and rat teratology studies, and the 3-generation rat reproduction study.

If the data from studies utilizing washed technical chemical are to be considered valid and applicable, the formulations to be marketed must also be manufactured from comparable technical chemical washed in a similar manner. The applicant has submitted their current manufacturing procedure for technical ethalfluralin, and data indicate the nitrosamine content to be similar to that used in these studies, or less. The current manufacturing procedure includes the washing procedure employed on the technical chemical used in the toxicology studies. Residue Chemistry Branch acknowledges that the technical chemical used in the toxicology studies and that of current manufacture would be expected to be comparable in terms of nitrosamine content.

PLANT METABOLITE RESIDUES:

Residue Chemistry Branch defers to Toxicology Branch regarding concern over levels and identity of residues of plant metabolites.

A tolerance of 0.05 ppm is requested for residues of the chemical ethalfluralin. Radiostudies demonstrate residues up to 0.16 ppm unidentified metabolites in dry beans, peas, soybeans, and lentils. Radioautograms indicate at least 5 to 8 metabolites are present, none of them being predominant.

Toxicology Branch needs to have some assurance that plant metabolites and their relative quantities are the same or comparable to the animal metabolites, already studied.

Review:

Three Month Oral Toxicity Study of Ethalfluralin (94961; EL-161) in Mice: (Study M-9286): Conducted by E.R. Adams, F.O. Gossett, D.G. Hoffman, N.V. Owen, J.L. Emmerson, and D.M. Morton. Lilly Research Laboratories. [illegible], 1978. Accession 070678.

Material Tested: Ethalfluralin. [Lilly Compound 94961; EL-161. N-ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl) benzenamine]. Technical chemical of 94% purity. The N-ethyl-2methyl-N-nitroso-2-propene-1-amine (nitrosamine) content of the ethalfluralin used in this study was 92 ppm.

Procedure: B6C3F$_1$ hybrid mice obtained from Charles River Breeding Laboratories, Wilmington, Mass. were randomly assigned to test groups to receive ethalfluralin at dietary levels of 0, 0.056, 0.11, 0.225, 0.4, or 0.8% (0, 560, 1100, 2250, 4000 or 8000 ppm) in pelleted mash ration for 3 months. The mice were caged 3/sex/cage of stainless steel mesh and housed in a separate room maintained at 77 $\pm$ 2°F, with a minimum relative humidity of 45%. Automatic timers provided 12 hours of artificial light and 12 hours of dark. There were 15 males and 15 females in each treatment group. At the beginning of the test the mice were approximately 7 weeks old. The mean weights of the males was 18.5 $\pm$ 0.2 g; of females, 16.6 $\pm$ 0.2 g. The test diets were analyzed for chemical content 1 month prior to the study, during the 4th week of the test, and at the termination of the study. (The test chemical was present at theoretical levels and was stable in the diet for 7 months.)

All mice were observed twice daily for physical signs of toxicity, and at least once each week each mouse was closely examined.

All mice were weighed once each week on a self-taring, self-recording balance. Food consumption was not measured because of excessive spillage by the mice.

At the end of the 3-month treatment period the mice were fasted overnight and bled before being killed for necropsy. Terminal hematology tests included determination of hematocrit; hemoglobin; erythrocyte, total, and differential leucocyte counts, and erythrocyte morphology, using the Model S Coulter Counter for all but erythrocyte morphology.

Terminal clinical chemistry determinations included serum concentrations of glucose, urea nitrogen (BUN), creatinine, bilirubin, alkaline phosphatase, and glutamic pyruvic transaminase (SGPT).

The mice were killed with CO_2. The thoracic and abdominal viscera were examined and sections of the following organs taken for microscopic examination: adrenal, bone marrow, colon, duodenum, heart, ileum, jejunum, kidney, liver, lungs, lymph node, mammae, ovary, pancreas, parathyroid, pituitary, prostate, salivary gland, skin, spleen, stomach, striated muscle, testis, thymus, thyroid, urinary bladder, and uterus. Terminal weights were recorded and organ weight/body weight ratios were calculated for the following organs: heart, kidney, liver, spleen, testis, and uterus.

Tissues were fixed with formalin, stained with H & E, and evaluated by pathologist Norris V. Owen, D.V.M., Ph.D. Dunnett's test was used for statistical analysis of differences between control and treated groups.

Results:

Chemical Purity and Stability in Feed: The purity of the technical chemical is approximately 94%. At 2-months in the mash there appears to be no loss in potency. In pelleting the same mash there appears to be a potency loss of 6 to 8%; however, this apparent "loss" may be due to inadequate mixing, sampling, or assay, since at 7 months the "apparent loss" in potency is much less. However, at 4 months after mixing it was noted that some pellets were dark in color (cause not determined). These dark pellets assayed only 54 to 60% of theoretical, whereas the normal light colored pellets assayed 82-88% of theoretical potency.

From the report it appears the dark colored pellets were detected and therefore were not fed. It also is noted that dark colored pellets were found in the control diet, as well as in the diet containing the chemical. At this 4-month period following mixing (actually week 4 of feeding), even the light colored pellets contained only 82-88% of the theoretical level of chemical, the lowest level assayed during the study.

The dark pellets were discussed with Elizabeth R. Adams of Elanco via telephone. Ms. Adams verified that none of the dark pellets were fed, and that they were dark because of scorching in pelleting at too high a pressure and temperature. In some instances pelleting, while not destroying the chemical, increases binding to the feed components, thereby reducing recovery in the assay process.

At 7 months (after completion of the study) the pellets averaged 90.5% of theoretical potency. Since the theoretical potency of the technical chemical is 94%, and since effect levels of the chemical were demonstrated, it can be concluded that dosing was adequate.

Mortality: No deaths occurred during the study.

Toxic Signs: No clinical signs of toxicity were seen. Urine of treated mice was yellow-orange colored, particularly at higher dose levels, on a dose related basis. Analysis demonstrated that the coloration was due to ethalfluralin metabolites; ethalfluralin per se was not present.

Mean Body Weight and Mean Weight Gain: The mean body weights at 3 months of both males and females were significantly less at the 0.4 and 0.8% dosage levels. The mean weight gains (in grams) were significantly less at the 0.225, 0.4, and 0.8% dietary levels in the males, but only at the 0.8% dietary level in the females.

Hematology: There was no apparent effect of treatment on mean white blood cell counts, red blood cell counts, hemoglobin, or hematocrit values.

Blood Chemistry: Compared with controls, the blood urea nitrogen was statistically high in females (21.19 mg%) and statistically low in males (20.98 mg%) at the 0.8% dietary level.

The bilirubin values are statistically low in the males on the 0.110, 0.225, 0.4, and 0.8% dietary levels. (Values are 0.191, 0.183, 0.157, and 0.165 mg %. Control values are 0.230 mg %).

Alkaline phosphatase values were significantly high in males at the .225, .4, and .8% dietary levels, with values of 77.7, 104.3, and 148.5 mU/ml, compared with control values of 50.9 mU/ml. The females at .225 and .8% dietary levels were significantly high with values of 97.9 and 108.1 mU/ml compared with a value of 88.6 mU/ml for control females.

The serum glutamic-pyruvic transaminase (SGPT) values were high (250 ± 101.4 mu/ml) for males at the 0.8% dietary level, and also were higher than controls in the females at this level. This high SGPT also partially correlated with liver necrosis.

Mean BODY WEIGHTS were statistically lower than controls in both males and females at the 0.4% and 0.8% dietary levels. Mean LIVER WEIGHTS were statistically greater than controls in males and females at the 0.8% dietary level and in males at the 0.4% dietary level. Mean KIDNEY WEIGHTS (with adrenals attached) were statistically lower than controls in males and females at the 0.8% dietary level, and also in males at the 0.11%, 0.225%, and 0.4% dietary levels. Mean HEART WEIGHTS were statistically lower than controls at the 0.8% dietary level in both males and females. Mean SPLEEN WEIGHTS were statistically lower than controls in males at the 0.8% dietary level. Mean TESTES WEIGHTS (males) and UTERUS WEIGHTS (females) were statistically lower than controls at the 0.8% dietary level.

Relative Organ Weights:

The RELATIVE ORGAN WEIGHTS of males and females were significantly greater than controls at the 0.4% and 0.8% dietary levels.

RELATIVE HEART WEIGHTS were significantly greater than controls in females at the 0.225% dietary level only.

RELATIVE TESTES WEIGHTS were significantly greater than controls in males at the 0.8% dietary level.

RELATIVE UTERUS WEIGHTS were significantly lower than controls at the 0.8% dietary level.

GROSS NECROPSY findings were normal in all males on the control diet and at the 0.056%, 0.110%, 0.225% and 0.4% dietary levels of ethalfluralin, and in females at the 0, 0.056%, and 0.110% dietary levels.

Histologically, two male mice at the 0.4% level showed slight centrilobular hypertrophy of the hepatocytes, and one male mouse showed two small foci of necrosis.

Gross necropsy of the males on the 0.8% dietary level showed small white areas on livers of 4 of the 15 mice.

Histologically, 8 of the 15 males on the 0.8% dietary level showed enlarged hepatocytes, with a few of the cells being necrotic. In some instances the necrosis spots appeared to be of possible infectious etiology. Two additional livers showed slight centrilobular hypertrophy of hepatocytes.

In gross necropsy of the females, one mouse on the 0.225% dietary level showed an enlarged red ovary. However, microscopic examination of this ovary revealed it to be normal. Another female on this dietary levels of chemical had a ruptured left eyeball. One female on the 0.4% dietary level had acute focal interstitial pneumonia.

Gross necropsy of the females on the 0.8% dose showed all to be normal. However, histologically, 12 of the 14 mice showed enlarged centrilobular hepatocytes with eosinophilic "ground glass" cytoplasm, with necrosis of some of the cells. (One mouse in the group was found to be a male, having been missexed when the groups were formed; therefore, this mouse was removed from the study). One mouse was completely normal, and one showed only slight centrilobular hypertrophy of hepatocytes.

Conclusions:

When ethalfluralin was fed to mice for 3 months at levels of 0.8% and below, no clinical signs of toxicity were seen, and there were no effects on hematology. Weight gains were depressed at the 0.8% dietary level in males and females, and also at the 0.225% and 0.4% levels in males. Hepatic injury, apparently compound related, was also seen at the 0.8% level, as was an increased SGPT value, associated with this hepatic injury.

Relative liver weights were increased at both 0.4 and 0.8% dietary levels, as were also the alkaline phosphatase serum levels.

The NOEL was 560 ppm, or about 84 mg/kg/day, based on low bilirubin values at 1110 ppm. Data are Core-Minimum.

One-Year Dietary Toxicity Study with Ethalfluralin in the Fisher 344 Rat. Study R-257 conducted by Elizabeth R. Adams, M.S. (Project Leader) et al. Lilly Research Laboratories, Greenfield, Indiana. August, 1979. Accession#070678.

Ethalfluralin (EL-161; Lilly Compound 94961) was administered to Fisher 344 rats in the diet for 1-year to evaluate the chronic toxicity of the chemical. Dietary concentrations of the chemical were 0, 100, 250, and 750 ppm, (0.0, 0.01, 0.025 and 0.075 percent), and were based on 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 the 0.11 percent (1100 ppm) dietary level. 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 (750 ppm) was a dose interpolated to cause a 10% increase in liver weight. The low dose, 100 ppm, was selected to provide a 100x multiple if residue levels were as great as 1 ppm. The middle dose, 250 ppm, was chosen as an acceptable intermediate.

Fifteen rats/sex/dose were used in the study. All rats were housed in one room.

Test Material: MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

Ethalfluralin, chemically is N-ethyl-N(2-methyl-2 properyl)-2,6-dinitro-4-(trifluoromethyl)-benzenamine. The lot used was B30-Y64-35B, and [redacted] to reduce the amount of nitrosamine contaminant, N-ethyl-2-methyl-N-nitroso-2-propene-1-amine. After this treatment, 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 0.05 ppm initially, with a maximum value of 0.07 ppm in the periodic assays.

Animals:

Fisher 344 rats, 4-5 weeks of age, were supplied as litters by Harlan Industries, Inc., Cumberland, Indiana. 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.

Rats were held 1 week 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 mesh cages 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 ± 3°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, and 9 months after the start of the tests.

Samples of each diet level were assayed for ethalflu in 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 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.

Hematology and Clinical Chemistry were conducted at the end of the 1-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, and erythrocyte morphology.

Clinical chemistry determinations performed on rats at 1-year included serum concentrations of glucose, urea nitrogen, creatinine, bilirubin, 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 to body weight ratios were calculated.

The following organ and tissues were collected and fixed in 10% buffered formalin: 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 statistical method was used to analyse differences between control and treated group means for parameters which are generally distributed normally.

Results:

Nitrosamine Content of Ethalfluralin Lot Used in Study: The initial assay for N-Ethyl-2-Methyl-N-Nitroso-2-Propene-1-Amine gave as assay value of 0.05 ppm. In 4 subsequent assays at 2 and 3 month intervals, no nitrosamine could be detected.

Ethalfluralin Assay of the Feed gives values of 80 to 96% of theory, with an overall of 85% of theory. Part of the "loss" apparently is due to the chemical having a purity or "potency" of 94.5%. Registrant states ethalfluralin was stable in the diet for up to 2 weeks.

Survival was 100% for all rats in this 1 year study.

The mean weight gain was statistically less in the females at the 750 ppm level at 12 months.

Feed Consumption and Feed Efficiency were unaffected by treatment.

Mean Daily Intake of Ethalfluralin from consumption of treated diets decreased on a mg/kg body weight basis over the 12 months as follows:

100 ppm in diet: from 7.5 mg/kg/day to 3.7 mg/kg/day (average of males and females)

250 ppm in diet: from 19.1 mg/kg/day to 9.3 mg/kg/day (average of males and females)

750 ppm in diet: from 55.2 mg/kg/day to 29 mg/kg/day (average of males and females)

Physical Signs of Toxicity attributable to treatment were not seen.

Yellow stained coats attributed to urinary metabolites of the chemical were seen in all ethalfluralin groups. Physical conditions noted in low incidence but not related to treatment or dose level included chromodacryorrhea, and head tilt (probable middle ear infection). These were noted in controls as well as treated rats.

Hematology

There were no differences between groups related to ethalfluralin administration in white blood cell counts, red blood cell counts, hemoglobin or hematocrit. Males on 250 ppm had significantly lower mean hemoglobin than controls ($p \leq 0.05$, Dunnetts two tailed "t" test). The hemoglobin value of this group (14.77 g) can be compared with the hemoglobin value for the controls (15.19 g). This was an isolated occurrence not related to the test compound.

Blood Chemistry (See Tables 1 & 2)

Statistically significant differences ($p \leq 0.05$) between controls and rats on the 750 ppm dietary level include: blood glucose increase in males; alkaline phosphatase decreased in females; and total bilirubin reduced in males. Creatinine and BUN were increased significantly in females on the 750 ppm and 250 ppm levels.

