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

OFFICE OF
PESTICIDES AND TOXIC
SUBSTANCES

MEMORANDUM

SUBJECT: Oryzalin - Update on Decisions of RfD/Peer Review Committee - Revised DER's

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THRU: Yiannakis M. Ioannou, Ph.D., Section Head
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and

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Registrant: DowElanco

Action Taken: 1) Meeting of the RfD/Peer Review Committee on August 26, 1993; 2) Re-review of the following studies: 1) 2-year feeding/carcinogenicity study in the rat; 2) carcinogenicity study in the mouse; 3) 1982 developmental toxicity study in the rabbit; and 4) 1978 developmental toxicity study in the rabbit.

Conclusion: The RfD/Peer Review Committee has revised the Reference Dose (RfD) level. Updated DER's are attached. The one-liners should be revised/ corrected.

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Conclusions of RfD Peer Review Committee

The RfD/Peer Review Committee met on August 26, 1993, to discuss oryzalin. The Reference Dose (RfD) level had previously been based on the NOEL (5 mg/kg) from the one-year dog study (MRID # 40024801). The committee reviewed this study and concluded that the NOEL should be 50 mg/kg/day rather than 5 mg/kg/day. Therefore, the RfD will be based on the NOEL from the rat two-year feeding/ carcinogenicity study which is 12.16 mg/kg/day in males and 13.82 mg/kg/day in females.

Summaries of Revised DER's

1) Two-Year Feeding/Carcinogenicity Study in the Rat
"The Toxicological Evaluation of Oryzalin (Compound 67019) Given to Fischer 344 Rats in the Diet for Two Years" - MRID #'s 00026779, 00044337 & 00070569

Technical oryzalin was administered in the diet to Fischer 344 rats at dosages of 0, 300, 900 or 2700 ppm for two years. There was significant evidence of chronic toxicity and carcinogenicity. (See summary under Revised One-liners).

Systemic NOEL = 300 ppm (12.16 mg/kg/day - males; 13.82 mg/kg/day - females)
Systemic LEL = 900 ppm (36.86 mg/kg/day - males; 42.69 mg/kg/day - females)

Oryzalin was carcinogenic at doses of 300 ppm or higher in female rats (mammary tumors) and 900 ppm or higher in male rats (combined benign skin tumors).

Classification: Core Minimum

2) Carcinogenicity Study in the Mouse
"The Toxicological Evaluation of Oryzalin (Compound 67019) Given to B6C3F, Mice in the Diet for Two Years" - MRID #'s 00026780 & 00068079

Technical oryzalin was administered in the diet to B6C3F, mice at dosages of 0, 500, 1350 and 3650 ppm for two years. Body weight gain was decreased in the 3650 ppm group males and females. There was no evidence that oryzalin was carcinogenic when administered at levels as high as 3650 ppm.

Systemic NOEL = 1350 ppm - males & females
Systemic LEL = 3650 ppm - males & females
(These levels are revised from a previous DER.)

Classification: Core Minimum

3) 1982 Developmental Toxicity Study in the Rabbit
"A Replicated Teratology Study on Oryzalin (EL-119, Compound 67019) by the Oral Route in Dutch Belted Rabbits" - MRID # 00098461

Pregnant Dutch Belted rabbits received either 0, 10, 25, 55 or 125 mg/kg/day of technical oryzalin by oral gavage on gestation days 6 through 18, inclusive. Minimal maternal toxicity was observed at the mid and high dose groups as reduced food consumption during the dosing period. There was no evidence of developmental toxicity.

Maternal Toxicity NOEL = 25 mg/kg/day
Maternal Toxicity LEL = 55 mg/kg/day
Developmental Toxicity NOEL > 125 mg/kg/day
Developmental Toxicity LEL ≥ 125 mg/kg
(These levels are revised from a previous DER.)

Classification: Core Minimum

4) 1978 Developmental Toxicity Study in the Rabbit
"A Teratology Study on Lilly Compound 67019 (Oryzal'n) in the Rabbit" - MRID #'s 00026785, 00052557 & 00073552

Pregnant Dutch Belted rabbits received either 0, 25, 55 or 125 mg/kg/day of technical oryzalin by oral gavage from gestation days 6 through 18, inclusive. Maternal toxicity was observed in the form of decreased body weight gain and decreased food consumption in the 55 and 125 mg/kg/day groups. Developmental toxicity was observed in the form of reduced mean litter size, increased resorptions and increased postimplantation loss.

Maternal Toxicity NOEL = 25 mg/kg/day
Maternal Toxicity LEL = 55 mg/kg/day
Developmental Toxicity NOEL = 25 mg/kg/day
Developmental Toxicity LEL = 55 mg/kg/day

Classification: Core Minimum

<u>Revised One-Liners</u>

1) Citation: 83-1(a) and 83-2(a) Feeding/oncogenic - 2 yr. - rat

Reason for revision: 1) Revised in accordance with new DER; 2) Correction - oncogenic LOEL was based on mammary tumors observed at 300 ppm, not skin tumors.

Revision:

Technical oryzalin was administered to Fisher 344 rats at dosages of either 0, 300, 900 or 2700 ppm for two years. Survival was significantly decreased in the 2700 ppm group males and females. Mean body weight and body weight gain were reduced in the 2700 ppm group males and females. Feed efficiency was decreased in the 2700

ppm females. Decreases in RBC parameters (HGB, HCT, RBC count and MCHC) were significantly decreased in the 900 and 2700 ppm group females and the 2700 ppm group males. Increases in weight were observed in the following organs: absolute and/or relative weights of the liver in the 900 and 2700 ppm group males and females; absolute and relative weights of the thyroid in the 2700 ppm males; and relative weight of the kidney in the 900 and 2700 ppm males and females. There was an increased incidence of cystic follicles, C-cell hyperplasia and focal follicular hyperplasia of the thyroid in the 2700 ppm group males and females. There was an increased incidence of neoplasms of three organs, the skin, thyroid and mammary gland. It was concluded that the thyroid tumors resulted from oryzalin's interference with thyroxin formation and the compensatory overstimulation of the gland. The combined tumor incidence of benign mammary tumors (adenomas, fibroadenomas and adenocarcinomas) was increased at all of the dosage levels. The combined tumor incidence of skin papillomas, keratoacanthomas, and squamous cell carcinomas was significantly increased in the 2700 ppm group males and females. The combined tumor incidence of skin basal cell adenomas, preputial gland adenomas, sebaceous gland adenomas, Zymbal's gland adenomas and trichoepitheliomas was significantly increased in the 900 and 2700 ppm males and females.

Classification: Core Minimum

2) Citation: 83-1 (a) and 83-2 (b) Feeding/oncogenic - 2 yr - mouse

Reason for revision: Revised in accordance with new DER

Revision:

Technical oryzalin was administered in the diet to B6C3F$_1$ mice at dosages of 0, 500, 1350 and 3650 ppm for two years. Body weight gain was decreased in the 3650 ppm group males and females. There was no evidence that oryzalin was carcinogenic when administered at levels as high as 3650 ppm.

Systemic NOEL = 1350 ppm - males & females
Systemic LEL = 3650 ppm - males & females

Classification: Core Minimum

3) Citation: 83-1(b) Feeding - 1 year oral capsule - dog

Revision for revision: The RfD/Peer Review Committee concluded that the NOEL should be 50 mg/kg/day.

Revision:

Technical oryzalin was administered in oral capsules to beagle dogs at dosages of either 0, 1.5, 5, 15/250/500* or 50 mg/kg/day for one year. (* 15 mg/kg/day for weeks 0-14; 250 mg/kg/day for weeks 15-32

and 500 mg/kg/day for weeks 47-52). Males and females in the 75/225/500 group had decreases in RBC parameters (RBC count, HCT and HGB) at various testing periods during the study. There was no other evidence of systemic toxicity. Serum thyroxine (T_4) and TSH stimulation tests were within normal limits for the treated animals.

Systemic Toxicity NOEL = 50 mg/kg/day
Systemic Toxicity LEL = 500 mg/kg/day

Classification: Core Minimum

4) Citation: 83-3(a) Developmental Toxicity Study - rat - MRID # 41163801

Reason for revision: At the RfD/Peer Review Committee meeting, it was concluded that the DER for this study was in error. The reduced fetal weight of the pups in the mid (255 mg/kg/day) and high (1000 mg/kg/day) dose groups was overlooked in assigning the developmental toxicity levels.

Revision:

Pregnant CD rats received either 0, 50, 225 or 1000 mg/kg/day of technical oryzalin by oral gavage from gestation days 6 through 17, inclusive. There was evidence of maternal toxicity in the form of significant reductions in mean daily food consumption and mean body weight gain in the 225 and 1000 mg/kg/day dose groups. Developmental toxicity was evidenced as significantly reduced fetal weight in the pups of the 225 and 1000 mg/kg/day dose groups. There was also an increased incidence of incomplete ossification of the forepaw metacarpal bones in pups of the 1000 mg/kg/day dose group.