Organ Weights (See Tables 3 & 4)

Organ and organ/body weight ratios are significantly greater than controls for adrenals and prostates in males on the low (100 ppm) dietary level and for mean thyroid weights in males at this level. Mean and relative prostate weights also are significantly greater than controls in males on the high (750 ppm) dietary level.

TABLE 1: MEAN BLOOD CHEMISTRY VALUES OF MALE RATS GIVEN ETHALFLURALIN

Ethalfluralin % in Diet	Number of Rats	Mean and Standard Error					
		Glucose mg%	BUN mg%	Creatinine mg%	Total Bili. mg%	Alk. Ph. mU/ml	SGPT mU/ml
0.0	15	148.3	15.08	0.49	0.342	113.2	74.9
		3.9	0.29	0.04	0.012	3.5	3.8
0.01	15	153.3	15.35	0.43	0.322	105.5	76.3
		5.9	0.20	0.04	0.010	2.7	4.8
0.025	15	160.3	15.35	0.39	0.318	106.5	72.2
		6.1	0.44	0.04	0.009	4.0	3.8
0.075	15	174.0[a]	15.84	0.43	0.307[a]	102.9	78.4
		9.2	0.25		0.010	2.3	5.7

[a]Significantly different from the control, $P \leq 0.05$, two-tailed Dunnett's "t".

TABLE 2: MEAN BLOOD CHEMISTRY VALUES OF FEMALE RATS GIVEN ETHALFLURALIN

Ethalfluralin % in Diet	Number of Rats	Mean and Standard Error					
		Glucose mg%	BUN mg%	Creatinine mg%	Total Bili. mg%	Alk. Ph. mU/ml	SGPT mU/ml
0.0	15	130.3	14.60	0.46	0.426	91.2	55.7
		4.6	0.44	0.04	0.023	14.1	2.8
0.01	15	133.5	15.27	0.51	0.406	83.2	59.0
		5.0	0.53	0.02	0.023	5.5	4.0
0.025	15	133.6	16.47[a]	0.57[a]	0.437	67.6	64.1
		6.5	0.44	0.02	0.032	2.9	3.7
0.075	15	148.9	17.41[a]	0.57[a]	0.433	60.6[a]	60.5
		9.0	0.50	0.03	0.032	2.7	4.7

[a]Significantly different from the control, $P \leq 0.05$, two-tailed Dunnett's "t".

TABLE 3: MEAN TERMINAL BODY WEIGHTS AND RELATIVE ORGAN WEIGHTS FOR MALE RATS

Ethalfluralin % in Diet	Body (g) and S.E.	Mean Organ Weight Per 100 Grams Body Weight and Standard Error		
		Thyroid mg	Adrenals mg	Prostate g
0.0	436	6.31	11.50	0.11
	5	0.16	0.47	0.01
0.01	443	6.99	13.37[a]	0.15[a]
	6	0.29	0.38	0.01
0.025	444	6.59	11.82	0.12
	4	0.16	0.30	0.01
0.075	435	6.42	12.47	0.14[a]
	5	0.25	0.19	0.01

[a]Significantly different from the control, $P \leq 0.05$, two-tailed Dunnett's "t".

TABLE 4: MEAN TERMINAL BODY WEIGHTS AND ORGAN WEIGHTS FOR MALE RATS

Ethalfluralin % in Diet	Body (g) and S.E.	Mean and Standard Error		
		Thyroid mg	Adrenals mg	Prostate g
0.0	436 5	27.5 0.7	50.0 1.9	0.50 0.04
0.01	443 6	30.9[a] 1.3	59.1[a] 1.7	0.67[a] 0.03
0.025	444 4	29.2 0.7	52.5 1.4	0.55 0.04
0.075	435 5	27.9 1.2	54.3 1.2	0.63[a] 0.03

[a]Significantly different from the control, $P \leq 0.05$, two-tailed Dunnett's "t".

There was an isolated decrease in mean heart weight in females at the 250 ppm dose level; because of this isolated occurrence it is not considered compound related.

Relative adrenal weights are statistically greater than for controls in the 250 ppm dietary level females.

The relative adrenal weights are 26.33 mg in the females compared with 22.25 mg in control females. No statistically significant increase is present at the high dose levels.

The relative liver weight is significantly greater than controls in females on the high (750 ppm) dietary level, being 2.62 grams, as compared with 2.32 grams for the controls.

Gross and Microscopic Pathology:

There was no gross or microscopic pathology of any type found in males on the high dietary level (750 ppm). Pathology in the control males included a Leydig cell tumor in the left testis of one male; and in another male atrophic tubules in the left testis, skin abscesses, and unilateral purulent otitis media.

In the low dose (100 ppm) males, very mild glomerulonephritis was found in one male, scattered areas of atrophy of pancreas acinar cells in another male, some atrophic tubules in the right testis of another male (probably the result of edema), and a preputial gland adenoma in another male.

In the mid dose (250 ppm) males, a papilloma was found on the nose of one rat. And as previously stated, no gross or microscopic lesions were found in the high dose (750 ppm) males.

In the high dose females, one lobe of the thyroid was small in one rat (probably congential and of no toxicologic significance). In another high dose female, a small mass was noted in one uterine horn on gross necropsy, but was not found on the slide, tissue block, or wet tissue.

In the mid dose (250 ppm) females, a 0.1 cm adenoma was found in 1 rat; a uterine polyp and abscess in another rat; an endometrium of pseudopregnancy in another rat; and mild purulent endometritis in another rat.

In the low dose (100 ppm) females, focal bronchiectasis was found in a left lung lobe of 1 rat; pyometra in the uterus of another; and a 0.1 cm pituitary adenoma in another.

In the control females a uterine abscess was found in one rat; urolithiasis, and moderate urinary bladder epithelial hyperplasia in another rat; mild glomerulonephritis in two rats; uterine polyps in two more rats; and a 0.1 cm pituitary adenoma in another rat.

In summary, there was no gross or microscopic pathology that could be related to treatment with ethalfluralin.

Conclusions:

1) Nitrosamines of ethalfluralin were absent, or were present near the limits of detection in the chemical used in these tests.

2) Ethalfluralin had no adverse effect on survival. Survival was 100% for all groups.

3) Mean weight gain was statistically less in females at the 750 ppm dose level.

4) Feed consumption and feed efficiency were unaffected by treatment.

5) There were no physical signs of toxicity which could be attributed to the chemical.

6) Statistically significant blood chemistry effects include increased blood glucose in males, decreased alkaline phosphatase in females, and decreased total bilirubin in males, all at the high (750 ppm) dietary level. Also, creatinine and BUN were increased significantly in females on the 750 ppm and 250 ppm levels.

7) Treatment produced an apparent increase in relative liver weights in females on the high (750 ppm) dietary level. Increases in adrenal, prostate, and thyroid weights were noted. However, these appear to be not dose-related effects. This also appears to be the case with the single decreased mean heart weight of females on the 250 ppm dose level.

8) Blood was taken only at the termination of the study; interim hematology and blood chemistry values were not determined. Therefore, the blood chemistry values from this one-year rat feeding study will be inspected with the values from the 2-year feeding study.

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

9) The NOEL is 100 ppm based upon significant creatinine and BUN effects at 250 ppm. The data are equivalent to Core-Minimum for a subchronic rat feeding study. (25 rats/sex/dose are needed for a chronic study).

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: MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

Ethalfluralin, chemically is N-ethyl-N(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl)-benzenamide. The lot used was B30-Y64-35B, and [redacted] to reduce the amount of nitrosamine contaminant, N-ethyl-2-methyl-N-nitroso-2-propene-1-amine. After this treatment, 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 0.05 ppm initially, with a maximum value of 0.07 ppm in the periodic assays.

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 dur 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 16 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 ± 3°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 analyse 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. The initial assay for N-Ethyl-2-Methyl-N-Nitroso-2-Propene-1-Amine gave an assay value of 0.05 ppm. In 5 subsequent assays conducted between 7/19/77 and 6/13/78 no nitrosamine was detected at assay sensitivities as low as 0.01 ppm. The last 2 assays 4 and 11 months later demonstrated assay values of 0.06 and 0.07 ppm.

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 adminstration. 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 R257 1-Year	MALES R267 2-Year	MALES R277 2-Year	FEMALES R-257 1-Year	FEMALES R267 2-Year	FEMALES 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 R257 1-Year	MALES R267 2-Year	MALES R277 2-Year	FEMALES R257 1-Year	FEMALES R267 2-Year	FEMALES 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

[a]Mean 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 R257 1-Year	MALES R267 2-Year	MALES R277 2-Year	FEMALES R-257 1-Year	FEMALES R267 2-Year	FEMALES 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 R257 1-Year	MALES R267 2-Year	MALES R277 2-Year	FEMALES R257 1-Year	FEMALES R267 2-Year	FEMALES 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 R257 1-Year	MALES R267 2-Year	MALES R277 2-Year	FEMALES R-257 1-Year	FEMALES R267 2-Year	FEMALES 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:

Control		Low Dose		Mid Dose		High Dose		Replicate
Male	Female	Male	Female	Male	Female	Male	Female	Number
0/30	6/30	0/30	4/30	0/30	12/30	1/30	15/30	267
0/30	3/30	1/30	6/30	0/30	9/30	0/30	13/30	277
TOTAL	9/60		10/60		21/60		28/60	
	15%		16.7%		35%		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	[illegible]	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	30	4	13.3%
	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.)

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.

A One-Year Dietary Toxicity Study with Ethalfluralin (Compound 94961) in the B_6C3F_1 Mouse. Study M-9157. Conducted by Elizabeth R. Adams, M.S. (Project Leader) et. al., Lilly Research Laboratories; Greenfield, Indiana. April, 1981. Accession#070679.

Ethalfluralin (Compound 94961, EL-161) was administered to B_6C3F_1 mice in the diet for 1 year to evaluate its chronic toxicity.

The 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. Fifteen animals/sex/dose were used.

Ethalfluralin is N-ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl)-benzenamine. The lot used was B30-Y64-35B, and [redacted] to reduce the amount of nitrosamine contaminant. The purity of the lot was 94.5 percent. The nitrosamine content after "cleansing" was 0.05 ppm initially, with none detected subsequently in the periodic assays. Mash diet containing the 3 ethalfluralin concentrations was pelleted every 2-months.

The mice were supplied by Charles River Breeding Laboratories, Inc.; Wilmington, Massachusetts. Mice were held for 2-weeks housed 3 or 4/sex/cage to permit adaptation to the caging, housing, and watering systems of the laboratory. Mice which were significantly different in weight from the general population, or which were in poor physical condition were not used. 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 weighed 22.8 $\pm$ 0.2 g and the females 19.9 $\pm$ 0.2 g.

The test mice were housed 3 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°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 1-year treatment period all 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 (MVC), 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

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

These histologic preparations were examined by a certified veterinary pathologist.

Statistical analysis of differences ($p \leq 0.05$) between control and treated group means for parameters which are generally distributed normally was by Dunnett's method.

Results:

The mean assay percent of theory of all the pellets assayed were: low-level, 103.4%; mid-dose level, 91.7%; and high dose level, 78.7%. Since these are mean values, some of the individual assays fall above these 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 in the final batches of the study, and gave values of 77% and 75% of theory, respectively. Assays for other pelleting batches near or exceed 100% for the low and mid dose levels. Since a single pelleting batch would not be fed for a period exceeding 2 months, the effect of feeding pellets of lower ethalfluralin content would be minimized.

However, it appears from the available data that the high dose group received approximately 80% of the intended dose.

Survival

Feeding ethalfluralin had no apparent effect on numbers of mice surviving for one year. One control, two low-dose, and three high-dose mice died during the study; none due to treatment.

Toxic Signs

No clinical signs of toxicity were seen. Urine of treated mice was yellow-orange colored, particularly at higher dose levels, on a dose related basis. Analysis demonstrated that the coloration was due to ethalfluralin metabolites; ethalfluralin per se was not present.

Weight Gain

Mean body weights and mean weight gains did not differ significantly among the various treatment groups.

Ethalfluralin Intake

The time-weighted average ethalfluralin intake over the year study for males was 11.6, 47.0, and 173.4 mg/kg/day; for females it was 11.5, 48.8, and 183.9 mg/kg/day for the low, mid, and high dietary levels, respectively; based on estimated food intake values.

There calculations also did not consider the reduced ethalfluralin levels of technical material (~ 80% of theoretical) present at the high dose level. Correcting for assay values, at the high dose level, the time-weighted average intake would be 138.7 mg/kg/day for males and 147.1 mg/kg/day for females. Analyses demonstrated that ethalfluralin in the low and mid dose levels was consistent with the theoretical intended concentrations.

Hematology

There are no statistically significant differences in hematology to be seen in males on the various dose levels. In females, the mean red blood cell count of mice on the high dose level was statistically lower than controls.

Blood Chemistry

Alkaline phosphatase values were statistically higher than controls in males at the mid (400 ppm) and high (1500 ppm) dietary levels ($p \leq 0.05$, Dunnetts' "t" test). Mean values were 58.6 mU/ml for controls, 76.3 mU/ml for the mid dose level, and 84.0 mU/ml for the high dose level. (Alkaline phosphatase values for treated females were between 116.7 and 140.7 mU/ml, female control values being 120.1 mU/ml).

Mean SGPT values were statistically higher (56.3 mU/ml) for the high dose males than controls (30.1 mU/ml).

In females, BUN and creatinine values were significantly lower in mice on the high dietary level.

Body and Organ Weights

Mean relative liver weights were slightly, but significantly greater than controls in both males and females on the high dietary level. These were the only differences seen in relative organ weights.

Pathology

There was no pathology which could be considered to be treatment related.

Conclusions:

1. - Alkaline phosphatase values were increased in males at the mid and high dose levels (400 ppm and 1500 ppm).

2. - SGPT values were increased in the high dose males.

3. - BUN and creatinine values were decreased in high dose females.

4. - Relative liver weights were increased in both males and females on the high dietary level.

5. - Clinical chemistry & hematology were determined only at 1 year. No urinalysis determinations were performed.

- Based on the above, the NOEL is 100 ppm.

- The data are classified CORE minimum for a subchronic mouse feeding study. (25 animals/sex/dose should be used in a chronic study.)

A Two-Year Dietary Evaluation of Ethalfluralin in the B_6C3F_1 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 B_6C3F_1 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 120 mice on test. The dietary concentrations of ethalfluralin and the resultant estimated time-weighted average exposures (average of males and females), were as follows:

Ethalfluralin, % in diet	Ethalfluralin; mg/kg/day (time-weighed average)
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, and [redacted] to reduce the amount of nitrosamine contaminant[1]. The purity of the lot was 94.5 percent. The nitrosamine content after "cleansing" was 0.05 ppm initially, with a maximum of 0.07 ppm in the perodic assays. Mash diet containing the 3 ethalfluralin concentrations was pelleted every 2-months.

[1]N-ethyl-2-methyl-N-nitroso-2-propene-1-amine; ethyl-methylallyl-nitrosamine; EMANA.

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 $\pm$ 0.1 g and the females 20.3 $\pm$ 0.1 g; the males of M-9177 weighed 23.6 $\pm$ 0.1 g and the females 19.7 $\pm$ 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°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-t-ring 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 We_ghts

We_e 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.