Maternal Toxicity NOEL = 50 mg/kg/day
Maternal Toxicity LEL = 225 mg/kg/day
Developmental Toxicity NOEL = 50 mg/kg/day
Developmental Toxicity LEL = 225 mg/kg/day

Classification: Core Guideline

5) Citation: 83-3(b) Developmental Toxicity Study - rabbit - MRID #'s 00026785, 00052557 & 00073552

Reason for revision: The one-liner is consistent with the findings of the new DER, however there is an error in the levels tested on the one-liner.

Revision:

It should read 0, 25, 55, & 125 mg/kg/day rather than 0, 25, 75 & 125 mg/kg/day.

Classification: Core Minimum

6) Citation: §3-1(b) Developmental Toxicity Study - rabbit - MRID # 00098461

Reason for revision: Revised in accordance with new DER.

Revision:

Pregnant Dutch Belted rabbits received either 0, 10, 25, 55 or 125 mg/kg/day of technical oryzalin by oral gavage on gestation days 6 through 18, inclusive. Minimal maternal toxicity was observed at the mid and high dose groups as reduced food consumption during the dosing period. There was no evidence of developmental toxicity.

Maternal Toxicity NOEL = 55 mg/kg/day
Maternal Toxicity LEL = 125 mg/kg/day
Developmental Toxicity NOEL > 125 mg/kg/day
Developmental Toxicity LEL ≥ 125 mg/kg/day

Core Minimum: Core Minimum

Reviewed by: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 7/24/93
Section I, Toxicology Branch II (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. [signature] 7/24/93
Section I, Toxicology Branch II (H7509C)

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REVISED DATA EVALUATION REPORT[1]

STUDY TYPE:	Combined Carcinogenicity/Chronic Toxicity/Rats (83-5)
EPA I.D. NUMBERS:	P.C. CODE: 104201 MRID NUMBERS: 00026779, 00044332 and 00070569
TEST MATERIAL:	Oryzalin (Technical)
STUDY NUMBER:	Replicate Studies, R-167 and R-177
TESTING FACILITY:	Lilly Research Laboratories, Greenfield, Indiana
SPONSOR:	Elanco Products Company Indianapolis, Indiana
TITLE OF REPORT:	The Toxicological Evaluation of Oryzalin (Compound 67019) Given to Fischer 344 Rats in the Diet for Two Years
AUTHOR(S):	J. L. Carter
REPORT ISSUED:	March 1980
CONCLUSIONS:	In two replicate studies, technical oryzalin was administered to Fisher 344 rats at dosages of either 0, 300, 900 or 2700 ppm for two years. The actual mean mg/kg/day dosages received in the 300, 900 and 2700 ppm groups were 12.16, 36.96, and 112.46, respectively, for the males and 13.82, 42.89 and 135.86, respectively, for the females. Survival was significantly decreased in the 2700 ppm group males and females at Month 24 of the study. Mean body weight was significantly decreased in the 2700 ppm group males and females. Body weight gain was decreased in the 2700 ppm group females throughout the study; it was significantly decreased in the males only at the end of the study. Food consumption was comparable between the treated and control groups. Feed efficiency was decreased in the 2700 ppm group females. Decreases in RBC parameters (HGB, HCT, RBC count and MCHC) were significantly decreased in the 900 and 2700 ppm group females and in the 2700 ppm group males. Increases in the absolute weight of the liver, thyroid gland and heart were seen in the 2700 ppm group males;

[1] The original DERs are numbered 000959, 000961 and 004962.

the weight of the adrenal glands was increased in the 2700 ppm group females; increased liver weight was seen in the 900 ppm group males. Increases in the relative weight of the liver, thyroid, heart, kidney and testes were seen in the 2700 ppm group males; increases in the liver, heart, kidney, spleen and adrenal glands were seen in the 2700 ppm group females; increases in the liver and kidneys were seen in the 900 ppm group males and females. At necropsy, the incidence of three non-neoplastic thyroid lesions (cystic follicles, C-cell hyperplasia and focal follicular hyperplasia) was increased in the 2700 ppm group males and females. There was an increased incidence of neoplasms of three organ systems, the skin, thyroid and mammary gland. The combined tumor incidence of skin fibromas and fibrosarcomas was significantly increased in the 900 and 2700 ppm group males. The combined tumor incidence of skin papillomas, keratoacanthomas, and squamous cell carcinomas was significantly increased in the 2700 ppm group males and females. The combined tumor incidence of skin basal cell adenomas, preputial gland adenomas, sebaceous gland adenomas, Zymbal's gland adenomas and trichoepitheliomas was significantly increased in the 900 and 2700 ppm males and females. The combined tumor incidence of thyroid follicular cell adenomas and carcinomas was significantly elevated in the 2700 ppm group males and females. The combined tumor incidence of mammary gland adenomas, fibroadenomas and adenocarcinomas was increased in the 300, 900 and 2700 ppm group females.

The study demonstrated that oryzalin is carcinogenic at doses of 300 ppm and higher in female Fisher 344 rats and 900 ppm (36.86 mg/kg/day) and higher in male Fisher 344 rats.

The systemic No Observed Effect Level (NOEL) was 300 ppm (males: 12.16 mg/kg/day; females: 13.92 mg/kg/day)

The systemic Lowest Observed Effect Level (LEL) was 900 ppm (males: 36.86 mg/kg/day; females: 42.89 mg/kg/day) based on decreases in hematology parameters in the females and increases in the weight of the liver and kidneys in both sexes

The Maximum Tolerated Dose (MTD) was less than 2700 ppm and greater than 900 ppm based on decreased survival in the 2700 ppm group and minimal signs of toxicity in the 900 ppm group.

CLASSIFICATION: Minimum - This study satisfies the guideline requirements (83-5) for a carcinogenicity/chronic toxicity study in rats.

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I. MATERIALS

A. Test Material

Name: Oryzalin
Chemical Name: 3,5 dinitro-N⁴,N⁴-dipropyl-sulfanilamide
Lot Numbers and Purity: Lot X23607: 96.5%; Lot 9SY47: 96.0%
Description: Not Stated
Storage Conditions: Not Stated

B. Administration: dietary

C. Test Animals

Species: Fisher 344 rats
Source: Harlan Industries, Cumberland, Indiana
Age: 5 to 6 weeks
Initial body weight: Study R-16, - males: 144.6 g; females: 113.5 g;
Study R-17 - males: 140.4 g; females: 110.7 g

D. Animal Husbandry and Acclimation

The rats were group housed by litter and sex for one week (acclimation period) after which they were housed individually in stainless steel wire mesh cages. Animals for both of the replicates were housed in one room where the temperature was 25 ± 1.1°C with a minimum relative humidity of 45% and a 12 hour light/dark cycle. The test chemical was mixed with a mash ration, Lilly ANIORT, which was prepared every 2 to 4 weeks. Food and water were available *ad libitum*.

E. Administration and Dietary Preparation

Dosage Groups

A computer-generated randomization table was used to assign animals to the following groups (the number of animals/group was identical in the replicates)

Dosage Level (ppm)	Number of Males	Number of Females
0 (Control)	30	30
300 (Low)	30	30
900 (Mid)	30	30
2700 (High)	30	30

Diet Preparation

The procedure for the mixing of the test material in the ration is not described. Samples of each dietary level were analyzed at the beginning of the study and after aging for one or two weeks in the animal rooms. Thereafter, samples were assayed every four months.

F. Experimental Design

The following observations and examinations were made at the indicated times or frequencies.

observations for mortality and moribundity - once daily
"close observation" - once a week
body weight - once a week
food consumption - once a week
hematology and clinical chemistry - at end of two-year treatment period
necropsy - gross and histopathology - all animals

G. Pathological Parameters

Hematology

At the end of the treatment period, the surviving animals were fasted overnight and blood was drawn before necropsy. The following (CHECKED) parameters were evaluated:

X Hematocrit (HCT)*
X Hemoglobin (HGB)*
X Leukocyte count (WBC)*
X Erythrocyte count (RBC)*
__Platelet count*
X Prothrombin Time (50% of animals)
__Total plasma protein (TP)
X Leukocyte differential count
__Mean corpuscular HGB (MCH)
__Mean corpuscular HGB conc. (MCHC)
__Mean corpuscular volume (MCV)

* EPA Guideline Requirement

Clinical Chemistry

The following (CHECKED) parameters were analyzed:

Electrolytes:
__Calcium*
__Chloride*
__Magnesium*
__Phosphorus*
__Potassium*
__Sodium*

Enzymes:
X Alkaline phosphatase
__Cholinesterase
__Creatine phosphokinase*
__Lactic acid dehydrogenase
X Serum alanine aminotransferase (also SGPT)*
__Serum aspartate aminotransferase (also SGOT)*

Other:
__Albumin*
X Blood creatinine*
X Blood urea nitrogen*
__Cholesterol*
__Globulins
X Glucose*
X Total Bilirubin*
__Total Protein*
__Triglycerides

* EPA Guideline Requirement

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Post-mortem Examinations

All those animals which died during the study or were sacrificed because of their poor condition or the size of their neoplasm were subjected to gross and histopathology examinations. At the end of the treatment period, the surviving animals were killed with carbon dioxide and immediately necropsied. The following CHECKED (X) tissues were collected for histological examination. The (XX) organs) in addition were weighed.

Digestive System
__Tongue
X Salivary glands*
__Esophagus*
X Stomach
X Duodenum*
X Jejunum*
X Ileum*
X Cecum*
__Colon*
X Rectum*
XX Liver*
__Gall bladder*
X Pancreas*

Respiratory System
__Trachea*
X Lung*

Cardiovasc./Hemat. System
__Aorta*
XX Heart*
__Bone marrow*
X Lymph nodes*
XX Spleen*
X Thymus*

Urogenital System
XX Kidneys*
X Urinary bladder*
XX Testes*
__Epidic mides
XX Prostate
__Seminal vesicle
XX Ovaries
XX Uterus*

Neurologic System
X Brain*
__Periph. nerve*
__Spinal cord (3 levels)
X Pituitary*
X Eyes (Optic n.)*

Glandular
XX Adrenals*
__Lacrimal gland
X Mammary gland*
XX Parathyroids*
XX Thyroids*

Other
X Bone*
X Skeletal muscle*
X Skin
X All gross lesions and masses

H. Statistical Analyses

The Dunnett's two-tailed t-test was used to test for statistical significance in the following parameters: weight gain, hematology, clinical chemistry, terminal body weights, organ weights and relative organ weights. Tumor and survival data were analyzed for linear trend using an arc sin transformation of proportions.

I. Compliance

The study was not done in compliance with the GLP regulations. A final study review was conducted by the Quality Assurance Unit of the testing facility. No Statement of No Data Confidentiality Claims or FIFRA Flagging Statement has been submitted.

.I RESULTS

The study report states that there were no toxicologically important differences between the replicate studies, therefore the results were presented together.

A. Diet Analyses and Actual Dosages

Diet Analyses

Analyses of samples stored for one and two weeks at room temperature showed that the concentrations of oryzalin in the diets were relatively stable. Analyses at quarterly intervals showed that the concentration of the chemical in the diet ranged from 81% to 120% of the theoretical value with the exception of one

analysis (after about 18 months of treatment) that was 155% of the theoretical value for the high dose group. Another diet was prepared that approximated the theoretical value.

Actual Dosages

The mean mg/kg dosages achieved during the replicate studies were as follows:

	Dosage Groups					
	Low		Mid		High	
Study Number	Males	Females	Males	Females	Males	Females
R-167	12.23	13.86	36.74	42.39	111.89	139.19
R-177	12.08	13.77	36.98	43.39	113.02	132.52
Mean	12.16	13.82	36.86	42.89	112.46	135.86

Calculated by the reviewer from Tables 11 and 12 (pages 58 and 59 of the study report).

B. Survival and Clinical Observations

Survival

Survival was decreased in the high dose groups at two years in both studies. The number and percentage survival is summarized in Table 1.

Table 1
Cumulative Survival (Number and Percentage) of Mice Treated with Oryzalin in the Diet for Two Years*

	Dosage Levels (ppm)							
	Males				Females			
Month of Study	0	300	900	2700	0	300	900	2700
Month 1								
Study R-167	30 100%	30 100%	30 100%	30 100%	30 100%	30 100%	30 100%	30 100%
Study R-177	30 100%	30 100%	30 100%	30 100%	30 100%	30 100%	30 100%	30 100%
Month 3								
Study R-167	30 100%	30 100%	30 100%	29 97%	30 100%	30 100%	30 100%	30 100%
Study R-177	30 100%	30 100%	30 100%	30 100%	30 100%	30 100%	30 100%	30 100%
Month 6								
Study R-167	30 100%	30 100%	30 100%	29 97%	30 100%	30 100%	30 100%	30 100%
Study R-177	30 100%	30 100%	30 100%	30 100%	30 100%	30 100%	30 100%	30 100%
Month 12								
Study R-167	30 100%	30 100%	30 100%	29 97%	30 100%	30 100%	30 100%	29 97%
Study R-177	28 93%	29 97%	29 97%	30 100%	30 100%	30 100%	30 100%	30 100%
Month 18								
Study R-167	29 97%	30 100%	29 97%	23 77%	27 90%	29 97%	29 97%	26 87%
Study R-177	25 83%	29 97%	24 80%	27 90%	29 97%	30 100%	27 90%	27 90%
Month 24								
Study R-167	24 80%	19 63%	20 67%	16 53%	23 77%	21 70%	19 63%	16 53%
Study R-177	22 73%	21 70%	16 53%	17 57%	21 70%	19 63%	18 60%	15 50%

* Extracted from Tables 3 and 4 (pages 40 and 41 of the study report).

C. Clinical Observations

The study report states that there were no treatment-related changes in physical appearance in the rats but that the treated rats had yellow stained pelage due

to excretion of the compound. No data on clinical observations have been submitted.

D. Body Weight and Body Weight Gain

Body Weights

There were no statistically significant differences in body weight in either of the replicate studies until the end of the studies. In Study R-167 at 24 months, mean body weights were significantly decreased in the 2700 ppm group males and females. In Study R-177, there were significant decreases at 24 months in the 900 and 2700 ppm group females.

Body Weight Gain

Mean body weight gain was significantly decreased in the 2700 ppm group females throughout both replicate studies. The decreases in males were significant only at the end of the study. Table 2 summarizes the body weight gain data at selected times.

Table 2
Mean Weight Gain (G) in Rats Treated with Oryzalin in the Diet for Two Years[a]

	Dosage Levels (ppm)							
	Males				Females			
	0	300	900	2700	0	300	900	2700
0 - 3 Months								
Study R-167	202.6	201.3 (99)[b]	205.6 (101)	195.3 (96)	81.1	75.9 (96)	76.5 (94)	69.5* (86%)
Study R-177	203.8	199.4 (98)	207.0 (102)	198.2 (97)	83.6	81.4 (97)	80.3 (96)	71.7* (86%)
0 - 12 Months								
Study R-167	314.6	313.7 (98)	318.4 (101)	300.6 (96)	156.5	151.8 (97)	147.3 (94)	118.6* (76%)
Study R-177	322.6	317.3 (98)	329.2 (102)	307.7 (95)	168.6	153.1* (91%)	150.2* (89%)	123.4* (73%)
0 -18 Months								
Study R-167	335.7	327.2 (97)	337.3 (100)	308.1* (92%)	207.6	195.0 (94)	197.0 (95)	162.8* (78%)
Study R-177	343.2	336.6 (98)	358.5 (104)	304.9* (89%)	211.0	198.9 (94)	191.8* (91%)	162.5* (77%)
0 -24 Months								
Study R-167	289.6	292.4 (101)	277.2 (96)	241.4* (83%)	204.1	199.1 (98)	189.5 (93)	134.6* (66%)
Study R-177	287 3	274.7 (96)	286.4 (100)	259.5 (90%)	208.7	201.4 (97)	182.0 (87)	143.3* (69%)

[a] Extracted from Tables 5 and 6 (pages 42-45 of the study report).
[b] Percentage of control value
* Significantly different from control, $p \leq 0.5$

E. Food Consumption and Feed Efficiency

Food consumption was comparable between the treated and control groups in both of the replicates. Feed efficiency was decreased in the 2700 ppm group females in both replicates. No statistical analyses were done on this parameter.

F. Clinical Pathology

Hematology

Decreases in RBC parameters (HGB, HCT and RBC count) were seen in the 900 and 2700 ppm group females and in the 2700 ppm group males in both of the replicates. Decreases in MCHC were seen in the 2700 ppm males (ore replicate) and females. Table 3 summarizes these data.