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.

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. (32.9% vs 36.35% for HCT; 12.44 g vs 13.84 g for HGB; 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%).

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.

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*	116.9*		140.7	242.8	321.4*
0.225%	77.7				97.9*			
0.400%	104.3*				95.6			
0.800%	148.5*				108.1*			

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

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.
$p \leq 0.05$.

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.
$p \leq 0.05$.

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	[illegible].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.
$p \leq 0.05$.

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).

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.

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.

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.

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%

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.

002251

Guinea Pig Sensitization Studies of Ethalfluralin, Studies G-9530 and G-00370. Conducted by Elizabeth R. Adams, C.L. Pierson, et. al.; Lilly Research Laboratories. September, 1981.

Study G-9530 was conducted according to the modified Buehler topical patch method. (This also is the method suggested in the Core Guidelines.)

Material: Ethalfuralin technical chemical, 94.5% purity, Lot B30-Y64-35B.

Test Animals:

Female guinea pigs, Hartley strain, obtained from Murphy Breeding Laboratories, Plainfield, Indiana. After 7 weeks acclimation 45 physically acceptable animals were assigned to test and control groups. When placed on test, the animals were 10 to 14 weeks old and weighed 354 ± 3 g.

Housing:

Guinea pigs were housed in groups of 5 in stainless steel cages having a floor area of 3097 cm^2 and a height of 17.8 cm, suspended over stainless steel traps which were flushed with water. They were kept in rooms at a temperature of 21° ± 3°C with a relative humidity of 45%. An automatic timer provided a 12 hour light/dark cycle. They were fed Wayne Guinea Pig Diet.

Dose Levels:

I Induction and Challenge: 70% ethanol in water

II Challenge Control: 70% ethanol in water

III Induction and Challenge: 0.1% dinitrochlorobenzene (DNCB) in 70% ethanol

IV Challenge Control: 0.1% DNCB in 70% ethanol

V Induction and Challenge: 5% ethalfluralin in 95% ethanol

VI Challenge Control: 5% ethalfluralin in 95% ethanol

There were 10 animals in each group receiving induction and challenge; 5 animals in each challenge control group.

The 5% dilution of ethalfluralin in 95% ethanol was based on pilot studies designed to determine the minimal erythema dose. The 5% dilution was shown to be non-irritating in guinea pigs.

As a positive control, dinitrochlorobenzene (DNCB) was administered at a concentration of 0.1% w/v in a 70% ethanol in water solution.

Administration:

The study was conducted in 2 phases: induction and challenge. Induction was conducted 3 times a week for 2 consecutive weeks in Groups I, III, and V. The guinea pigs were clipped of hair in the nuchal area and the exposed area was swabbed with acetone. 0.2 ml of the test solution was applied to the nuchal area of each pig. The application site was occluded with a 1 1/2 inch square patch held in place with adhesive tape wrapped around the torso of the animal. The bandage was removed after 6 hours.

Guinea pigs assigned to a control group (Groups II, IV, and VI) were left untreated during the induction period.

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

Observations:

Twenty-four, 48, and 72 hours after the challenge application, treated areas were graded for dermal response.

Body Weights: Recorded weekly during the test.

Results:

There was light erythema at 24 hours in 1 of the 10 guinea pigs receiving 5% induction with ethalfluralin. No reactions were seen in any of the guinea pigs of this group at the 48- or 72-hour observations. Guinea pigs not receiving ethalfluralin induction did not respond to challenge.

All guinea pigs receiving an induction dose of DNCB reacted to challenge with DNCB, the reactions of erythema and edema persisting beyond the 72-hour observation period.

None of the vehicle control guinea pigs receiving induction doses of 70% ethanol in water responded to a challenge dose of 70% ethanol in water. All guinea pigs gained weight during the study.

Conclusions:

1) Ethalfluralin is non-sensitizing to guinea pigs when tested by a modified Buehler topical patch method.

2) Data meet Core-Minimum standards.

Study G-00379 was conducted according to the Guinea Pig Maximization Test of Magnusson and Kligman. This is a test which is exquisitely sensitive and valuable for screening purposes, but which may not predict sensitization to humans.

Materials:

The ethalfluralin technical chemical was of 94.5% purity and was from Lot B30-Y64-35B. Benzocaine (ethyl aminobenzoate) Lot WCRN, obtained from Mallinckrodt Chemical Works, was used as a positive control.

Test Animals:

Female albino guinea pigs of the Hartley strain, obtained from Sweetwater Farm, Hillsboro, Indiana, were used for the study. After 2 weeks of acclimation, 42 physically acceptable animals were randomly assigned to four test groups. When placed on test, the animals were approximately 10 to 14 weeks old and weighed 361.2 $\pm$ 5.9 g.

Housing:

Guinea pigs were housed in groups of 5 in stainless steel cages having a floor area of 3097 cm^2 and a height of 17.8 cm, suspended over stainless steel trays which were flushed with water. They were kept in rooms at a temperature of 21° $\pm$ 3°C with a relative humidity of 45%. An automatic timer provided a 12-hour light/dark cycle. They were fed Wayne Guinea Pig Diet.

Dose Groups:

I Induction and Challenge: benzocaine

II Challenge Control: benzocaine

III Induction and Challenge: ethalfluralin

IV Challenge Control: ethalfluralin

There were 15 animals in each group receiving induction and challenge. Each challenge control group contained six animals.

Induction Procedure:

Took place in 2 phases. First, 3 paired intradermal injections were given in the clipped nuchal region of the back as follows:

1) 0.1 ml of Freund's complete adjuvant diluted 1:1 with sterile distilled water.

2) 0.1 ml of a solution containing 5% ethalfluralin in acetone.

3) 0.1 ml of an emulsion containing 5% ethalfluralin in a vehicle of Freund's adjuvant diluted 1:1 with water.

The second phase of induction took place one week after the injections. The nuchal region was again clipped and 0.2 ml of petrolatum containing 25% ethalfluralin was applied to an area measuring 2 x 4 cm. The treated site was covered with a 2 x 4 cm occlusive patch which was secured with zonas adhesive tape wound around the torso of the animal. The dressing was left in place 48 hours. The six challenge control animals were subjected to the same induction procedure without ethalfluralin.

The 15 animals in the benzocaine group received phase one induction similar to the above with a benzocaine concentration of 5%. The topical treatment in phase two was 25% benzocaine in petrolatum. The six control guinea pigs received phase one and phase two induction without benzocaine.

Challenge Procedure:

The 15 guinea pigs subjected to induction with ethalfluralin and the 6 control animals were challenged topically 2 weeks after the second induction with 0.2 ml of a petrolatum ointment containing 25% ethalfluralin. A previously untreated area on the flank of each animal was selected as the site of application and was prepared for treatment by removal of the hair. Application sites were occluded for 24 hours.

The benzocaine-treated and control animals were similarly challenged with benzocaine at a 25% concentration in petrolatum.

Observations:

Twenty-four, 48, and 72 hours after the challenge application, treated areas were observed and graded.

Body Weights: Recorded weekly during the test.

Results:

Animals receiving induction and challenge with ethalfluralin demonstrated moderate erythema and slight edema at 24 hours, persisting for 72 hours (to a lesser degree in many instances). Guinea pigs receiving ethalfluralin challenge without the induction dose showed slight erythema.

The animals receiving induction and challenge with benzocaine showed slight to moderate erythema and slight edema. A couple of the animals receiving benzocaine challenge without an induction dose showed a slight transient erythema at the application site.

Body Weights: Animals gained weight during the study.

Mortality:

There were 5 deaths, none attributed to treatment. (4 in benzocaine group and 1 in the ethalfluralin group).

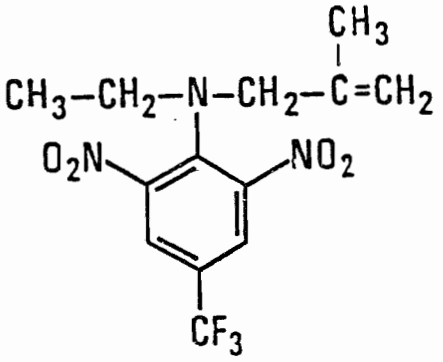
Conclusions:

1) Under the conditions of the Magnusson and Kligman Guinea Pig Maximization Test, ethalfluralin is positive for sensitization in the guinea pig by combined intradermal and topical routes.

2) The study is not a standard or required test for registration of a pesticide, but it is a graded Core-Minimum. In view of the positive finding in this test the labeling should include dermal sensitization precautionary statements.

Metabolism and Disposition of Ethalfluralin Male Wistar Rats. Unnumbered study conducted by Elizabeth R. Adams, G.K. Hanasono, et. al.. Lilly Research Laboratories; Greenfield, Indiana. December, 1981. Accession#070683.

Test Material: Ethalfluralin. N-ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4(trifluoromethyl)benzenamine. Lot 800-B35-011, 97.0% purity and Lot 721-109A-25-1, 98.8% purity were used. Also, uniformly ring-labeled ^{14}C-ethalfluralin was also used (Lot 553-B34-136, initial sp. act. 3.94 in Ci/mg and Lot 553-B97-294, initial sp. act. 8.36 u Ci/mg). Purity of the radiolabeled lots was in excess of 99.6%.



ETHALFLURALIN
MW 333

Excretion and Tissue Distribution: Male Wistar rats (Harlan Industries, Inc., Cumberland, Indiana) in the weight range of 220 to 320 g were fasted overnight prior to receiving single 100 mg/kg doses of ^{14}C-ethalfluralin (specific activity, 0.25 to 0.50 uCi/mg) by gastric intubation. The labeled compound was suspended in a 10 percent (w/v) aqueous solution of acacia to a final concentration of about 10 mg/ml. The animals were placed in individual stainless steel metabolism cages designed to permit the separate collection of urine and feces. Urine and fecal samples were taken at various times during a 7-day period after dosing.

Food (Lilly Mill Diet) was provided for consumption ad libitum throughout the collection period. Aliquots of urine, and cage rinse (methanol) were each added to 15 ml of a scintillation solution, and the radioactivity was quantified by liquid scintillation spectrometry. The radioactivity excreted in feces was measured by combustion techniques. The fecal excretion of radiocarbon was corrected for the recovery of radioactivity (93.9 percent) by the combustion procedure. The counting efficiencies for urinary and for fecal radioactivity were determined by the channels ratio method and the internal standards method, respectively.

Radiocarbon Tissue Distribution and Plasma Concentrations: Male rats were fasted overnight and given single 100 mg/kg doses (p.o.) of [^{14}C]-ethalfluralin (sp. act. 0.125 uCi/mg). The animals (4 per group) were sacrificed at 0.5, 4, 8, 16, 24, and 36 hours, and the concentrations of radioactivity in plasma, brain, peripheral fat, kidney, and liver were measured by solubilizing the tissue samples (in duplicate) in 3 ml of Soluene-350 (Packard Instrument Co. Inc., Downers Grove, Illinois). The samples were counted in glass counting vials which contained 15 ml of a scintillation solution (Dimelume-30, Packard Instrument Co., Inc.). Counting efficiency was determined by the method of internal standards.

In separate experiment, male rats were given single oral doses of [^{14}C]-ethalfluralin (100 mg/kg) as described above and sacrificed at various times during a 4-hour period (4 rats per time point) to measure plasma levels of radioactivity and the parent compound. Duplicate 0.2-ml aliquots of plasma were each added to counting vials containing 15 ml of a scintillation solution (Insta-Gel, Packard Instruments Co., Inc.) and counted. Counting efficiency was determined by the channels-ratio method. Plasma concentrations of ethalfluralin were quantified by a gas-liquid chromatography procedure. Duplicate one-ml aliquots of plasma were each placed in glass test tubes followed by the successive additions of an aliquot of the internal standards, i.e., N-propyl-N-(2-methypropyl)-2,6-dinitro-4-(trifluoromethyl) benzenamide, and 1 ml of a pH 7.5, 0.5 M potassium phosphate buffer. Each sample was extracted with two successive 5-ml volumes of distilled ether. The ether extracts were combined and evaporated to dryness in vacuo in silanized glass tubes at room temperature. The residue was redissolved in 50 ul of acetonitrile. The samples were eluted on a 6-foot silanized glass column (1/4 in. OD x 2 mm i.d.) packed with 1 percent Carbowax-20 m coated on

a chromosorb W (HP), 80/100 mesh support (Supelco, Inc., Bellefonte, Pennsylvania). Hewlett Packard Model 7610A gas chromatograph equipped with a ^{63}Ni electron capture detector (ECD) was used. Standard curves were obtained from control plasma containing known concentrations of ethalfluralin (10 to 100 ng/ml) plus the internal standard. The standard curve for peak area ratios (ethalfluralin:IS) vs. plasma concentrations of ethalfluralin were linear in the concentration range examined.

Analyses of Glucuronide-Conjugated and Non-Conjugated Metabolites in the Urine: Several parameters were measured to characterize the radiolabeled compounds excreted in the urine. The "free" metabolite fraction served as an estimate of the non-conjugated fraction of ethalfluralin metabolites.

A second parameter, the glucuronide fraction, was an estimate of the fraction of ethalfuralin metabolites present in urine as conjugates of glucuronic acid.

The net percent of ether-extractable urinary radioactivity, i.e., corrected for the percent of radioactivity due to the "free" metabolite fraction, was then determined and used to estimate the percent of non-enzymatically hydrolyzed conjugates.

The presence of beta-glucuronidase activity in urine from ethalfuralin-treated rats (100 mg/kg) was measured using a modification of the methods described by Fishman. Phenolphthalein glucuronide was used as the substrate. This procedure was also used to determine if urine from treated rats contained inhibitors of ketodase hydrolysis.

Biliary Excretion: Male rats were fasted overnight prior to the test in this and subsequent experiments described below. The animals were anesthetized with ether and prepared surgically with a common bile duct cannula (PE-50 polyethylene tubing). The cannula was brought out through the sutured abdominal wound, passed forward subcutaneously, and exteriorized through the skin at the back of the neck. The external portion of the cannula was sheathed in a 9-inch length of a flexible coil spring attached at

one end to the skin on the animal's neck. The other end of this spring was secured to the cage top to serve as a tether allowing the animal sufficient mobility for access to food and water. Following recovery from ether-induced anesthesia, the rats were given single oral 100 mg/kg doses of [^{14}C]-ethalfluralin, and bile samples were collected at various times during a 48-hour peri d to quantify the excretion of radioactivity.

Enterohepatic Circulation (EHC): A group of animals (donor rats) prepared with bile fistulas (see above) were dosed orally with 100 mg/kg of [^{14}C]-ethalfluralin, and bile was collected for 24 hours in glass vessels chilled in dry ice. A second group (N=4) of animals (recipient rats) were prepared with bile duct cannulas; in addition, each animal in this group was also prepared with a second cannula (PE-50 tubing) which was inserted caudad into the common bile duct until the tip entered the duodenum. The intraduodenal cannula was exteriorized with the bile duct cannula (see procedure described above).