Table 3
Selected Hematology Parameters in Rats
Treated with Oryzalin in the Diet for Two Years*

	Dosage Levels (ppm)							
	Males				Females			
	0	300	900	2700	0	300	900	2700
Hematocrit								
Study R-167	40.27	38.79	37.62	33.11*	37.59	36.64	32.91**	30.92**
Study R-177	40.51	37.52	38.22	33.96*	37.[illegible]	36.39	31.80**	30.25**
Hemoglobin								
Study R-167	15.07	14.33	13.97	11.83*	14.21	13.78	12.29**	11.16**
Study R-177	15.33	14.05	14.27	12.78	14.12	13.71	12.06**	11.00**
Red Blood Cell Count								
Study R-167	7.838	7.501	7.558	6.338*	6.980	6.818	6.039*	5.599**
Study R-177	7.888	7.196	7.471	6.959	6.897	6.750	5.797**	5.427**
MCHC								
Study R-167	37.40	36.60	37.29	35.08**	37.88	37.65	37.16	35.79**
Study R-177	37.75	37.07	37.09	37.51	37.97	37.69	37.87	36.17*

* Extracted from Tables 13.01-13.02 (pages 52-53 of the study report) and Tables 15.01-15.02 (pages 70-71).
* Significantly different from control, $p \leq 0.5$
** Significantly different from control, $p \leq 0.1$

The study report indicates that the mean erythrocytic parameters were within expected normal clinical range for rats of this age. Several rats in the 2700 ppm group had marked decreases in RBC parameters which may have been compound-related. No data on normal values were provided in the study report. It is noted that the incidence of polychromasia (indicative of young RBC's) is increased in the 2700 ppm group males and females in both replicates. Therefore, evidence exists that these decreases were significant enough to cause an erythrocytic response and were likely treatment-related.

Clinical Chemistry

There were statistically significant increases in BUN and SGPT values in the 2700 ppm group females in Study R-167 and BUN in the 900 ppm group females in Study R-177. There was also a significant decrease in creatinine in the 2700 ppm group males in Study R-167. The study report indicates that all these values were within normal limits for rats of this age but no data on normal ranges were provided. Data on individual animals show that the increases in the mean values were due to large increases in a few animals in each group and were probably not treatment-related.

G. Post-mortem Findings

Organ Weight

Absolute Organ Weight

The mean weight of the following organs was increased:

liver - 2700 ppm group males - both replicates
900 ppm group males - one replicate
thyroid gland - 2700 ppm group males - one replicate
heart - 2700 ppm group males - one replicate
adrenal gland - 2700 ppm group females - one replicate

Relative Organ Weight

The mean relative weight of the following organs was increased:

liver - 900 ppm group females and 2700 ppm males and females - both replicates
900 ppm group males - one replicate
thyroid gland - 2700 ppm group males - one replicate
kidney - 900 and 2700 ppm group females - both replicates
900 and 2700 ppm group males - one replicate
spleen - 2700 ppm group females - one replicate
adrenal - 2700 ppm group females - both replicates
testes - 2700 ppm group males - one replicate

The data on the selected organs is summarized in Table 4.

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Table 4
Weight of Selected Organs at Necropsy in Rats
Treated with Oryzalin in the Diet for Two Years*

		Dosage Levels (ppm)							
		Males				Females			
		0	300	900	2700	0	300	900	2700
Body Weight (g)									
Study R-167		438	436	417	387**	319	312	300	251**
Study R-177		431	412	430	403	321	310	292*	259**
Liver (g)									
Study R-167	A	10.11	10.75	11.60*	12.20**	7.11	7.44	7.79	7.62
	R	2.31	2.47	2.79**	3.19**	2.23	2.39	2.59**	3.03**
Study R-177	A	10.57	11.07	11.26	11.98*	7.30	7.76	7.96	8.12
	R	2.46	2.75	2.63	2.99**	2.28	2.50	2.72**	3.13**
Thyroid (mg)									
Study R-167	A	46.7	42.1	79.6	42.4	31.8	30.4	49.5	35.8
	R	10.61	9.59	19.27	10.97	9.98	9.80	16.67	15.14
Study R-177	A	40.0	37.5	56.5	136.8*	52.5	30.5	40.7	42.9
	R	9.40	9.11	12.99	35.28*	17.51	10.03	13.85	16.67
Heart (g)									
Study R-167	A	1.13	1.19	1.18	1.25*	.88	.85	.87	.83
	R	.26	.28	.29	33*	.28	.27	.23	.34**
Study R-177	A	1.25	1.17	1.30	1.19	.88	.87	.89	.85
	R	.29	.29	.30	.30	.28	.28	.31	.33**
Kidney (g)									
Study R-167	A	2.81	2.84	2.93	3.04	1.90	1.90	2.00	1.92
	R	.64	.65	.70*	.79**	.60	.61	.67**	.78**
Study R-177	A	3.07	2.86	2.82	2.93	1.99	1.93	2.02	1.94
	R	.71	.70	.66	.74	.63	.62	.71*	.75**

* Extracted from Tables 21 - 28 (pages 108-148 of the study report).
A = absolute organ weight; R = relative organ weight (organ weight per 100 grams of body weight)

n-neoplastic Lesions

both replicates, the most frequently reported non-neoplastic changes were bile t hyperplasia, progressive glomerulonephritis and degeneration of the testis. e incidence of these lesions was comparable between the treated and control groups. In Study R-167, the incidence of extramedullary hematopoiesis was increased in the 2700 ppm group females (5/30 vs 0/30 in the control group). In Study R-177, the incidence of skin lesions (abscess, edema, keratin cyst and suppurative dermatitis) were increased in the 2700 ppm group (2/30 in male and female control animals vs 10/30 and 5/30 in the 2700 ppm group males and females, respectively). In this study, purulent endometritis was also increased in the 2700 ppm group females (7/30 vs 2/30 in the control group).

In both replicates, there was an increased incidence of three non-neoplastic thyroid lesions (cystic follicles, C-cell hyperplasia and focal follicular hyperplasia) in the 2700 ppm group. The incidence of these lesions is summarized in Table 5.

Table 5
Incidence[a] of Non-neoplastic Thyroid Lesions in Rats
Treated with Oryzalin in the Diet for Two Years[b]

	Dosage Levels (ppm)							
	Males				Females			
	0	300	900	2700	0	300	900	2700
Cystic Follicles								
Study R-167	2	3	5	9	2	6	6	9
Study R-177	1	2	7	10	0	2	7	6
C-cell Hyperplasia								
Study R-167	1	0	0	1	5	4	0	2
Study R-177	2	0	4	0	1	3	1	0
Focal Follicular Hyperplasia								
Study R-167	2	1	2	7	0	0	3	6
Study R-177	0	1	2	8	0	0	2	8

[a] Thirty animals were examined in each group.
[b] Extracted from Tables 29 (pages 149-156) and 31 (pages 161-168 of the study report).

Neoplastic Lesions

Statistical Analysis

According to the statistical analysis (Appendix I, pages 768-772 of the study report), there was a significant increase in females with benign tumors due to increases in skin and mammary tumors. If these tumors are omitted from the analysis, there is a decrease in benign tumor-bearing males and no effect of treatment in females. The overall incidence of malignant tumors was not affected

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in males or females. The number and percentage of animals with benign and malignant tumors is provided in Table 6.

Table 6
Incidence of Benign and Malignant Tumors in Rats
Treated with Oryzalin in the Diet for Two Years*

	Dosage Levels (ppm)									
	Males					Females				
	0	300	900	2700	Z stat.*	0	300	900	2700	Z stat.
Benign Tumors	39/60 (65.0)	47/60 (78.3)	41/60 (68.3)	50/60 (83.3)	1.815	36/60 (59.3)	42/60 (70.0)	52/60 (86.7)	49/60 (81.7)	3.457
Malignant Tumors	8/60 (13.3)	13/60 (21.7)	13/60 (21.7)	15/60 (25.0)	1.557	11/60 (18.3)	10/60 (16.7)	11/60 (18.3)	12/60 (20.0)	296

* Extracted from Table 1 (Appendix I, page 770 of the study report).
b Statiatically significant if Z-statistic is outside the range -2.48 to +2.48

Dermal Neoplasms

The incidence of benign skin and adnexal neoplasms (fibroma, keratoacanthoma, trichoepithelioma, papilloma, sebaceous gland adenoma, preputial gland adenoma, and Zymbal's gland adenoma) was increased in the treated groups. The incidence of fibrosarcomas and squamous cell carcinomas was also increased in the treated groups. According to the study report, none of these lesions were considered life-threatening nor were they clinically important. The data are summarized in Table 7.

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Table 7
Incidence[a] of Benign and Malignant Skin and Adnexal Lesions in Rats Treated with Oryzalin in the Diet for Two Years[b]

	Dosage Levels (ppm)							
	Males				Females			
	0	300	900	2700	0	300	900	2700
Basal Cell Adenoma								
Study R-167	0	2	1	0	0	1	0	0
Study R-177	2	2	1	0	0	0	0	1
Fibroma								
Study R-167	2	2	4	4	1	1	1	1
Study R-177	1	1	5	7	1	1	1	2
Keratoacanthoma								
Study R-167	3	1	2	9	1	0	0	5
Study R-177	1	2	2	7	0	1	3	3
Papilloma								
Study R-167	1	3	0	4	0	1	0	0
Study R-177	0	0	1	3	0	0	0	1
Preputial gland adenoma								
Study R-167	1	5	8	2	-	-	-	-
Study R-177	3	1	4	6	-	-	-	-
Sebaceous gland adenoma								
Study R-167	1	1	3	1	1	2	3	3
Study R-177	2	2	1	4	1	2	4	2
Zymbal's gland adenoma								
Study R-167	0	0	1	2	0	0	1	0
Study R-177	0	1	1	0	0	0	0	4
Trichoepithelioma								
Study R-177	0	0	0	3	0	0	0	0
Total Benign Tumors	17	23	34	52	5	9	13	22
Fibrosarcoma								
Study R-167	1	1	2	2	0	1	1	0
R-177	0	1	2	0	0	1	1	2
Squamous cell carcinoma								
Study R-167	0	0	1	1	0	0	1	0

[a] Thirty animals were examined in each group.

[b] Extracted from Tables 30 (pages 157-160) and 32 (pages 169-172 of the study report).

Thyroid Neoplasms

The incidence of hyperplastic and neoplastic lesions of the thyroid follicular cells and cystic follicles was increased in the 2700 ppm groups. Four types of thyroid follicular lesions were observed - cystic follicles, focal hyperplasia, adenomas and carcinomas. A histological description of each lesion is given in the study report (page 21). According to the statistical analysis, there was an increase in follicular cell tumors when the sexes are combined. The incidence of the lesions is summarized in Table 8.

Table 8
Incide of Neoplastic Thyroid Lesions in Rats
Treate. Oryzalin in the Diet for Two Years[a]

	Dosage Levels (ppm)							
	Males				Females			
	0	300	900	2700	0	300	900	2700
C-cell Adenoma								
Study R-167	2	6	0	3	0	1	5	2
Study R-177	4	6	1	1	4	1	0	3
C-cell Carcinoma								
Study R-177	0	0	0	0	1	0	0	0
Follicular Cell Adenoma								
Study R-167	1	4	3	1	0	0	1	3
Study R-177	0	2	2	3	1	1	2	5
Follicular Cell Carcinoma								
Study R-167	0	0	0	0	0	0	0	1
Study R-177	0	0	0	3	0	0	0	0
Total Follicular Neoplasms	1	6	5	7	1	1	3	14

[a] Thirty animals were examined in each group.
[b] Extracted from Tables 30 (pages 157-160) and 32 (pages 169-172 of the study report).

Mammary Gland Neoplasms

The incidence of mammary gland tumors in females, especially fibroadenomas, was increased in all the treated groups as compared to the control group, although the increases were not dose-responsive. Table 9 summarizes the data.

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Table 9
Incidence* of Mammary Gland Tumors in Female Rats
Treated with Oryzalin in the Diet for Two Years*

	Dosage Levels (ppm)			
	0	300	900	2700
Adenoma				
Study R-167	0	0	0	2
Study R-177	0	1	1	0
Fibroadenoma				
Study R-167	6	12	15	12
Study R-177	4	8	22	17
Adenocarcinoma				
Study R-167	0	0	0	1
Study R-177	0	0	1	0
Total	10	21	39	32

* Thirty animals were examined in each group.
* Extracted from Tables 30 (pages 157-160) and 32 (pages 169-172 of the study report.)

III. DISCUSSION

Three Peer Review Meetings have been held on this chemical. The first meeting assessed the adequacy of the carcinogenicity studies. At the second meeting on September 16, 1985, the following conclusions were drawn about this study:

(1) The highest dose (2700 ppm) exceeded the maximum tolerated dose (MTD), however the mid-dose level (900 ppm) did not exceed the MTD.

(2) A statistically significant positive trend occurred in female rats for thyroid follicular cell adenomas and carcinomas combined. The combined tumor incidence was significantly elevated at the 2700 ppm level in both males and females due primarily to an increase in adenomas.

(3) A statistically significant positive trend for skin fibromas and fibrosarcomas combined occurred in male rats. The combined tumor incidence was significantly elevated at dose levels of 900 and 2700 ppm in male rats primarily due to an increase in fibromas.

(4) A statistically significant positive trend for skin papillomas, keratoacanthomas, and squamous cell carcinomas combined occurred in both male and female rats. The combined tumor incidence was significantly elevated at 2700 ppm in both males and females, primarily due to increases in papillomas and keratoacanthomas in males and to an increase in keratoacanthomas in females.

(5) A statistically significant positive trend for skin basal cell adenomas, preputial gland adenomas, sebaceous gland adenomas, Zymbal's gland adenomas and

trichoepitheliomas combined occurred in both male and female rats. The combined tumor incidence was significantly elevated at doses of 900 and 2700 ppm in both males and females and was due primarily to increases in preputial gland adenomas, sebaceous gland adenomas and trichoepitheliomas in males and sebaceous gland and Zymbal's gland adenomas in females.

(6) A statistically significant positive trend for mammary gland adenomas, fibroadenomas and adenocarcinomas combined occurred in female rats. The combined tumor incidence was significantly elevated at doses of 300, 900 and 2700 ppm and was due to an increase in fibroadenomas.

(7) A statistically significant positive trend for hepatic adenomas and bile duct adenomas combined occurred in male rats.

The Peer Review Panel concluded that there was presumptive evidence that the thyroid tumors were in response to the anti-thyroid action of the chemical. Oryzalin, like other para-substituted aniline derivatives, inhibits the formation of thyroxin, resulting in positive feedback stimulation of the pituitary to release TSH thereby causing thyroid gland hypertrophy and the ensuing hyperplasia and tumor formation. However, based on the occurrence of the other tumors, oryzalin was classified as a Category C (possible human) carcinogen.

At the June 15, 1990 Peer Review Meeting, it was concluded that the mammary gland tumors (adenomas, fibroadenomas, and adenocarcinomas combined) would be used for the risk extrapolation.

IV. STUDY DEFICIENCIES

1. No data on clinical observations were submitted.

2. Clinical pathology examinations were conducted only at the end of the study rather than at six-month intervals as required by the Guidelines.

3. Optnalmological examinations were not done.

V. CONCLUSIONS

In two replicate studies, technical oryzalin was administered to Fisher 344 rats at dosages of either 0, 300, 900 or 2700 ppm for two years. The actual mean mg/kg/day dosages received in the 300, 900 and 2700 ppm groups were 12.16, 36.86, and 112.46, respectively, for the males and 13.82, 42.89 and 135.86, respectively, for the females. Survival was significantly decreased in the 2700 ppm group males and females at Month 21 of the study. Mean body weight was significantly decreased in the 2700 ppm group males and females. Body weight gain was decreased in the 2700 ppm group females throughout the study; it was significantly decreased in the males only at the end of the study. Food consumption was comparable between the treated and control groups. Feed efficiency was decreased in the 2700 ppm group females. Decreases in RBC parameters (HGB, HCT, RBC count and MCHC) were significantly decreased in the 900 and 2700 ppm group females and in the 2700 ppm group males. Increases in the absolute weight of the liver, thyroid gland and heart were seen in the 2700 ppm

group males; the weight of the adrenal glands was increased in the 2700 ppm group females; increased liver weight was seen in the 900 ppm group males. Increases in the relative weight of the liver, thyroid, heart, kidney and testes were seen in the 2700 ppm group males; increases in the liver, heart, kidney, spleen and adrenal glands were seen in the 2700 ppm group females; increases in the liver and kidneys were seen in the 900 ppm group males and females. At necropsy, the incidence of three non-neoplastic thyroid lesions (cystic follicles, C-cell hyperplasia and focal follicular hyperplasia) was increased in the 2700 ppm group males and females. There was an increased incidence of neoplasms of three organ systems, the skin, thyroid and mammary gland. The combined tumor incidence of skin fibromas and fibrosarcomas was significantly increased in the 900 and 2700 ppm group males. The combined tumor incidence of skin papillomas, keratoacanthomas, and squamous cell carcinomas was significantly increased in the 2700 ppm group males and females. The combined tumor incidence of skin basal cell adenomas, preputial gland adenomas, sebaceous gland adenomas, Zymbal's gland adenomas and trichoepitheliomas was significantly increased in the 900 and 2700 ppm males and females. The combined tumor incidence of thyroid follicular cell adenomas and carcinomas was significantly elevated in the 2700 ppm group males and females. The combined tumor incidence of mammary gland adenomas, fibroadenomas and adenocarcinomas was increased in the 300, 900 and 2700 ppm group females.

The study demonstrated that oryzalin is carcinogenic at doses of 300 ppm and higher in female Fisher 344 rats and 900 ppm (36.86 mg/kg/day) and higher in male Fisher 344 rats.