Approximately 6 to 10 ml of bile containing the labeled products excreted by the donor rats was infused into the duodenum of the conscious recipient rats during a 4-hour period. Subsequently, bile obtained from non-treated animals was infused intraduodenally to replace bile lost through the fistula. The recipient rats were placed in stainless steel metabolism cages to collect bile, urine, and feces. The animals were given access to food 6 hours after the start of the infusion. Bile from the recipient rats were collected at 1, 2, 3, 4, 6, 9, 12, 15, 18, 21, and 24 hours to quantify the excretion of radiocarbon and thereby assess the extent to which EHC occurred. At the end of the experiment the animals were sacrificed and the intestinal tract from the duodenum caudad was removed and flushed with water to quantify the content of radioactivity in the gut lumen. The radiocarbon excreted in the urine and feces was measured.

Radiocarbon Tissue Distribution in Bile Fistula Rats: In other experiments the tissue distribution of radioactivity was compared among control (intact) rats, sham-operated rats, and bile duct-cannulated rats. The sham-operated rats were anesthetized with ether and were subjected to laparotomy only. Animals from each group were given single oral doses of [^{14}C]-ethalfluralin (sp. act. = 0.167 uCi/mg) equal to 100 mg/kg. Bile was collected from rats with fistulas. All animals (4 per group) were sacrificed 24 hours after dosing, a ıe radiocarbon levels in plasma, lung, kidney, fat. lung, and bile were quantified by the procedures described above. The statistical analyses of tissue distribution data were performed using orthogonal comparisons ($P \leq 0.05$). The urinary excretion data were analyzed statistically by the Mann-Whitney U test ($P \leq 0.05$).

Identification of Urinary and Biliary Metabolites: Urine excreted by rats during the first 24 hours after single 100-mg/kg oral doses of [^{14}C]-ethalfluralin was collected in glass tubes chilled in dry ice. The urine sample from each rat was acidified to pH 1 with 1N HCl and extracted twice with 20-ml aliquots of distilled ether. In some experiments, the urine samples were partially purified in a column containing 80 g of Amberlite XAD-2 resin (Rohm and Haas Co., Philadelphia, Pennsylvania) prior to acidification and extraction. Two bed volumes of water were passed through the XAD-2 colum before elution of the radiolabeled compounds with 2 bed volumes of methanol. The methanolic eluate was evaporated to dryness, and the residue was dissolved in ca. 5 ml of 0.1 N HCl and extracted with ether as described above.

The ether-extractable radioactive compounds were isolated by preparative thin-layer chromatographic procedures on 1000-u thick silica gel G plates (Quantum Industries, Fairfield, New Jersey). The solvent system consisted of a 9:1 mixture of ethylacetate and methanol. The urinary metabolites of ethalfluralin in each chromatographic zone were scraped and eluted with 0.1 N HCl and subsequently extracted into ether. Major urinary metabolite C was derivatized with diazomethane.

Each metabolite fraction was subsequently analyzed by combined gas chromatography-mass spectrometry (GCMS).

Ethalfluralin metabolites (urinary and biliary) were also analyzed by a Varian-MATS Model 731 double-focusing, high resolution mass spectrometer.

The fraction of total biliary metabolites conjugated with glucuronic acid was also quantified by thin-layer radiochromatographic procedures.

Results:

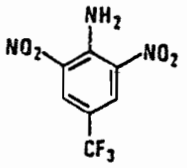
Eighty-eight to 95% of the ^{14}C was excreted via the urine and feces within 7 days; most of it within the first day. About 1/4 was excreted in the urine, and about 3/4 in the feces.

Urinary Metabolites:

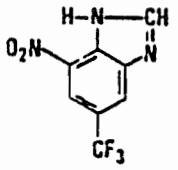
The uncojugated metabolites of the urine accounted for 31% of total urinary radioactivity. The glucuronide-conjugated metabolites accounted for 14% of urinary radioactivity.

Of the urinary radioactivity, ethalfluralin was not detected. Four urinary metabolites were detected: 2 each accounting for 7% of total urinary radioactivity, one 25 to 35%, and the fourth about 10%.

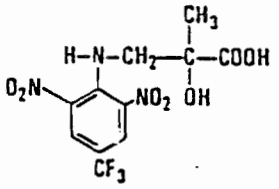
Urinary metabolite A is thought to be as follows: 002251



Urinary metabolite B is thought to be as follows:

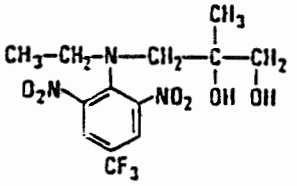


Urinary metabolite C is thought to be as follows:



Urinary metabolite D is still of unknown structure.

The biliary metabolite (glucuronide conjugate) is thought to be similar to the following:



Plasma and Tissue Concentrations:

Following a single oral dose of 100 mg/kg ^{14}C-ethalfluralin, plasma radioactivity rose rapidly within 30 minutes and remained high through the 4 hour observation period. Ethalfluralin plasma levels did not reach a peak until about 1 hour after dosing, remained at the peak for about one hour, declined during the third hour, and nearly disappeared by 3 1/2 hours. This indicates rapid metabolism of the ethalfluralin.

Tissue concentrations of radioactivity reached a peak in the liver at 1/2 hour, and again at about 8 hours. All other tissues examined showed radio-active peaks at about 8 hours. Radioactivity of the liver, fat, and kidney remained higher than plasma. Radioactivity of the lung and brain were below that of plasma.

Biliary excretion accounted for 41% of the radioactivity within 48 hours, as determined from rats with bile fistulas. All radioactivity was found in metabolites as conjugates of glucuronic acid, no parent compound being recovered.

Conclusions:

1. Ethalfluralin is rapidly and extensively metabolized following oral administration to rats.

2. Peak tissue concentrations of metabolites are highest in liver, fat, and kidney, as determined by radioactivity.

3. Major storage depot of ethalfluralin, which is fat soluble, is the body fat.

4. Ninety-five percent of ethalfluralin radioactivity was excreted via feces and urine by 7 days; 70% via feces.

5. Biliary metabolism, especially glucuronide conjugation, plays a major role in ethalfluralin metabolism.

6. The data are Core-Minimum.

Three-Week Subchronic Dermal Toxicity Study in Rabbits with Ethalfluralin Emulsifiable Concentrate Formulation. Study B-7400. By Elizabeth R. Adams, N.V. Owen, and C.L. Pierson. Lilly Research Laboratories. November 1981. Accession No. 070683.

Test Material:

An emulsifiable concentrate formulation containing 3 pounds per gallon of ethalfluralin technical chemical of 95.5% purity obtained from lot B30-Y64-35B. The formulation is composed of ethalfluralin technical chemical, 37.74% by weight; [redacted]

Test Animals: INERT INGREDIENT INFORMATION IS NOT INCLUDED

New Zealand White rabbits supplied by Dutchland Laboratories, Denver, Pa., were used for this study. When received, each animal was weighed and eartagged with a unique identification number. After two weeks of acclimation, each rabbit was examined for physical and ophthalmic abnormalities. Forty acceptable rabbits were randomly assigned to four test groups of five males and five females.

When placed on test, the animals were approximately 12 to 16 weeks old and weighed 2.98 $\pm$ 0.04 kg and 2.87 $\pm$ 0.04 kg for males and females, respectively.

Animal Housing and Care:

Rabbits were housed individually in stainless steel cages having a floor area of 0.28 m^2 and a height of 41 cm. Cages were suspended over stainless steel trays which were flushed with water every 32 minutes.

Rabbit rooms were maintaind at a temperature of 20 $\pm$ 3°C with a minimum relative humidity of 40 percent. An automatic timer controlled a 12 hour light/dark cycle.

The diet was Purina Certified Rabbit Chow, No. 5322.

Dose Levels:

Three concentrations of ethalfluralin 3 EC were prepared by dilution with water. The four dose groups were assigned as follows:

1) Water, 2 ml/kg

2) 0.5 percent ethalfluralin 3 EC, 2 ml/kg (equivalent to approximately 3.6 mg/kg ethalfluralin)

3) 1.0 percent ethalfluralin 3 EC, 2 ml/kg (equivalent to approximately 7.2 mg/kg ethalfluralin)

4) 5.0 percent ethalfluralin 3 EC, 2 ml/kg (equivalent to approximately 36 mg/kg ethalfluralin)

The dose levels were selected on the basis of one-week pilot dermal irritation study in rabbits. A concentration of 0.5 percent ethalfluralin 3 EC in water was expected to be a non-irritating dose level. A 2 ml/kg dose of 5.0 percent ethalfluralin 3 EC was considered the highest dose that could be administered to rabbits without eliciting a dermal response which would interfere with the evaluation of systemic toxicity. The dose volume of 2 ml/kg was the maximum that could be applied to the dorsal skin surface without runoff. This dose was sufficient to cover ca 10 percent total body surface area.

Administration:

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

To increase skin permeability, the treated areas of one-half the animals in each group were abraded once a week prior to treatment. The abrasions, administered with a stiff nylon brush, were sufficiently deep to penetrate the stratum corneum without causing bleeding.

Each dose was measured and applied with a syringe. A gauze pad was placed over the application site to hold the formulation in place.

After each application, the torsos of all rabbits were wrapped with an elastic bandage lined with an occlusive rubber dam. The occlusive dressings were removed after six hours and the application sites were rinsed with tap water and dried with a cloth towel.

Dermal treatments were performed five days a week for three consecutive weeks.

Observations:

All animals were observed daily for changes in behavior or appearance. Dermal irritation was graded daily using an eight point scale for erythema and edema.

Body Weight and Food Consumption:

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

Hematology:

Hematologic evaluations were conducted for each rabbit prior to study initiation and at termination of the study. Blood samples were drawn from the medial artery of the ear. Hematology determinations included hemoglobin (HGB); mean corpuscular volume (MCV); and erythrocyte (RBC); leukocyte (WBC), differential leukocyte counts, and erythrocyte morphology. Hematocrit (HCT), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were calculated from the above determinations.

Clinical Chemistry:

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

Pathology:

All animals were necropsied following death. The necropsy was an examination of the animal's general physical condition, body orifices, external and internal organs and tissues. The following organs and tissues were collected, placed in a fixature, and processed for histopathologic evaluation: kidney, liver, skin, and application site. The following organs were weighed and organ weight/body weight ratios were calculated: kidneys, heart, liver, ovaries, spleen, testes, thyroids, and adrenals. The gross and histopathologic examinations were performed by a board certified pathologist.

Statistics:

Hematology and clinical chemistry parameters were analyzed statistically using a two-tailed Dunnett's "t" test at each time point-pretreatment and terminal. Mean relative organ weights and body weight gains were also analyzed using a two tailed Dunnett's "t" test. All comparisons were made to the control group. The parameters for which statistically significant differences were found ($p \leq 0.05$) were examined further for a dose related trend for biologically abnormal values.

Results:

Mortality:

Six rabbits died on test: one water control, two on the 1% dose, and 3 on the 5% dose. Death was associated with severe acute diarrhea. Two other rabbits (one water control and one on the 0.5% dose) also developed diarrhea, but recovered.

Body Weight:

One control female and one male on the 5% dose level lost weight. The mean body weight gain of rabbits on the high dose level was statistically less than controls.

Food Consumption:

Was less for treated rabbits, especially the females, but not statistically significant nor dose related.

Dermal Irritation:

The high dose was irritating to the skin in males and females, producing slight hyperkeratosis and acanthosis at the application site in 8 rabbits. Figure 1 was to have charted irritation scores for all groups, but charts only the mean scores for the high dose groups. Report narrative states that there was no important difference in the dermal response of abraded and non-abraded skin.

Hematology:

There were no statistical differences related to treatment in any of the hematological parameters.

Clinical Chemistry:

The only difference from controls noted were decreased mean alkaline phosphatase values in treated females, particularly in those on the low dose. The decreases were statistically significant at the low and mid dose levels.

Other differences which occurred, but do not appear to be dose related or of toxicologic significance are initial low BUN and creatinine values in the mid dose males at week 0; high creatinine values in the low and high dose females in week 3 (the controls are extremely low).

Organ Weights:

Absolute mean adrenal weights of treated rabbits were less than controls for all treatment levels. Whether due to stress-induced adrenocortical depletion or to the high values for controls is not known. The same is true for absolute mean ovarian weights in the females.

Absolute mean thyroid weights were increased over controls in the mid dose males; not being dose-related or seen in the other treatment groups, this appears to be an aberrant finding.

The mean relative organ weights follow the trend shown in the absolute weights.

In looking at the absolute and relative organ weights of individual rabbits, we find a wide variation in weights in both treated and control animals, but some of the control animals have a larger proportion with heavier adrenals and ovaries, resulting in exceptionally heavy mean organ values. Control adrenal and ovary weights from two other studies are shown below. These show organ weights which are much less than for the controls in this study.

ORGAN WEIGHTS FOR CONTROL FEMALE RABBITS
IN STUDIES B-7021, B-7300, and B-7400

Organ Weight Per 100 Grams Body Weight

Study	Statistic	Body Weight (kg)	Adrenals (mg)	Ovaries (mg)
B-7021	Mean	3.280	9.07	8.61
	STD	0.507	1.45	2.66
	N	5	5	5
B-7300	Mean	3.280	8.93	7.54
	STD	0.581	1.44	1.20
	N	5	5	4
B-7400	Mean	3.450	9.93	15.36
	STD	0.289	1.93	4.34
	N	4	4	4

Additionally, ovarian weights vary greatly with the estrus cycle.

Pathology:

As stated previously, one control, 2 mid-dose, and 3 high-dose rabbits died with severe diarrhea. In addition 1 control and 1 low-dose rabbit developed diarrhea, but recovered. It is difficult to associate the diarrhea with ethalfluralin toxicity, per se.

The only pathology which could be associated with the formulation (not necessarily with ethalfluralin, per se) is the dermatitis, hyperkeratosis, and acanthosis at the application site in the high dose rabbits. This probably is a local and not a systemic effect.

Discussion:

One of the chief indicators of systemic toxicity one would expect to see due to absorption of topically applied ethalfluralin in a subchronic dermal toxicity test would be increased liver weights on a dose related basis. This was not seen in this study. The only sign of possible systemic toxicity was decreased weight gain in rabbits on the high dose level. This could have been due either to systemic toxicity of ethalfluralin (and "inerts" in the formulation), or it could be due to the stress of daily application of the irritant formulation - or the stress of the irritation.

Conclusions: INERT INGREDIENT INFORMATION IS NOT INCLUDED

1. The NOEL for topical irritation is 2 ml/kg of the 1% dilution of the formulation, or approximately 20 mg formulation per kg (equivalent to 7.2 mg/kg ethalfluralin). (Formulation contains 37.7% ethalfluralin, [redacted]

2. If lowered body weight gain was a systemic effect due to absorbed ethalfluralin formulation in this study, then the NOEL for systemic effect is 2 ml/kg of the 1% dilution of the formulation, or 20 mg formulation/kg (equivalent to 7.2 mg/kg ethalfluralin).

3. Dermal irritation scores for all groups should be furnished.

4. The data are core supplementary because there were only 5 animals per sex per group and dermal irritation scores were not provided.

A Teratology Study (I) with Ethalfluralin in the Dutch-belted Rabbit. Study B-7079. Conducted by Elizabeth R. Adams, et al., Lilly Research Laboratories, Greenfield, Indiana. October, 1980. Accession No. 070682.

Test Chemical: MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

Ethalfluralin. Compound 34961. N-ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl)-benzenamine. Technical chemical from Lot B30-Y64-35B. [redacted] to reduce contamination with the nitrosamine N-ethyl-2-methyl-N-nitroso-2-propene-1-amine (ethyl-methylallyl-nitrosamine, EMANA). After treatment the chemical was 94.5% pure. Dosage was not adjusted for purity.

Animals:

Virgin female Dutch-belted rabbits, 3 to 4 months old, were obtained from Johnson's Bunny Ranch, Wilkinson, Indiana, and were kept in the laboratory for several months prior to use. On arrival the rabbits were treated for 2 weeks with drinking water containing 0.085% sulfaquinoxaline for possible coccidiosis.

Dose:

Fifteen pregnant Dutch-belted rabbits per dose level were given daily oral doses of 0, 250, 500, or 750 mg/kg ethalfluralin in 10% aqueous acacia base by gavage on days 6 through 18 of gestation.

Housing:

The rabbits were caged individually in stainless steel units 47 x 53 x 34 cm suspended over neomycin-treated cardboard covered dropping trays. The cages were kept in a room, with similar animal studies, maintained at 20 ± 3°C with a relative humidity of 45%. A cycle of 12 hours light was maintained.

Identification:

Rabbits were identified by a 4 digit ear tag number combined with the study number.

Ration:

They were fed Purina Rabbit Chow HF (pellets containing 25% fiber).

Body Weights:

Were recorded initially (gestation day [illegible] 2.40 ± 0.05 kg, mean ± S.E.), on the first day of treatment [illegible]station day 6) and every third day there after (only data [illegible]estation days 0, 6, 12, 18, 21 and 27 are reported). Dosing was adjusted according to body weight.

Food Consumption:

Was measured daily and is reported as a mean for pre-treatment, treatment, and post-treatment periods.

Terminal Measurements:

Observation day 28 or after aborting, the rabbits were killed with I.V. barbiturate and examined for gross tissue changes and reproductive status. Rabbits that died were similarly examined. Ovaries were examined for number of corpora lutea and the uterus for the number and distribution of fetuses, resorptions, or empty implantation sites. Resorptions were categorized as early or late to indicate relative time of fetal death.

Individual progeny weights were recorded. Each fetus was examined for sex, viability, and external abnormalities. The progeny were killed and placed in a 1% NaCl solution. After 24 hours the viscera, including the brain, were examined and the carcasses were prepared for skeletal examinations.

Mean values of the reproduction parameters were determined for each group. In calculating the mean fetal weight, the number of litters was considered the number of independent sampling units. The following reproduction indices were reported:

Fertility - the proportion of females that were pregnant.

Gestation Survival - the proportion of fetuses that were alive at cesarian.

Resorption - the proportion of implanted conceptuses that resulted in resorption.

Implantation - the proportion of implantations to the number of corpora lutea.

Statistical Analysis:

Percentage change in weight during gestation, fetal weight data, and live litter size were analyzed by the method described by Dunnett. For resorption data, the percentages of resorptions occurring in each female, based on the number of implantations was determined. An arcsin square root transformation of the percentage of these data was conducted to normalize the distribution. Student's "t" statistic was then calculated using the transformed data. Due to the interrelationship between resorption occurrence and litter size, a Bonferroni-type argument was used, and the experimental error rate of 0.05 was apportioned between the two parameters.

Dosing:

The pregnant Dutch-Belted rabbits were given daily oral doses of 0, 250, 500, or 750 mg/kg ethalfluralin in 10% aqueous acacia base by gavage on days 6 through 18 of gestation. These doses were based on results of two pilot studies. In the pilot studies, doses of 10 to 250 mg/kg showed no signs of maternal toxicity. In these same pilot studies one of 3 on 500 mg/kg and all three receiving 1000 mg/kg aborted. Since it was thought the rabbit aborting on the 500 mg/kg dose could be a chance occurrence, 750 mg/kg was chosen as the high dose in order insure a maternally toxic dose level. 250 mg/kg was chosen as the low dose since, it was expected to be a NOEL for maternal toxicity.

Sixty female rabbits were selected and randomly assigned 15 to each control and treatment groups. The females were artificially inseminated.

Results:

Food Consumption:

Mean daily food consumption was markedly decreased during the treatment period and following, even in the controls. But the decrease was greater as the dose of chemical increased. Most of the rabbits at the 500 and 750 mg/kg levels became almost totally anorectic during and following the treatment period and as a result many of them died or aborted. Approximately one-half the rabbits on the 250 mg/kg dose level and one control rabbit also was so affected.

Mean Body Weights of Dams:

The controls had a mean weight loss of 0.3 ± 2.1% and the 250 mg/kg dose level had a mean weight loss of 3.7 ± 2.7% from day 0 to day 27 of gestation. These weight losses were not statistically significant. Statistically significant weight losses occurred in dams at the 500 and 750 mg/kg/day dose levels.

Dose (mg/kg/day)	No. Pregnant	No. not dying or aborting by Gestation day 28	Mean Live Fetuses	Mean Implantation	Mean C.L.
0	14	14	5.7 ± 0.6	6.2 ± 0.5	7.9 ± 0.5
250	13	9	5.3 ± 0.7	6.3 ± 0.8	7.8 ± 0.3
500	15	6	4.3 ± 1.1	6.0 ± 0.9	7.8 ± 0.7
750	13	2	0	5.0 ± 1.0	7.0 ± 0.0

Resorptions

Dose mg/kg/day	Mean Percent ± S.E	Early:Late Distribution	Females Resorbing	Resorptions per litter
0	10.3 ± 4.0	7:0	5/14	0.5
250	15.8 ± 5.7	8:1	4/9	1.0
500	32.5 ± 11.8	10:0	5/6	1.7
750	70.7 ± 9.3	10:0	2/2	5.0

Dose (mg/kg/day)	Fertility Index (Females Pregnant)		Proportion of Fetuses* Alive at Cesarean (Gestation Survival)		Proportion Resorbed Per Total Number Implantation Sites (Resorption Index) Resorbed	Implantation Sites	Implantation Index Implantation/C.L.
0	0.93	14/15	1.00	(80/80)	0.08	(7/87)	0.79 (87/110)
250	0.87	13/15	1.00	(48/48)	0.16	(9/57)	0.81 (57/70)
500	1.00	15/15	1.00	(26/26)	0.28	(10/36)	0.77 (36/47)
750	0.87	13/15	1.00	(0/0)	1.00	(10/10)	0.86 (6.7)

The 500 mg/kg and 750 mg/kg dose level dams had mean weight losses of 10.3 ± 3.2% and 18.4 ± 1.3% from day 0 to day 27 of gestation. These weight losses were significantly different from controls ($p = 0.05$), using Dunnett's two-tailed "t" test.

The accompanying charts demonstrate maternal and/or fetal toxicity at all dose levels, as follows:

1) Ethalfluralin treatment resulted in deaths or abortions of the females on a dose-related basis.

2) There were no live fetuses from dams on the 750 mg/kg dose level.

3) The percent of implantations which are resorbed increased in relation to dose level, 70.7 ± 9.3% of the fetuses in the high dose dams resorbing early in the gestation period. (This value includes rabbits that died or aborted, when implantation sites could be accounted for. Statistically significant at $p = 0.01$).

Mean fetal weights were 30.17 ± 1.47 g for controls; 31.75 ± 2.35 g for the 250 mg/kg dose level; and 28.95 ± 3.89 g for the 500 mg/kg/day dose level. These means are not statistically different. There were no live fetuses from the 750 mg/kg/day group.

Fetal Abnormalities:

No visceral abnormalities were seen in fetuses from either treated or control rabbits in this study. While historical control data were provided for external and skeletal abnormalities, the only historical control data given for visceral abnormalities were 15 cases of internal hydrocephalus in 5127 control rabbits.

In external abnormalities, 1 of 26 fetuses at the 500 mg dose level was dwarf-like; and of 26 fetuses from 2 of 6 litters (including the dwarf) had open eyelids and cleft palate. One control fetus (of 80) had varus limbs and a myelocele. Historical data provided in Table 9 of the study indicates an incidence of 1/4664 (.02%) for dwarf fetuses; 4/4664 (.085%) for open eyelids; and 8/4664 (.17%) for cleft palate.

In skeletal abnormalities, 13 ribs bilaterally were seen in 1/80 (1.3%) of controls; 2/48 (4.2%) at 250 mg/kg dose; and 3/26 (1.5%) at the 500 mg/kg dose. 13 ribs bilaterally were found in 565/4664 (12.1%) of cumulative control rabbit fetuses of the laboratory.

Thirteen ribs unilaterally were found in 2/80 (2.5%) of controls; and 1/26 (3.8%) at the 500 mg/kg dose. 13 ribs unilaterally were found in 366/4664 (7.8%) of cumulative control rabbit fetuses of the laboratory.

There were no fetuses to examine at the 750 mg/kg dose level.

Fused sternebrae and misaligned sternal bars occurred in 1 of 48 fetuses from the 250 mg/kg/day dose level.

Mortality:

Four rabbits, all on 750mg/kg/day, died during the study. The deaths occurred on days 21, 23, 25, and 26 of gestation. In addition, four, nine, and seven rabbits from the 250, 500, and 750 mg/kg dose levels aborted and were killed.

Toxic Signs:

For a matter of days preceding abortion blood was seen on the dropping trays. And the urine of the treated rabbits was a bright yellow (probably due to ethalfluralin metabolites), especially at the higher doses. There were no other physical signs of toxicity besides the death and abortions.

Conclusions:

1) Under the conditions of this test, no apparent teratogenic response was evident at any dose level.

However, 2/6 litters demonstrated unusual terata for this strain (open eyelids and cleft palate). These findings were not demonstrated at other data levels but due to the few litters available for examination no conclusions can be drawn from this study.

2) Maternal toxicity occurred at all dose levels:

a. Abortions occurred at all treatment levels on a dose-related basis.

b. Decreased food consumption was evident in treated rabbits; almost complete anorexia at upper two dose levels.

c. Significant weight losses occurred in dams on the high two dose levels.

d. Four high dose rabbits died.

3) Fetotoxicity occurred in the form of increased resorptions and decreased live litter sizes, particularly at mid- and high-dose levels.

4) A NOEL was not established in this study for maternal or fetotoxicity.

This study, by itself, is classified Core Supplementary since too few litters were available for examination.

A Teratology Study II with Ethalfluralin in the Dutch-Belted Rabbit. Study B-7160. Conducted by Elizabeth R. Adams, et al. Lilly Research Laboratories, Greenfield, Indiana. October, 1980. Accession No. 070682.

A similar study (see preceding study, Number B-7079), was conducted one year previous to this study. Dosing for study B-7079 was based on two pilot studies. The dose levels were 750, 500, and 250 mg/kg/day, with the expectation that 250 mg/kg/day would be the NOEL. However, maternal toxicity was observed at 250 mg/kg/day, and 750 mg/kg/day was excessively toxic to both the dams and the fetuses to the point that no live fetuses were obtained.

Therefore, this present study was conducted with treatment levels of 250 mg/kg/day and 75 mg/kg/day, utilizing 15 pregnant rabbits per dose level with the anticipation that 75 mg/kg will be the no-effect level.

The study protocol, test chemical, source of animals, housing, and identification were as for the previous study except that a different Ralston Purina Rabbit Chow (Certified Rabbit Chow 5-322) was fed.

Results:

Toxic Signs:

There were no physical signs of toxicity related to ethalfluralin.

Mortality:

Seven rabbits died during the test: 3 controls, 3 low dose (75 mg/kg), and 1 high dose (250 mg/kg). It is suspected that some of the deaths may have been related to trauma during daily intubation for dosing. And one control was anorectic for 14 days, possibly related to a hairball in the stomach.

Food Consumption:

Mean daily food consumption decreased and varied during the study, in controls as well as in treated rabbits. It does not appear that this decrease is related to ethalfluralin toxicity. The mere procedure of intubation may have had a role in suppressing food consumption in both treated and control rabbits. Hairballs were also present in treated and control rabbits.

Body Weights:

Body weights of individual rabbits appeared to fluctuate. Mean body weights of rabbits on the 250 mg/kg level appeared to decrease somewhat during the treatment period, and then partially recover after discontinuance of treatment. No statistically significant changes were in evidence, however.

Mortality and Abortions:

Controls: 3 aborted (days 20, 24, and 26). 3 died (days 14, 17, and 28)

75 mg/kg: 1 aborted (day 27). 3 died (days 10, 12 and 16)

250 mg/kg: 2 aborted (days 26 and 27). 1 died (day 10)

Necropsy findings in aborting rabbits:

Control: Rabbit 4782 (day 24): GI tract empty. 6 implantation sites, 9 c.l.

Rabbit 4890 (day 20); Acute URI. 4 live fetuses, 4 implantation sites, 7 c.l.

Rabbit 4956 (day 26); Acute URI. Hairballs; and GI tract empty. 11 live fetuses, 11 implantation sites, 11 c.l.

75 mg/kg: Rabbit 4954 (day 27): Hairball; and GI tract empty.

3 live fetuses, 1 dead fetus, 2 early resorptions, 6 implantation sites, 7 c.l.

250 mg/kg: Rabbit 4892 (day 26); Hairball; and GI tract empty.

7 live fetuses, 8 implantation sites, 8 c.l.

Rabbit 4974 (day 27): Hairball; and GI tract empty.

7 live fetuses, 7 implantation sites, 11 c.l.

Necropsy findings in dead fetuses.

Control: Rabbit 4962 (day 17): Pale and fatty liver. Pale and ecchymotic kidneys. Purulent pericarditis with septicemia. 9 implantation sites; 9 c.l.
Rabbit 4965 (day 14): Acute URI. 9 implantation sites; 8 c.l.

75 mg/kg: Rabbit 4891 (day 12): Hydrothorax; fibrinous pericarditis. 6 implantation sites.

Rabbit 4897 (day 16): Aspiration pneumonia; hydrothorax. 7 implantation sites; 7 c.l.

Rabbit 4976 (day 10): Collapsed red lungs; diarrhea; 8 implantation sites; 8 c.l.

250 mg/kg: Rabbit 4958 (day 10): Collapsed red lungs. Fibrin and purulent exudate in thorax. Diarrhea.

The above abortion and mortality data do not indicate any mechanism by which ethalfluralin may have caused abortion or mortali

Reproduction Values:

There were essentially no differences in mean numbers of live fetuses, implantations, or corpora lutea between control and treated groups. The data also fail to demonstrate any dose-related effects of the chemical on resorptions.

Fetal Values:

Male/Female Distribution:

There were 60% male fetuses with the control group, 48% males in the 75 mg/kg group, and 54% males in the 250 mg/kg group.