The systemic No Observed Effect Level (NOEL) was 300 ppm (males: 12.16 mg/kg/day; females: 13.82 mg/kg/day)

The systemic Lowest Observed Effect Level (LEL) was 900 ppm (males: 36.86 mg/kg/day; females: 42.89 mg/kg/day) based on decreases in hematology parameters in the females and increases in the weight of the liver and kidneys in both sexes

The Maximum Tolerated Dose (MTD) was less than 2700 ppm and greater than 900 ppm based on decreased survival in t e 2700 ppm group and minimal signs of toxicity in the 900 ppm group.

VI. Classification - Minimum - See STUDY DEFICIENCIES

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY 000758 01061

DATE: January 7, 1981

SUBJECT: Oryzalin, TB/HED evaluation of 2-year feeding/oncogenicity study in the rat, submitted in support of requested tolerances at 0.05 ppm on certain fruits/nuts.

FROM: Dr. Mary L. Quaife, TB/HED (TS-769) M.L.Q. 1/7/81

TO: Mr. R. Taylor, PM Registration Division (TS-767)

THRU: Mr. William Burnam, Acting Chief Toxicology Branch/HED (TS-769) W.L.Burnam

ALG for R.B. Jaeger 1/29/81

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PP No. 6F1859
EPA Registration Nos. 1471-96, 112

Eli Lilly and Company
Indianapolis, Indiana 46206

SUMMARY:

1. The two-year feeding study (Acc. Nos. 099517 and 099518) is judged acceptable to support tolerances (Core-minimum rating) with a "No-observed-effect level" of 300 ppm in the diet (= 15 mg/kg body weight/day) with respect to chronic (non-oncogenic) effects.

2. TB/HED requests the following information with respect to the two-year rat feeding study (Acc. Nos. 099517 and 099581) to enable TB/HED to evaluate the oncogenicity potential of oryzalin in the rat:

   a. Tables of incidence of each skin and each thyroid tumor for each of the following time-intervals of the study:
      15 months (test day 452),
      18 months (test day 543),
      20 months (test day 599, Study R-167; test day 598, Study R-177),
      and 22 months (test day 662) - as in Tables 30 and 32 (24 months).

      (Time intervals are chosen to correspond to those of Tables 3 and 4, which give survival data.)

   b. Resolution of apparent discrepancies in giving purity of test sample of oryzalin used:

      i. On p. 19 of this report, purity of lots X28607 and 9SY47 is given as 96.5 and 96.0%, respectively.

      ii. In Appendix C of the report, p. 761, purity of lots X28607 and 9SY47 is given as 97.4 and 98.9 mole %, respectively.

      iii. Report of rat reproduction study (Acc. No. 241742, Appendix C, p. 13), gives purity of lot X28607 (Sodyeco production lot 86343) as 99.0% (although a preliminary figure of 96.5% purity was used in protocols). P. 65 of the reproduction study report (Acc. No. 24172) states that the lot of test material used in the rat reproduction study was used in "chronic/oncogenic tests," also.

   c. Chemical identity of following impurities of test sample of oryzalin used in the 2-year rat study, noted on p. 759, Appendix C, Acc. No. 099518: Compounds 68761, 69469, 32507, and 36001.

EPA Form 1320-6 (Rev. 3-76)

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NEW TOX STUDIES:

1. "The toxicological evaluation of oryzalin (compound 67019) given to Fischer 344 rats in the diet for two years." by James L. Carter et al., Toxicology Division, Eli Lilly & Co., Greenfield Indiana, March 1980, Acc.# 094017.

Test substance. Oryzalin* (compound 67019; EL-119; 3,5-dinitro-N^4,N^4-dipropylsulfanilamide, lots X28607 (96.5% pure, used until 6/21/78) and 9SY47 (96.0% pure, used until end of study). Assay for content of N-nitroso-di-n-propylamine of first sample, eleven times, and of second sample, six times (monthly), showed contents varying in random order from 0.12 to 0.78 ppm.

Procedure. Groups of 30 M and 30 F rats received in the diet either 0, 300, 900, or 2,700 ppm oryzalin (uncorrected for purity) for two years. Two identical experiments were conducted at the same time. Animals were 5 to 6 weeks old at start, and mean weights were approximately 140 (males) and 110 (females) g. Rats were kept individually in stainless steel, wire mesh cages. The basal diet was a natural food mash ration. Samples of control and test diets were assayed at start and after one or two weeks' aging. Every four months, new samples were assayed for content of test compound.

Animals were checked daily for dead or moribund ones and were observed carefully as to physical condition once a week. Rats were weighed and food consumption determined weekly. Two-year survivors were fasted overnight and blood samples collected for hematologic and clinical chemical testing. Packed cell volume, hemoglobin content, red cell count, total and differential white cell count, and erythrocyte morphology were determined on all rats; while the prothrombin time was measured on one-half of them. On all rats, the serum concentrations of glucose, urea nitrogen (BUN), creatinine, bilirubin, alkaline phosphatase, and glutamic-pyruvic transaminase were each determined.

All animals dying on test, killed while moribund, or killed after two years on test were autopsied grossly "by qualified pathologists with experience in laboratory animal diseases and pathology." Following organs and tissues were collected and fixed in 10% formalin: Gross lesions, nodules, tumors, skin, mammary gland, salivary gland, lung, heart*, thyroid (paratyroid)*, stomach, duodenum, jejunum, ileum, colon, mesenteric lymph node, liver*, skeletal muscle, thymus, pancreas, spleen*, kidney*, adrenal*, urinary bladder, prostate*, testis* ovary*, uterus*, cerebrum, cerebellum, brain stem, pituitary, eye, bone, and bone marrow. Organs starred were weighed. Fixed tissues were stained with hematoxylin and eosin. Evaluation was made by Pathologist (with experience in diagnosis of lesions from rats on long-term toxicity studies") J. L. Carter, D.V.M., Ph.D. Other pathologists, including, Dr. W. Carlton of Purdue, were available for help. Criterion used in making diagnoses was "that of classical pathology." (Organs were weighed and relative organ weights calculated for two-year survivors, only.)

The following mean values were tested for statistical significance with the Dunnett's two-tailed "t" test: Weight gain; hematologic and blood chemical findings; terminal body weights; absolute and relative organ weights. Tumor and survival data were analyzed for linear trend using an arc sine transformation of proportions (Appendix I).

* Note: Appendix C lists the two as giving following assay results:
Lot X28607 96.5% pure and 97.4 Mole % (Assays of 2/26/80 and 1/22/80)
Lot 9SY47 96.0% pure and 98.9 Mole %

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Results. Test diets, fresh and aged, gave assay results for oryzalin content that were very close to theoretical; one exception was a high assay, and new test diet was made when the high result was discovered.

Survival. Inspection of tables for survival at monthly intervals reveals no apparent difference between the sexes in either (replicate) study. No dose-related difference is shown for survival at 12 months, the rate being over 95% for combined sexes in all dose-groups of both studies. Same at 18 months exceeded 80%. However, survival at 24 months showed a "linear trend;" the "Z" statistic exceeded the allowable limits to show this (Appendix I) (in each replicate study). Respective survival rates were, control to highest dose, 76.7, 66.7, 60.0, and 55.0 (first study), and 73.3, 66.7, 61.7, and 51.7% (second study), according to table for "Z" statistic calculation (Appendix I).

We point out that these values differ, in 6/8 cases, from corresponding survival rates at term (Tables 3 and 4 of report) by at least one percent. However, the general trend is decreased survival at term - in Appendix or Tables. ✓

According to Petitioner, there was no toxicologic or pathologic evidence to account for decreased survival rates of treated groups, which are dose-related.

Growth. During the first 12 months, males in either study showed no statistically significant growth retardation; while females in both studies gained one-fourth less than controls when ingesting 2,700 ppm oryzalin. However, both males (top-dose group in first study) and females (top-dose in both studies) had gained significantly less (females, one-third less) than corresponding controls by the end of the study. Body weight gain of mid-dose females was significantly decreased at intervals throughout both studies, also.

Food consumption. There was no evident effect of oryzalin ingestion on food consumption. Efficiency of food utilization was decreased, therefore, when growth was inhibited.

Clinical effects. No toxic effects were manifested overtly, according to the report. However, treated rats had yellow stained fur. ✓

Hematologic values. High-dose males and high- and mid-dose females in both studies showed pronounced decreases in red blood cell values, and in most cases they differed statistically significantly from corresponding control values: Hematocrit, hemoglobin content, and red blood cell count. (Mean corpuscular volume was unaffected, but either mean corpuscular hemoglobin or mean corpuscular hemoglobin concentration was significantly decreased in high-dose males or females.) Prothrombin time was not affected by test compound.

There were statistically significantly increased mean leukocyte counts in some groups. Most groups had individual animals with greatly increased W.B.C.'s, including controls. According to Petitioner, "....alterations in leukocyte differential counts usually supported the chronic or neoplastic disease status in the respective individual animal."

Individual animals in all groups had marked changes in "erythocytic and/or leukocytic parameters."