Mean fetal weights were 30.01 ± 1.69 g for controls, 25.51 ± 1.67 g for the 75 mg/kg group, and 26.47 ± 2.16 g for the 250 mg/kg group.

These fetal weights are 11.8 - 15% less for treated groups, but are not dose related or statistically significant.

Gross Abnormalities:

No external abnormalities were observed in 56 fetuses from 9 litters at the 75 mg/kg dose level. One of 45 control fetuses (from 8 litters with live fetuses) had a cleft palate. At the 250 mg/kg dose level, fetuses of 3 litters were affected: 4/63 were dwarf-like, 6/63 had cleft palates, 3/63 were edematous, 6/63 had open eyelids, 3/63 had varus hindlimbs, and 2/63 brachygnathia.

At the 250 mg/kg dose level, 6 fetuses from 3 litters had cleft palate (out of a total of 63 fetuses examined). Six fetuses out of two litters had open eyelids.

In one of these litters the following additional findings were noted:

4 fetuses were found to be dwarf-like;
3 were edematose, 3 had varus hindlimbs, and two had brachygnathia. Historical incidence for these terata are:

Open eyelids	4/5127	(0.08%)
Dwarf-like	1/5127	(0.02%
Edema	9/5127	(0.18%)
Varus limbs	10/5127	(0.2%)
Brachygnathia	0	

The above historical data were not submitted on a per litter basis. Therefore, comparisons of these findings to this study on a per litter basis was not possible.

Based on the total number of fetuses at this dose level, the incidence of cleft palate for 6 of a total of 63 fetuses give a 9.5% incidence. Others are:

dwarf-like	4/63	(6.3%)
edema	3/63	(4.8%)
Open eyelids	6/63	(9.5%)
varus limbs	3/63	(4.8%)
brachygnathia	2/63	(3.2%)

As evident in the above analysis cleft palate and open eyelids are present in multiple litters and therefore is of greatest concern. Certainly the incidences of these findings at the 250 mg dose level, i.e. - 3/12 litters for cleft palate (9.5% of fetuses) and 2/12 litters for open eyes (9.5% of fetuses) is not comparable to historical data even if litter #4964 were excluded.

The only visceral abnormality observed was internal hydrocephalus occurring in 1/56 fetuses at the 75 mg/kg dose level.

The skeletal abnormality occurring in greatest incidence are 13 ribs bilaterally, occurring in 23% of the fetuses at the 250 mg/kg dose level, and 11% in controls and at the 75 mg/kg dose level. The laboratory states this abnormality varies greatly in incidence in controls from one study to another, averaging 12%.

Abnormalities detailed by litter and dose are as follows:

Surviving Females	Litter#	Fetuses in Litter	Fetal Abnormalities
Vehicle Control			
8 litters	4884	7	Normal
	4896	5	1/5 - 13 ribs bilaterally
	4953	7	1/7 - 13 ribs bilaterally
	4950	4	Normal
	4959	5	1/5 - cleft palate, bipartite sternebrae, fused vertebrae, 13 ribs bilaterally
			1/5 - 13 ribs bilaterally
	4969	7	Normal
	4972	4	Normal
	4975	6	1/6 - 13 ribs bilaterally
75 mg/kg			
9 litters	4888	5	1/5 - 13 ribs left side
	4894	4	
	4951	5	2/5 - 13 ribs bilaterally
			1/5 - 13 ribs left side
			1/5 - missing rib
	4957	6	Normal
	4963	8	Normal
	4967	9	3/9 - 13 ribs bilaterally
			1/9 - Internal hydrocephalus
	4970	7	Normal
	4882	4	1/4 misaligned sternal bars
	4885	8	1/8 - 13 ribs bilaterally
			1/8 - 13 ribs right side
			1/8 - 13 ribs left side

Surviving Females	Litter #	Fetuses in Litter	Fetal Abnormalities
250 mg/kg			
12 litters	4883	5	1/5 - 13 ribs bilaterally
			1/5 - 13 ribs left side
	4886	1	1/1 - cleft palate; crooked ribs bilaterally
	4889	5	1/5 - bipartite sternebrae; fused sternebrae
			1/5 - 13 ribs right side
	4895	8	1/8 - 13 ribs bilaterally
	4898	3	1/3 - 13 ribs bilaterally
	4852	7	5/7 - 13 ribs bilaterally
			1/7 - 13 ribs right side
	4955	8	4/8 - 13 ribs bilaterally
	4961	5	1/5 - Open eyelids; fused sternebrae
			1/5 - Open eyelids; cleft palate
	4964	4	2/4 - Cleft palate, open eyelids, edema, brachygnathia, varus hindlimbs, crooked ribs bilaterally, fused sternebrae, shortened thickened ribs bilaterally, 13 ribs bilaterally, dwarf-like, all limb bones shortened, incomplete development of metacarpals, metatarsals, and phalanges.

Surviving Females	Litter #	Fetuses in Litter	Fetal Abnormalities
	4964 (continued)	4	1/4 - Cleft palate; open eyelids; varus hindlimbs; crooked ribs bilaterally; dwarf-like; all limb bones shortened; incomplete development of metacarpals, metatarsals, and phalanges. Fused sternebrae.
			1/4 - Cleft palate; open eyelids; edema; fused sternebrae; crooked ribs bilaterally; shortened thickened ribs bilaterally; dwarf-like; all limb bones shortened; incomplete development of metacarpals, metatarsals, and phalanges.
	4968	2	Normal
	4971	8	1/8 - Fused sternebrae
	4980	-	2/7 - 13 ribs bilaterally
			2/7 - 13 ribs right side
			1/7 - 13 ribs left side

We have an increased incidence of terata on a per litter and per fetus basis at the 250 mg dose level. It is noted, however, at the 75 mg dose level, although no incidences of these same terata occurred, only 9 litters were available for examination. Hence, the teratogenic potential at this level cannot be properly evaluated.

Conclusions:

1) Due to the extremely adverse effects and trauma which appear to be due at least in part to the dosing procedure, it is recommended that the study be repeated and an attempt be made to eliminate complications from the mechanical dosing procedure. It is apparent from the available data that an inadequate number of litters were available for examination, especially at the 75 mg/kg dose level.

2) Significant maternal toxicity was not evident at the 250 mg/kg/day dose level.

It is apparent at the 250 mg dose level that there is a positive teratogenic response. However, due to the high mortality, abortion rate, and numbers of non-pregnant animals utilized in this study and the resultant reduced number of litters available for examination, a meaningful assessment is not possible. Furthermore, the stress apparently induced by the dosing procedure may have played a part in the teratogenic response in the rabbits in this study. Hence, the study is classified as Supplementary and a final determination as to the actual teratogenic potential should be based on a repeat study.

A Teratology Study with Ethalfluralin in the Wistar Rat. Study R06880. Conducted by Elizabeth R. Adams, et. al. Lilly Research Laboratories, Greenfield, Indiana. November, 1980. Accession No. 070682.

Test Chemical: MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

Ethalfluralin. Compound 94961. EL-161. N-ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl)-benzenamine. Technical chemical from Lot B-30-Y64-35B. [redacted] to reduce contamination with the nitrosamine N-ethyl-2-methyl-N-nitroso-2-propene-1-amine (ethyl-methylallyl-nitrosamine, EMANA). After treatment the purity was 93.5%. Doses were not adjusted for purity.

Doses:

0, 10, 75, or 250 mg/kg on gestation days 6 through 15 by oral gavage.

Animals:

Virgin adult female Wistar-derived rats were obtained from Harlan Industries, Cumberland, Indiana. Male rats, not part of the test population were used for breeding. The females were acclimated for 3 days prior to being mated. The females were caged with the males, one-to-one, until a copulatory plug was found (gestation day 0). Each day the females for which plugs were found were randomly assigned to the control and ethalfluralin treatment groups. The mating period continued until 25 plugged females had been assigned to each test group.

Housing:

After mating the bred females were placed individually in stainless steel wire mesh cages (17 x 20 x 36 cm) suspended over cageboard covered trays. They were kept in a room maintained at 24 ± 3.0°C with relative humidity of 45% and a light-dark cycle of 12 hours light.

Identification:

ID number denoted study, animal number, and treatment. Females were ear punched to identify dose group.

Body Weights:

Recorded initially (gestation day 0), and on gestation days 7, 14, and 20. Dosing was based on gestation day 0 body weight (229.8 ± 2.0 g, mean ± S.E.)

Food Consumption:

Purina Laboratory Chow, ad libitum, measured at same intervals as body weight.

Procedure:

The pregnant Wistar-derived female rats were given daily doses of 0, 10, 75, or 250 mg/kg ethalfluralin on gestation days 6 through 15. There were 25 females to each dose group. Rats were observed daily for toxic signs. Dosing was by oral gavage of the 0.2, 1.5, or 5% w/v suspension of ethalfluralin in a 10% w/v aqueous acacia solution. Control rats received only the 10% acacia solution. Fresh test material was prepared daily.

Terminal Evaluation:

On gestation day 20 the rats were killed with CO_2. A gross internal examination of each was performed. Ovaries were examined for the number of corpora lutea and the uterus for the number of fetuses and resorptions. Resorptions were classified as early or late to indicate the relative time of mortality. The fetuses were examined for external abnormalities, viability, and sex; weights were recorded individually. Approximately one-third of the fetuses of each litter were fixed in Bouin's solution for visceral examination (Wilson modified technique), and the remainder were cleared for skeletal examination.

The following reproduction parameters were determined for each dose group:

Fertility Index - proportion of females that were pregnant

Gestation Survival - proportion of fetuses that were alive

Resorption Index - the proportion of implanted conceptures that resulted in resorptions.

Implantation Index - no. of implantations/no. of corpora lutea

Statistical Analyses:

Percent weight gain during gestation was analyzed by method of Dunnett. Dunnetts' "t" statistic was also calculated for live litter size and mean fetal weight. The mean weight of each litter was used as the independent sampling unit.

For resorption data, the percent of resorptions occurring in each female based on the number of implantations was determined.

Results:

Mortality and Toxic Signs:

There were no deaths or physical signs of toxicity. Urine of treated rats was yellow-orange colored. Previous analysis relative to another study demonstrated that the coloration was due to ethalfluralin metabolites; ethalfluralin per se was not present.

Food Consumption:

No differences between groups.

Weight Gain: No differences between groups.

Reproduction Values:

Live Fetuses:

Greatest number (13.1 ± 0.4) at 75 mg/kg/day dose. Lowest number (10.6 ± 0.9) for controls.

Implantations:

Greatest number (14.3 ± 0.4) at 75 mg/kg/day dose. Lowest number (12.7 ± 4.33) for controls.

Resorptions:

Greatest mean % (20.34 ± 4.33) for controls; least resorptions (10.84 ± 2.45%) at 250 mg/kg/day dose.

Females resorbing:

Greatest number (71.4%) in controls; least females resorbing (54.2%) at 250 mg/kg/day dose.

Fetal Values:

Male/Female Distribution:

Males = 51%, 51%, 50%, and 53% for 0, 10, 75, and 250 mg/kg/day. No treatment effect.

Mean fetal weight:

3.89, 3.95, 3.96, and 4.25 g for 0, 10, 75, and 250 mg/kg/day.

External Abnormalities:

None found at any treatment level.

Although no treatment related effects are detected for the following parameters: (live fetuses, implantations, resorptions, females resorbing, and mean fetal weight), it is noted that control values were consistently the poorest, and therefore useful comparison to the dose groups is limited.

There appears to be a dose related increase in incidence of hydronephrosis in litters and fetuses associated with ethalfluralin administration as noted in the table below:

Incidence of Hydronephrosis	Control	10 mg/kg/day	75 mg/kg/day	250 mg/kg/day
Litters with hydronephrosis per number of litters examined	11/22 (50%)	12/23 (52%)	14/24 (58%)	16/24 (67%)
Fetuses showing eitner unilateral or bilateral hydronephrosis per number of fetuses examined	17/86 (19.8)	22/97 (23%)	27/114 (23.7%)	31/108 (28.9%)

As a matter of interest, the combined incidences of unilateral and bilateral hydronephrosis in cumulative controls of this strain rat in the Lilly Laboratories is 12.1%, compared with the control incidence of 19.8% in this study.

Hernia:

There was 1/86 hernias (type unspecified) in controls and 1/108 (inguinal hernia) at the 250 mg/kg/day level.

Skeletal Abnormalities:

Rudimentary ribs were seen in 1/173 fetuses at the 10 mg dose level and in 1/108 fetuses at 250 mg/kg//day. Fourteen ribs bilaterally were seen in 2/173 fetuses at the 10 mg/kg/day level. Both were litter mates.

Conclusions:

In some parameters such as incidence of resorptions and mean fetal weights the treated groups tended to perform better than the controls; this may indicate poor performance on the part of the controls.

There also was a trend toward a higher incidence of combined bilateral and unilateral hydronephrosis in treated litters.

As stated on page 7 of the study report the highest dose in this rat study was based on reduced food consumption in the rabbit study. (Reduced food consumption was not seen at this dose level in the rats.) Selection of a dose level in one species (rat) based on reduced food consumption in another species (rabbit) is not valid for dosage selection. In this study no maternal toxicity was demonstrated. In a teratology study maternal toxic effects must be demonstrated at the highest dose level to assure adequate dosing.

While dose levels were chosen based on reduced food consumption in pregnant rabbits at 250 mg/kg/day, such effect was not seen at this dose level in rats. Therefore, the NOEL for maternal toxicity in this rat study is 250 mg/kg/day (HDT).

In this study there was a significant incidence of hydronephrosis (19.8% of the pups in the controls and 28.7% of the pups in the 250 mg/kg/day dose group). In contrast, the historical incidence of hydronephrosis is 12.1% in the same strain of rat in the registrant's laboratories. However, no instances of hydroureter were reported, although one often expects to find hydroureter associated with hydronephrosis.

In spite of the high incidence of hydronephrosis in this study, none was reported in the 3-generation reproduction study conducted with the same strain of rat. (See review page 95)

One might question whether this is true hydronephrosis. It is suggested that the study be repeated, allowing adequate numbers of pups to grow to maturity so it may be determined if the hydronephrosis noted in this study is a true teratogenic effect or a developmental retardation in growth of the kidney (apparent hydronephrosis).

Because of the control values being very poor for many parameters such as live fetuses, implantations, resorptions, females resorbing, and mean fetal weight, reliable assessment of the fetotoxic potential of this compound may be impaired. Therefore, it is concluded that the control values are abnormal and hence form a poor basis of comparison with the treated groups. In essence, the validity of the control data and comparisons made utilizing the control data must be considered questionable.

The study is classified as Core Supplementary due to the above listed concerns.

A 3-Generation Reproduction Study with Ethalfluralin in the Fischer 344 Rat. Conducted by Elizabeth R. Adams, et. al.; Lilly Research Laboratories, Greenfield Indiana. March, 1981. Studies R-68, R-738, and R-1248. Accession Number 070682.