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Some conditions which caused the "erythrocytic and/or leukocytic" changes in individual animals are identified as malignant lymphoma, purulent endometritis, purulent prostatitis, and extramedullary hematopoiesis.

Blood chemistry values. Blood urea nitrogen (BUN) and serum glutamic-pyruvic transaminase (S-GPT) mean values were significantly increased in high-dose females of first study (R-167). BUN mean value was significantly increased in mid-dose females of second study (R-177). Statistically significantly lower mean value for creatinine was found in high-dose males of first study.

Some individual rat values for AP, SGPT, and BUN were increased, according to report, due to chronic diseases, such as progressive glomerulonephritis, bile duct hyperplasia in the liver, and malignant lymphoma. Total bilirubin was also increased, according to report, in some cases. Inspection of tables of individual values did not reveal serious deviations.

Organ weights. There were statistically significantly increased mean liver weights in mid-dose and high-dose males of first study, along with a significantly increased mean heart weight in high-dose males of the study. Adrenal mean weight of high-dose females was significantly increased. Mean weights of livers and of thyroids of high-dose males were significantly greater than those of corresponding controls in second study. In both studies, high-dose females showed approximately twice as high mean ovarian weights as those of corresponding controls; although variance around the mean was so great in each case as to preclude statistical significance of the differences occurring.

Relative liver and kidney weights in mid-dose and high-dose males and females of first study and of mid- and high-dose females of second study were increased significantly in dose-related fashion.

Following additional increased organ-weight/body-weight ratios, compared to controls, occurred: For high-dose males of first study, heart; for high-dose females of first study, heart, spleen, adrenals; for high-dose males of second study, liver, thyroids, testes; and for high-dose females of second study, heart and adrenals.

According to the report, a cause for increased liver weights in mid- and high-dose groups is "not evident," but it is not due to any condition evident on microscopic examination. Increased thyroid weights in high-dose males in second study (R-177) are ascribed to "antithyroid action" of oryzalin. Other weight changes are not discussed.

Nor does this reviewer find noteworthy explanation of the organ weight changes, enumerated above, which can be related to ingestion of test compound.

Pathologic findings. No explanation, toxicologic or pathologic, is available for decreased survival rates in male and female rats of all test groups, according to report.

A myeloproliferative disease was diagnosed as malignant lymphoma due to lymphoid differentiation of many of the neoplastic cells; it has been described as a mononuclear cell leukemia (Coleman, G. L. et al.: Pathological Changes during Aging in Barrier-reared Fischer 344 Male Rats. J. Gerontol. 32, 258-78 (1977)) and occurs in aged Fischer 344 rats, report says.

The pathologist's summary describes an increase in the "total number of benign skin and adnexal tumors....in all dose groups." However, our Pathologist, Dr. L. Kasza, rejects this grouping as not being a sound one (told to M. Quaife, in discussion, 12/4/80).

The total number of thyroid follicular neoplasms for both studies shows dose-related increases (control, low-, mid-, and high-dose groups, respectively): Female, 1/60, 1/60, 3/60, and 9/60; male, 1/60, 6/60, 5/60, and 7/60. When the total is combined over sexes, and this is tested by "Z-statistic," for a "linear trend in tumorigenesis," a "Z-statistic" value (= 3.493) which exceeds a range of from -2.48 to +2.48 is calculated; this value is significant. Thus, there is an increase in total thyroid follicular cell tumors in all dose-groups. (Cf. Appendix I.) (We note that only 6 tumors for high-dose males are listed in Appendix I, Table 5; however, the tables in the text (p. 159 and p. 170, Tables 30 and 32) list 7 for them.)

This increased incidence of thyroid follicular neoplasms in the treated groups is ascribed by the study report (p. 25) to the "antithyroid activity of a sulfonamide with a para-substituted aminobenzene grouping, with or without aliphatic substitution of the amino nitrogen." This is believed due to increased pituitary thyrotropin release secondary to a deficiency of circulating thyroid hormone. Report claims sulfonamides have antithyroid actions in rats, but (they) "are not clinically goitrogenic in man."*

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In further statistical comparison (in Appendix I), female rats with benign tumors were significantly increased in all treated groups (Z = 3.407). However, female rats with malignant tumors and male rats with either benign or malignant tumors did not vary significantly with treatment (testicular tumors were not counted; evidently, they are very common in aged male rats of this strain (IARC monograph, Supplement 2, "Long-term and short-term screen assays for carcinogens: A critical appraisal," IARC, Lyon, 1980, p. 73)).

Twenty-four-month (but not 12-month) survival was found to be significantly decreased in males and females (Appendix I). For males, control to high-dose, in order, 24-month survival was 40, 40, 36, and 33 rats, each, out of 60, and for females, correspondingly, it was 44, 40, 37, and 31, each out of 60. Corresponding "Z-statistics" are -2.642 and -2.530; both are significant.

In further analysis (Appendix I), omission of skin and mammary tumors, results in decrease in benign tumor-bearing males, with no effect of treatment on females. The incidence of benign mammary tumors is increased (10, 21, 37, and 29, each out of 60 in female rats, controls on up, "Z statistic" being 4.554, which is significant.

Top-dose females (Appendix I) were classified as to mammary tumor positive or negative and follicular (thyroid) cell positive (hyperplasia, adenoma, carcinoma) or negative. However follicular cell problems were as prevalent in non- (9 positive, 21 negative) as in positive-mammary-tumor-bearing animals (10 positive, 20 negative.

* Refs. include: McLaren, E. H. and Alexander, W. D.: Goitrogens. Clin. End. Metabolism. 8, 129-44 (1979); MacKenzie, C. G. and McKenzie, J. B.: Effect of Sulfonamides and Thioureas on the Thyroid Gland and Basal Metabolism. Endocrinology 32, 185-209, (1943); (contd. on next page)

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In males, there is a significant (Z = -2.436) decrease in pituitary tumors (actual numbers, control to high-dose males, in order, being 16, 17, 5, and 9, each out of 60).

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Discussion. Petitioner's own analysis of the data shows that oryzalin is oncogenic to the Fischer 344 rat, having caused increased, dose-related incidence of thyroid follicular cell tumors in males and females and increased incidence of benign mammary tumors in females. The occurrence of the benign mammary tumors in females is apparently independent of the occurrence of the follicular thyroid tumors. In addition, two-year survival was decreased, significantly, in dose-related fashion, in both males and females.

Although Petitioner speaks of increased numbers of skin tumors, he does not provide statistical analysis of their occurrence for degree of significance. Because differences in occurrence of such tumors at two years could be masked by differences in survival among controls and treated rats, we ask Petitioner to provide further information on this point.

Specifically, Petitioner is asked to provide tables of incidence of all skin and all thyroid tumors at each of the following time-intervals of the study: 15 months (test day 452)
18 months (test day 543)
20 months (test day 599, Study R-167; test day 598, Study R-177)
and 22 months (test day 662)
The time intervals are chosen to correspond to those of Tables 3 and 4 which give survival data; so that we can calculate incidence in actual survivors, if we wish to do so. (The request for these data is based on discussions of the reviewer with Dr. L. Kasza, TB/HED Pathologist.) The tables will give incidence figures for all individual skin and thyroid tumors - at requested time-intervals - exactly like the tables on neoplastic lesions (Table 30, pp. 156 and 159, and Table 32, pp. pp. 168 and 170) do.

Petitioner's figures for tumor incidence in various parts of the report do not agree with each other. An example is given in second paragraph of the preceding page. For another example: Summing total incidence of benign mammary tumors from Table 30, p. 159, and Table 32, p. 170, gives, in order of control to high-dose groups, in females: 10, 21, 38, and 31, each out of 60; these comprise total incidence of mammary gland adenomas and fibroadenomas. Yet in Appendix I, Table 4, incidence for two top dose-groups is given as 37 and 29, respectively.

It appears, by inspection, that these slight differences in tumor incidences - in various parts of the report - do not affect the conclusions as to statistical significance of their occurrence in treated rats to an extent which will affect conclusions as to the biological effect of oryzalin ingestion on tumor production in these rats.

* Refs contd. from previous page: Astwood, E. B.: In: Pharmacological Basis of Therapeutics. Goodman, L. S. and Gilman, A. eds., 1483-7, Macmillan, New York, 1970; Swarm, R. L., Roberts, G. K. S., Levy, A. C., Hines, L. R.: Observations on the Thyroid Gland in Rats Following the Administration of Sulfamethoxazole and Trimethoprim. Toxicol. Applied Pharmacol. 24, 351-63 (1973); Astwood, E. B., Sullivan, J., Bissel, A., and Tyslowitz, R.: Action of Certain Sulfonamides and of Thiourea upon the Function of the Thyroid Gland of the Rat. Endocrinology 32, 210-25 (1943); Furth, J.: Pituitary Cybernetics and Neoplasia. Harvey Lect.: 63, 47-71 (1969).