Test Material: MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

Ethalfluralin. Compound 9461. EL-161. N-ethyl-N-(2-methyl-2-propenyl)-2,6-dinitro-4-(trifluoromethyl)-benzenamine. Technical chemical from B-30-Y-34B. Purity 94.5% [redacted] to reduce contamination with the nitrosamine N-ethyl-2-methyl-N-nitroso-2-propene-1-amine (ethyl-methylallyl-nitrosamine, EMANA). Doses not adjusted for purity.

Dose Levels:

0.01%, 0.025%, and 0.075% (100, 250, and 750 ppm) in diet.

Animals:

For the F_0 generation, Fischer 344 rats (4 to 5 weeks of age) of both sexes were supplied as litters by Harlan Industries, Inc., Cumberland, Indiana. They were held for 6 days in gang cages separated by litter and sex to permit adaptation to caging, feeding, and watering conditions of the laboratory. Rats which varied significantly from the general population, or in poor physical condition were not used. They then were distributed randomly by computer, and so that when possible animals of each litter were present in each test group.

In the F_1 and F_2 generations, the rats were separated from the female parent as weanlings, were housed in gang cages separately by sex and litter, and were maintained on the same diet concentration as their parents while awaiting the selection of offspring to be raised to adulthood. Since the F_1 and F_2 generation animals were the offspring of treated parents, animals in poor condition or of significantly different weight were not excluded from the population from which animals were selected. The selection was made randomly using representatives of all available litters within each test group.

At the start of study R-68 (F_0) the rats were 5 to 6 weeks old. The males weighed 95.5 ± 0.9 g and the females 82.2 ± 0.8 g. In the F_1 and F_2 generations, the age at initiation of the growth phases were about five ± one week due to a two-week period of mating.

Identification:

In the growth phase each rat was given an identification number that indicated study, animal, sex, and treatment. Also each rat was ear punched to identify dose group.

Offspring were not identified; the number of the dams was used for all rats within a litter.

Nitrosamine Assays:

These studies were conducted concurrently with the chronic/oncogenic studies and ethalfluralin from the same lot number was used for all the studies, and nitrosamine assays were applicable for all studies.

Diet Assays:

At the beginning of the 2-month growth phase of each parent generation, samples of each diet level were submitted for assay of ethalfluralin content.

Observations were made daily during the 2-month growth phases to determine if any rats were dead or moribund. At least once each week each rat was observed closely, noting muscle tone, pelage, eyes, teeth, secretions, and excretions. During the reproduction phases, females were closely observed near the time of parturition. Offspring also were observed closely at the time of the weekly weighing.

Adult Body Weight and Food Consumption:

During the 2-month growth phase, all rats were weighed once a week, using a self-taring balance connected to a recording computer. Food consumption was measured at the same time with the same equipment.

During the reproduction phase, the females were weighed on the day of separation from the male and on the day the pups were 21 days old. Food consumption was not measured during the reproduction phase.

Breeding:

At the end of the 2-month growth phase of each generation, males and females from corresponding diet levels were paired for mating, using a mating scheme which prevented sibling matings. Daily observations were made for the presence of copulatory plugs to establish the date of conception (gestation day 0). After a mating period of 2 weeks, the females were separated from the males and caged individually.

BREEDING SCHEME FOR THE MULTI-GENERATION STUDY
WITH ETHALFLURALIN IN FISCHER 344 RATS

F_0 (weanling rats)

Study R-68

F_1 - gross internal examination of one weanling/sex/litter

Study R-738

F_2 - gross internal examination of one weanling/sex/litter

Study R-1248

F_3 - gross internal examination of all weanlings; histopathology performed on one weanling/sex/litter

Reproduction Measurements:

Record was made of the date of parturition; the numbers of live and stillborn in each litter; and the number, weight, and condition of the survivors on days 1, 7, 14, and 21 after delivery. The progeny were weighed as litters on days 1, 7, and 14, and individually when 21 days of age. The sex of each pup was determined at this 21-day weighing.

Parental rats that died during either the growth or reproductive phases were submitted for gross necropsy. Offspring that died up through 21 days of age were given a gross external examination.

In the F_0 and F_1 parent generations, after selection of offspring to continue the generation study had occurred, one weanling pup per sex was given a gross internal examination. In the F_2 generation (F_3 progeny) all surviving pups were given a gross internal examination. The necropsy was an examination of the animal's general physical condition, body orifices, external and internal organs, and tissues. From one F_{3a} pup per sex per litter the following organs and tissues were collected, placed in a fixative, and processed for histopathological evaluation: kidney, liver, heart, spleen, thymus, lymph node, salivary gland, pancreas, stomach, duodenum, jejunum, ileum, colon, ovary, uterus, adrenal, thyroid, testis, prostate, skin, mammary gland, skeletal muscle, and urinary bladder.

In each generation, adult females failing to deliver were killed and examined for evidence of pregnancy. After completion of the offspring examinations in each generation, the males and the females which had been pregnant were given terminal eye and physical examinations and were killed.

Calculations and Indices:

Mean values of the individual parameters were determined for each dose group and generation including an indicator of overall performance, the mean number of offspring raised to weaning age per pregnant female. The following reproduction indices were calculated:

Fertility - proportion of females that were pregnant

Gestation Survival - proportion of newborn pups that were alive

Survival - proportion of offspring that survived 1, 7, 14, and 21 days.

Statistical Analyses:

Mean body weight gain of parent animals was analyzed by the method described by Dunnett[1]. In the analysis of progeny body weight data, the mean weight of each litter was used as the independent sampling unit. Statistical analysis was performed on mean progeny weight on day 21. The method of Dunnett was used; however, since nine comparisons were made at 21 days over the three generations, the critical value of "t" used in these analyses was a Bonferroni "t" ($p \leq 0.05$).

Results:

Nitrosamine Assays:

Assays indicated a virtual absence of nitrosamine in the test chemical. Of 5 assays, no nitrosamine was detected in two, and levels of 0.05, 0.06, and 0.07 ppm were found in the other 3.

Feed Assays:

Mean Ethalfluralin Content of Feed. Percent of Theory.

Theoretical Level	0.01%	0.025%	0.075%
F_0 Generation	85%	86%	90.7%
F_1 Generation	85%	80%	83.3%
F_2 Generation	95.9%	99%	100.1%

Ethalfluralin was demonstrated to be stable in the feed for at least two weeks. Assays which are less than 100% of theory may be due partly to assay losses (incomplete recovery on assay because of binding, etc.) and to the fact that the technical chemical is only 94.5% pure.

Toxic Signs:

No physical signs of toxicity were seen. Hair was stained yellow from ethalfluralin metabolites in the urine.

Mortality:

Mortality was very light and was not related to treatment. In the F_0 generation, 2 rats on the high dose level (0.075%, or 750 ppm) died; in the F_1 generation 1 rat on the low dose (0.01%, or 100 ppm) died; in the F_2 generation one control rat died.

In the high dose F_0 parents that died necropsy showed the tissues revealed no abnormalities; live pups were found in the uterine horns, but no pups were lodged in the body of the uterus or pelvis.

In the low dose F_1 parent the only observation recorded was marked dehydration.

The control F_2 parent was cannibalized, and advanced postmortem decomposition precluded critical evaluation.

Body Weight:

Mean weight gain in the males on the high dose was slightly less than controls in all 3 generations; the difference was significant at the 0.05 level in the F_2 generation males (196.5 g vs 182.6 g). This degree of difference was not evident in any of the females.

There were no consistent or significant differences in mean daily food consumption or efficiency of food utilization.

Fertility was comparable in all generations and at all dose levels. Also, treatment had no effect on litter size (live born), survival, mean progeny weights, or sex distribution in any of the generations. There may have been a questionable effect on progeny weights at 21 days in the F_0 generation at the high dose level.

There were no gross lesions in the progeny which could be related to treatment. The few gross lesions observed included ringtail, microphthalmia, anophthalmia, and small testes. As stated these appeared in progeny of various generations, and in controls as well as treated litters. (See table of gross lesions on following page.) Hydronephrosis was not reported in this study.

SUMMARY OF GROSS LESIONS IN WEANLINGS GIVEN ETHALFLURALIN IN THE DIET.
STUDIES R-68, R-738, AND R-1248.

Disgnosis	Ethalfluralin, % in Diet							
	0.0		0.01		0.025		0.075	
	M	F	M	F	M	F	M	F
Ringtail								
F_{2a}	0	1	1		0	0	0	0
F_{3a}	1	1	7	3	0	0	0	1
Microphthalmia								
F_{2a}	0	0	0	0	0	1	0	0
F_{3a}	0	0	0	1	0	0	0	0
Anophthalmia								
F_{2a}	0	0	1	0	0	0	0	0
F_{1a}	0	0	1	0	0	0	0	0
Small Testes F_{3a}	1	0	0	0	0	0	2	0
Moderate Hypoplasia of Kidney	1	0	0	0	0	0	0	0
Thin, Gastrointestinal Tract Empty								
F_{2a}	0	0	0	0	0	0	0	1
Thin, Diarrhea								
F_{1a}	0	0	0	0	1	0	0	0

In the teratology study a high incidence of hydronephrosis was reported (without corresponding hydroureter). An explanation of this possible discrepancy is desired. The attached table lists gross lesions of weanlings in the study.

Conclusions:

1) Administration of ethalfluralin at dose levels of 100, 250, and 750 ppm in the diet for 3 generations had no effects on the reproductive parameters examined in this study.

2) The NOEL is 0.025% (250 ppm) and the LEL is 750 ppm (HDT) based on the slightly depressed mean body weight gains in males in all 3 generations.

3) Data are Core Supplementary pending an explanation of the discrepancy in hydronephrosis findings between this study and stated historical incidence of hydronephrosis in this testing facility. (See discussion in the review of the rat teratology study p. 94.)

The Effect of Ethalfluralin (Lilly Compound 94961) on the Induction of Bacterial Mutation Using a Modification of the Ames Test. Study LBMS 1169. Conducted by J.K. Epp and C.Z. Thompson. Lilly Research Laboratories. Indianapolis, Indiana. November 1980.

Ethalfluralin obtained from Lot B30-Y64-35B was used in this study. Purity was 93.5%. Streptozotocin (STZ) and 2-acetylaminofluorene (2-AAF) were used as positive controls for the non-activated and activated assays, respectively. Ethalfluralin was tested with and without liver microsomal activation.

In this test Salmonella typhimurium and E. coli were treated with ethalfluralin to evaluate the induction of bacterial mutation, with and without metabolic activation. Salmonella L-2 strains used were: G46, TA1535, TA100, C3076, TA1537, D3052, TA1538, and TA98. E. coli strains used were: WP2 and WP2uvrA$^-$. Negative control plates were treated with DMSO, the solvent for compound solution.

Results:

"With and without microsomal activation, there was no evidence for bacterial mutation induced by ethalfluralin in the gradient plate assay at concentrations ranging between 1000-0.1 ug/ml".

Conclusions:

1. Ethalfluralin is reported to be not mutagenic in the Ames test.

2. Data are unacceptable in the absence of detailed data report and the failure to use maximal concentrations up to the level of toxicity or crystallization. Report should also cite their references. (Reference page apparently was omitted.)

The Effect of Ethalfluralin (Lilly Compound 94961) on the Induction of DNA Repair Synthesis In Primary Cultures of Adult Rat Hepatocytes. Study 791120-263 conducted by Gregory S. Probst and Steven B. Neal, Lilly Research Laboratories, Greenfield, Indiana. June, 1980.

Cultures of adult rat hepatocytes have been shown to be sensitive to both ultimate carcinogens and pre-carcinogens for the induction of DNA repair synthesis (unscheduled DNA synthesis), and selective against non-carcinogens for induction of unscheduled DNA synthesis. In this test, ethalfluralin was tested against the carcinogen N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and the pro-carcinogen 2-acethylaminofluorene (MNNG) and the pro-carcinogen 2-acetylaminofluorene (2-AAF) as positive controls, and DMSO as a negative control solvent.

Ethalfluralin from lot B30-Y64-35B was used in the study. This is the same lot used for all other toxicity studies. Using the GC assay, a purity of 93.5% purity was found, several impurity peaks being seen on the chromatogram.

All test chemicals were dissolved in reagent grade DMSO and serial dilutions were made in serum-free media to give 8 compound concentrations covering a range of 0.5 to 1000 nmoles/ml. The laboratories experience indicates that this is the range in which the potential of compounds to induce DNA repair synthesis will be demonstrated.

Primary cultures of adult rat hepatocytes were prepared from the liver of a 165 g male Fischer 344 rat. The liver was perfused at 37°C in situ via the hepatic portal vein for 4 to 6 minutes at a rate of 40 to 50 ml/minute with Ca^{2+}-Mg^{2+}-free Hank's balanced salt solution (HBSS) containing 0.5 mM EGTA and buffered with 0.05 M HEPES to pH 7.2. Cell disaggregation was accomplished by continued perfusion with Williams' medium E buffered to pH 7.2 with 0.05 M HEPES (WEH) containing 100 units/ml collagenase at 37°C for 10 to 15 minutes at a flow rate of 20 to 40 ml/min. Cells were detached by combing fresh WEH-collagenase medium followed by sequential filtration through 80 and 170 mesh nylon. The cells were washed once with WEH containing 10% fetal bovine serum (FBS) and 50 units/ml gentamycin by centrifugation at 50xg for 4 minutes. A yield 1.5×10^8 hepatocytes with 89.9% viability was obtained in this preparation.

Autoradiographic Assay for DNA Repair Synethesis:

Freshly prepared hepatocytes were plated at a density of 3.5×10^4 cells/cm^2 in 26 x 33 mm Multiplates containing 10.5 x 22 mm plastic coverslips in WEH containing 10% fetal bovine serum, 50 units/ml gentamycin and 100 units/ml each of penicillin and streptomycin. The cultures were incubated at 37°C in a humidified 95/5% air/CO_2 environment. Following a 90-minute attachment period, the cells were washed once with WEH, and serum-free WEH containing 10 uCi/ml ^{3}H-TdR and the appropriate test compound dilution was then applied to each culture. (Dilutions were 0.5, 1, 5, 10, 50, 100, 500, or 1000 nmoles/ml of either ethalfluralin, MNNG, or 2-AAF.) After incubation for 20 hrs., the media was removed and the cultures were washed and prepared for fixation. Hepatocytes grown on plastic coverslips were washed and fixed in the Multiplate chambers. The cultures were rinsed once with HBSS, allowed to stand for 10 minutes in 1% sodium citrate, and fixed by three 15 minutes washes with ethanol/acetic acid (3:1, v/v). Air-dried coverslips were glued to glass slides, then stained with 1 aceto-orcein for 4 to 5 minutes. Air-dried stained slides were individually dipped into undiluted NTB-2 liquid photographic emulsion sealed in light tight dessicated boxes, incubated 12 to 14 days at 4°C, developed with Kodak D-19 developer, and fixed with Kodak fixer. Cells were examined by oil immersion microscopy without coverslips.

DNA repair synthesis (unscheduled DNA synthesis, or UDS) in hepatocytes was quantified by counting silver grains over the cell nucleus using a Biotran II Automated Colony Counter adapted for oil immersion microscopy. Cytoplasmic background counts were determined by counting three nuclear-sized areas adjacent to the nucleus and subtracting the mean from the nuclear count. The net nuclear grain count represents the difference between the gross nuclear grain count and the mean cytoplasmic background count; thus a negative value for the net nuclear count is possible for treatments not inducing DNA repair synthesis. Nuclei of 20 morphologically unaltered cells, judged to be representative of the DNA repair synthesis responsiveness of the cell population and containing at least 4 grains, were counted for each treatment Autoradiographic gain counts were conducted for the highest compound concentration that did not produce pronounced cytotoxicity and continued through subsequently lower concentrations of test compound.

Criteria for a Positive Response for DNA Repair Synthesis:

In this study a compound was judged to have induced a positive response when at least two successive compound concentrations produced nuclear grain counts which exceeded those of the control by three standard deviations of the control value.

Results:

Induction of unscheduled DNA Synthesis was measured by autoradiography in primary cultures of adult rat hepatocytes after treatment with ethalfluralin, MNNG, or 2-AAF.

The two highest levels of ethalfluralin (1000 and 500 nmoles/ml) were cytotoxic, leaving no cells which could be evaluated for UDS.

The two highest levels of MNNG also were cytotoxic, but cells which did survive were positive for UDS. The same was true of the top level of 2-AAF.

The remaining six levels of ethalfluralin (100, 50, 10, 5, 1, and 0.5 nmoles/ml) were all negative for UDS.

The 100 and 50 mmole levels for MNNG (a direct carcinogen) were positive for UDS.

All levels 2-AAF (a pro-carcinogen) were positive for UDS.

Conclusions:

1) Even though cytotoxicity was seen in hepatocyte cultures treated with 500 and 1000 nmoles/ml of ethalfluralin, at no level did ethalfluralin demonstrate induction of unscheduled DNA synthesis.

2) Treatment of hepatocytes with the direct carcinogen MNNG in at least 2 dose levels and with the pro-carcinogen 2-AAF at all dose levels demonstrated induction of unscheduled DNA synthesis.

3) The study is acceptable as an assay for repair synthesis in rat hepatocytes.

Dominant Lethal Study with Ethalfluralin in the Rat. Study R-159 conducted by Elizabeth R. Adams, N.V. Owen, et. al., Lilly Research Laboratories; Greenfield, Indiana. December 1980.

Test Chemical and Dose Levels:

Ethalfluralin (Compound 94961; N-ethyl-N-(2-methyl-2-propenyl)-2, 6-dinitro-4-(trifluoromethyl)-benzenamine) was administered orally by gavage to 12 adult male rats in a single dose of 5.0 g/kg. The test material was a 25% (w/v) suspension of ethalfluralin in a 10% (w/v) aqueous acacia solution. This concentration resulted in a dose volume of 20 ml/kg or approx. 8.6 ml/rat (430 g rat). An equivalent volume of 10% aqueous acacia solution was given to 10 control males. Lot B30-Y64-35B of ethalfluralin, purity 93.5%, was used. The concentration of ethalfluralin was not adjusted for purity.

The lot of ethalfluralin used in this test was also used for chronic and oncogenic studies in rats and mice. The lot had been subjected to [redacted] to reduce the content of the nitrosamine contaminant, N-ethyl-2-methyl-N-nitroso-2-propene-1-amine (ethylmethylallyl-nitrosamine). [redacted] this contaminant was [redacted] The assay value in October 1978 was 0.06 ppm.

Animals:

Male and female Wistar-derived rats were supplied by Harlan Industries, Inc., Cumberland, Indiana. The males were young adults of proven fertility and the females were virgin adults. The entire population assigned to this evaluation was 180 animals (20 males, 160 females). The control animals, however were assigned to study R-149 (another study) and served as control animals for both R-149 and R-159. Two additional males were treated with ethalfluralin initially and held as spares in the event of a death in that group. One treated male did die during the first week of the test and one of the spares was substituted.

Acclimation:

The males were acclimated for several days in individual cages prior to the start of the test. The females were received from the supplier weekly on Mondays and were mated randomly with control or treated males upon arrival.

Caging:

The animals were housed in pairs during the mating period in stainless steel wire mesh cages (17 x 20 x 36 centimeters) that were suspended over trays containing neomycin cageboard. The females were placed individually in similar cages after the mating period.

Housing:

The animals were housed in a room maintained at 24 ± 3°C with minimum relative humidity of 45%. A light-dark cycle of 12 hours was maintained.

Identification:

Each rat was given a unique identification number consisting of a combination study number and animal number that specified sex and treatment. Females were given numbers with the same treatment-identifying number as the males with which they were mated, although the females received no treatment.

Breeding Protocol:

In studies of this type, the mating sequence is initiated on the day of dosing of the males. However, due to the large dose volume administered in this study, mating was delayed until three days after dosing. (No explanation was given for the delay in mating; dose volume wasn't excessive.) An untreated female rat was caged with each control and treated male for a one-week mating period. Gestation day 0 was defined as the date an expelled copulatory plug was found. After the mating period, the females of the first group were caged individually and a new group of females was mated. This cycle was repeated until eight groups of females had been mated.

Body Weights:

The male rats were weighed on the day of dosing (mean ± S.E., 428.9 ± 6.7 g), one and two weeks after dosing, and at termination of the study. The females were weighed at the time of mating (mean ± S.E., 190.4 ± 1.0 g), on gestation day 0 (if a copulatory plug was found) and on the day of sacrifice.

Physical Signs of Toxicity:

The males were observed closely for several days after dosing.

Terminal Evaluation - Females:

Females for which copulatory plugs were found were killed on gestation day 20, or if that day would occur on a weekend, on day 18 or 19. Females without copulatory plugs were killed two weeks after separation from the male.

The females were killed by carbon dioxide. A gross internal examination of each was performed. Ovaries were examined for the number of corpora lutea, and the uterus for the number and location of fetuses and resorptions. Fetuses were examined for viability; resorptions were classified as early or late to indicate the relative time of mortality.

Terminal Evaluation - Males:

Following completion of the female evaluations, the males were weighed, given an external physical examination, and were killed with carbon dioxide.

Calculations and Reproduction Indices:

For each set of ten females, mean values for the various reproduction parameters and the following reproduction indices were obtained:

Gestation survival - the proportion of fetuses that were alive.

Resorption - the proportion of implanted conceptuses that resulted in resorptions.

Implantation - the proportion of implantations to corpora lutea.

Statistical Analyses:

Since the end result of a dominant lethal mutation is an increase in pre-implantation loss and/or post-implantation death noted in females mated with males treated with a dominant lethal agent, statistical analyses of implantation occurrence and resorption incidence data were performed. In all analyses, the males was considered the experimental unit.

In order to increase the power of the statistical test, data for weeks 1 and 2, 3 and 4, 5 and 6, and 7 and 8 were combined. Pooling of these data is justified on the basis of studies conducted with a positive dominant lethal agent, TEM, in which consequences of the dominant lethal mutation were most evident during weeks one through four. Implantation and resorption data received similar statistical treatment: (1) For each male in each pair of weeks, the number of corpora lutea, implantation sites, and resorptions in the corresponding females were added. (2) The percentage of implantation sites to corpora lutea and the percentage of resorptions to implantation sites were determined. (3) An arcsin square root of the percentage transformation was conducted to normalize the distribution. (4) A one-way analysis of variance was used to compute an "F" value for each parameter for each of the four paired weeks. (5) Due to the fact that four comparisons were made for each parameter, the critical value of "F" used to determine statistical significance was a Bonferroni "F". The experiment-wise error rate was set at 0.05.

Results:

Mortality:

Male rat no. 101 of the ethalfluralin group was found dead 5 days after dosing. Necropsy revealed upper respiratory infection and ethalfluralin was not considered the cause of death. The dead rat was replaced by a comparable spare rat (number 110) obtained for such purposes.

Body Weights:

Ethalfluralin treatment had no body weight effect on the male rats at any time following treatment.

As stated in the procedure, to increase statistical significance, data for weeks 1 and 2, 3 and 4, 5 and 6, and 7 and 8 were combined for implantation and resorption data. On analysis, there were no significant differences in implantations and resorptions in females bred by control or ethalfluralin treated males. Also there were no significant differences in fertility, mean litter size, mean resorption sites, mean implantation sites, or mean corpora lutea between females bred to untreated males or to males treated once with 5 g/kg ethalfluralin. There also were no differences in pre-implantation losses or post-implantation deaths.

Discussion:

There were no signs of a dominant lethal effect under the conditions of this stduy. However, there were no clinical signs of toxicity in males related to treatment with 5 g/kg ethalfluralin or to administration of 20 ml/kg 10% solution.

Of concern relative to the conduct and findings of the study, the following points are noted: (per Irving Mauer, Ph.D.; Toxicology Branch Geneticist)

1) Of the single oral dose of 5 g/kg in 10% acacia, probably very little was absorbed due to the bolus effect. A dose volume of 20 ml/kg provides a dose of approximately 8.6 ml per 430 g rat, which doesn't seem excessive. Therefore, the absorbed dose probably is insufficient for transport to the gonads, which could be overcome with use of a vehicle permitting a larger concentration per dose, and perhaps better absorption.

Alternatively, the gavage dosage could have been administered over a 5-day schedule, permitting accumulation at levels up to 5 g/kg per day (with other carriers), and perhaps some toxicity.

2) No explanation was given why mating was delayed 3 days after dosing. If the compound were to have an effect (induction of dominant lethal toxicity) we then would be starting at 0.5 week and could miss a potential effect on mature (epididymal) sperm.

3) Too few males were treated, and too few females mated per treated male per week.

4) There is no positive control, preferably using a human mutagen by the same route of administration.

5) An alternative schedule would use treated males from subchronic studies (60 days), fed test compound in the diet at a level producing both clinical and laboratory evidence of toxicity (including reduction in fertility).

Conclusion:

The test protocol is both inadequate and inappropriate (especially as to the vehicle suspending agent) for generating meaningful results, and the study is unacceptable.

Pilot Teratology Study I with Ethalfluralin in the Dutch Belted Rabbit. Conducted by E.R. Adams, et al., Lilly Research Laboratories. Study B-7048. Acc. No. 070682. October, 1980.

Pregnant female Dutch Belted rabbits, 3-4 months old, 5 per group, were given orally by gavage 0, 10, 25, 75, or 150 mg/kg ethalfluralin on days 6 through 18 of gestation. The ethalfluralin was from Lot B30-Y64-35B and [redacted] to remove any nitrosamine contaminant (EMANA). Purity was 94.5%. The chemical was given in a 5% acacia solution, a dosage volume of 5.0 ml/kg being used at all dosage levels.

The rabbits were fed Purina Rabbit Chow pellets containing 25% fiber.

Body weights were recorded at gestation day 0 (mean 2.02 kg), on the first day of treatment (gestation day 6), and every 3 days through gestation day 27.

Food consumption was measured daily and is reported as group means.

The rabbits were observed daily for any signs of toxicity and especially for signs of abortion. On day 28 of gestation they were killed by IV injection and examined for gross tissue changes and reproductive parameters, including number of corpora lutea on ovaries and fetus number and distribution, resorptions, or empty implantation sites. Each fetus was examined for viability and external abnormalities.

Results:

There were no deaths or physical signs of toxicity.

Food consumption varied, but with the exception of the high dose group (15 mg/kg/day) showed no consistent relationship to dose. There also was no apparent effect on body weight, although the mean weight gains for groups receiving 10 mg/kg/day and 150 mg/kg/day gained less weight than the other groups during and following treatment.

Individual rabbits in various groups had pneumonia, diarrhea, hairball in stomach, and hydrothorax, thought to be related to the dosing procedure.

There were no abortions. The greatest number of abnormalities were in the controls, with 2 litters affected. One fetus had a missing tail and probable absence of lower vertebrae. Another fetus from this litter was dead on sacrifice and necropsy of dam.

In another control litter the fetus had a dome-shaped head and cleft palate. The doe had pitted kidneys. A third control doe also had pitted kidneys.

A doe in the 10 mg/kg/day group had pneumonia and diarrhea.

A doe in the high dose group had pitted kidneys; another had hairball in the stomach and an empty GI tract. A third doe had excessive fluid in the thoracic cavity.

There were no remarkable observations in the 25 and 75 mg/kg/day groups.

In none of the groups were there any apparent chemical-related effects on litter size or resorptions.

Conclusion:

Ethalfluralin had no apparent reproductive effect on rabbits at doses as high as 150 mg/kg/day on days 6-18 of gestation. Core Supplementary because only 5 does per dose group were used and no skeletal or visceral examinations were conducted.

Pilot Teratology Study II with Ethalfluralin in the Dutch Belted Rabbit. Conducted by E.R. Adams, et al., Lilly Research Laboratories. Study No. B-7438. Accession No. 070682. October, 1980.

Pregnant female Dutch Belted rabbits, 3-4 months old, 5 per group, were given orally by gavage 0, 250, 500, or 1000 mg/kg ethalfluralin on days 6 through 18 of gestation. The ethalfluralin was from Lot B30-Y64-35B and [redacted] to remove any nitrosamine contaminant (EMANA). Purity was 94.5%. The chemical was given in a 5% acacia solution in a dosage volume of 5.0 ml/kg for all dosage levels.

The rabbits were fed Purina Rabbit Chow pellets containing 25% fiber. Body weights were recorded at gestation day 0 (mean 2.06 kg), on the first day of treatment (gestation day 6) and every 3 days to day 27.

The rabbits were observed daily for any signs of toxicity and especially for signs of abortion. On day 28 of gestation they were killed by IV injection and examined for gross tissue changes and reproductive parameters, including number of corpora lutea on ovaries, and fetus number and distribution, resorptions, or empty implantation sites. Eacch fetus was examined for viability and external abnormalities.

Results:

Mortality: No rabbits died while on test.

Fertility: Of the 5 does in each group, 3 controls were not pregnant, one at 250 mg/kg/day was not pregnant, 2 at 500 mg/kg/day were not pregnant; and 2 at 1000 mg/kg/day were not pregnant.

Abortions: All 3 pregnant rabbits on 1000 mg/kg/day aborted; 2 on day 20, and one on day 22.

One of 3 pregnant rabbits on 500 mg/kg/day aborted on day 20.

Maternal Body Weight: All does on 1000 mg/kg/day lost approximately 15.9 grams of body weight. The does on 500 mg/kg gained about 8.6 grams, while the controls and 250 mg/kg does gained more than 10 grams.

Reproduction Data: No malformations were recorded for any of the fetuses. There also were no resorptions. Because of low fertility in nearly all groups and the abortions at the high dose, few litters were available for examination.

The abortions at 500 and 1000 mg/kg were attributed to these doses of ethalfluralin.

For the few pups examined externally, no terata were observed. NOEL for maternal coxicity is considered to be 250 mg/kg/day based upon abortions at higher levels. This is a dose screening study and is considered to be Core Supplementary. No visceral or skeletal examinations were performed.

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TS-769:th:TOX/HED:RAGessert:10-27-82:cards 1 & 2