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There are discrepancies in the chemical composition of the test compound given in varying places.

On p. 19 of this report, purity of lots X28607 and 9SY47 is given as 96.5 and 96.0%, respectively.

In Appendix C of this report, p. 761, however, the respective contents of oryzalin are listed as 97.4 and 98.9 "mole percent" for lot X28607 (Soydeco Corp.) and lot 9SY47 (Eli Lilly & Co.).

Appendix C, p. 759, lists four impurities in these two preparations, as follows:

Compound	Lot X-28607	Lot 9SY47
[redacted]	[redacted]	[redacted]

The impurities are not identified, and they should be. Petitioner is asked to do so.

Petitioner should also clear up the discrepancies between reported values for content of oryzalin and is asked to do so.

We note that it is very important that the test compound in this chronic/oncogenicity study be entirely representative of the grades produced commercially; else the toxicity of the latter is not truly being evaluated.

In addition, we note that the report of the reproduction study in rats, carried out on technical oryzalin (Acc. No. 241742), states (on p. 65) that the lot of material used in the reproduction study was also that used in chronic/oncogenic tests. (Whether that means the rat chronic/oncogenic test is not specified there.)

Acc. No. 241742, Appendix C, gives 99.0% purity for "lot X-28607," Soydeco production lot 86343, while stating (on p. 134, Appendix C) that a preliminary figure used in the protocols was 96.5% - for degree of purity.

Again, we ask Petitioner to tell whether that batch (lot X-28607, Soydeco production lot 86343) was used in this rat chronic/oncogenic study (Accession No. 099518), and, if so, to resolve the discrepancy between the 99.0% pure value given in the reproduction study report and the two values given in the chronic/oncogenic rat study report (cf. second and third paragraphs, this page.

Summary, oncogenicity study. Pending answering questions respecting occurrence of all individual skin tumors and thyroid tumors seen in the rats in this study at the specified intervals, the study cannot be evaluated, finally, as to oncogenic potential of oryzalin in the rat, nor can a CORE rating be assigned.

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Summary, chronic study. The study is judged to provide, with respect to chronic effects, a "no-observed-effect-level" though there was statistically significant, dose-related decrease in survival in the test rats (24-month survival) - cf. Appendix I, pp. 769-70. However, according to Mr. B. Litt, statistician, effect is not signif. at 300 ppm in the diet (= 15 mg/kg body weight/day). There was pronounced growth inhibition in rats on the top dose and some in mid-level rats, as well.

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MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

Also, such parameters as red blood cell count, hemoglobin concentration, and hematocrit were significantly decreased in high- and mid-dose groups. Also, relative and absolute liver weights were increased in high- and mid-dose rats (mean values of); although adverse effects were not seen on microscopic examination of livers from these rats.

Thus for rat chronic feeding (but not for oncogenicity - not yet evaluated), the NOEL is 300 ppm in diet or 15 mg/kg body weight/day, and the lowest effect level is 900 ppm (45 mg/kg BW/day), effects including decreased red cell count, hemoglobin content, and hematocrat and increased absolute and relative liver weight; some growth inhibition

Rat chronic (2-year) feeding study is rated Core-minimum.

1. Only terminal hematologic and blood chemical examinations (or assays) were made.

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

JUN 12 1981

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM

DATE: June 3, 1981

Caswell # 623A

SUBJECT: TB/HED comments on Eli Lilly responses of 3/6/81 and 2/24/81 to the EPA letter of 2/4/81 to Lilly, Oryzalin (Surflan®).

FROM: Mary L. Quaife, Ph.D. M.L. Quaife 6/4/81
Toxicology Branch, HED (TS-769)
RTSJC/11/81
RTS for CC13

TO: Mr. R. Taylor, PM
Registration Division (TS-767)

THROUGH: Mrs. Christine F. Chaisson, Acting Chief
Toxicology Branch, HED

PP No. 6F1859
Reg. Nos. 1471-96 and 112

Eli Lilly and Company
Indianapolis, Indiana
46206

SUMMARY:

(Numbers and letters of major headings, underlined, correspond to those in the EPA letter of 2/4/81 to Petitioner.) PM, please see footnote, below.

1. Two-year rat (chronic) feeding (and oncogenicity) study, 2. a.

A. Petitioner's requested new tables show that time distribution of occurrence of skin tumors is roughly similar for control and test rats.

B. Therefore, we compiled detailed tables of occurrence of certain tumors (and non-neoplastic thyroid lesions) in individual rats, including time of every rat on test.

C. These tables await TB/HED statistical evaluation for significance of results and estimate of "risk."

*** D. Until this is accomplished, TB/HED can make no final conclusions as to oncogenic potential of oryzalin.

Note: For convenience' sake, we have placed asterisks in front of all statements which relate directly to regulatory status of these TOX studies.

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E. This memo (pp. 5-10, inclusive) reviews some facets of tumor production by certain pesticide products, which - like oryzalin - are designated antithyroid agents.

Review is based on all Petitioner's cited references of this study; on certain "inhouse" documents, including WHO/FAO proceedings and R-PAR documents; and on results of a specific literature search made for us.

Following are points made, which relate to toxicology of oryzalin:

a. TB/HED agrees (with Petitioner), oryzalin causes thyroid follicular-cell tumors in the rat, probably, by indirect overstimulation of the thyroid gland.

b. Therefore, thyroid tumor "risk" estimates by a so-called one-hit model may be inappropriate.

c. We judge other (than thyroid) tumors rate inclusion when extrapolating oncogenic activity of oryzalin from test animals to man.

d. TB/HED finds (incidence of) certain non-neoplastic thyroid lesions in test rats important for (1) evaluating oryzalin's toxicologic potential in the rat and (2) extrapolating it to man.

e. We are unaware of any determination, experimentally, of the potency of oryzalin to affect thyroid function in the rat.

f. TB/HED finds this an important omission in Petitioner's studies on oryzalin, especially should this study be found to not support a clearcut "no-observed-effect" level (NOEL) with regard to thyroid (histopathologic) structure.

g. TB/HED judges that Petitioner's cited, indirect evidence (Refs. 5-14, inclusive) fails to establish the comparative antithyroid or oncogenic potencies of oryzalin in rat and monkey (or man).

h. Petitioner has not determined either comparative potency for oryzalin, experimentally.

i. TB/HED agrees, in the rat, thyroid tumor development due to oryzalin is probably reversible at an early stage (and before any such tumor appears).

2. Two-year rat (chronic) feeding (and oncogenicity) study, 2., b. and c.

A. TB/HED judges the newly identified impurities in technical oryzalin to be not of consequence in the trace amounts in which they occur.

B. Presence at less than 1 ppm in technical oryzalin (as affirmed by RCB) of N,N-di-n-propylnitrosamine (a carcinogen and mutagen) does not require further "risk" evaluation (in regard to its presence), apparently, according to proposed Agency policy.

C. Up to 0.8 ppm of this nitrosamine impurity was present in the test oryzalin samples of chronic/oncogenicity and reproduction studies; therefore, its presence in commercial oryzalin products was adequately taken into account in such testing.

D. Content of active ingredient in technical oryzalin varies by a factor of ca. 2% (PCB/HED); that degree of variation is, accordingly, judged not of consequence to TB.

*** E. TB/HED finds, specific deficiencies (2.b. and 2.c.) of rat two-year study are resolved.

3. Three-generation rat (reproduction) study, 5.

A. We judge Petitioner's additional evidence as showing that effects on eyes in the rat reproduction study were not caused by oryzalin.

*** B. TB/HED concludes that deficiency 5 of the three-generation rat study is resolved.

C. The study is upgraded to CORE-MINIMUM

*** D. The NOEL is set at 250 ppm.

4. Rat dominant-lethal study, 2.

A. Petitioner's response is judged insufficient to allow upgrading of the study (as found by two other prior TB/HED reviewers).

*** B. TB/HED concludes that the deficiencies (2. and 3.) of the study are not (and cannot be) resolved.

C. Thereforefore, the study remains CORE-SUPPLEMENTARY.

5. Petitioner has promised to run a DNA repair study on oryzalin. TB/HED did not request or approve (and was not asked to approve) this action

6. Final conclusion, No. 4 (p. 4 of 2/4/81 EPA letter to Lilly).

A. Petitioner has concluded that, "A general metabolism study on oryzalin is needed," (while stating that three previous metabolism studies have been submitted) (cf. p. 3, 3/5/81 letter, Lilly - R. Taylor).

*** B. We agree with Petitioner's proposal to do a new general metabolism study (on oryzalin technical).

*** C. Submission of a protocol for the study, for TB/HED comment, is requested.

TB/HED COMMENTS OF PETITIONER'S REPLIES:

Two-year feeding study, 2., a.

As we have noted previously, Petitioner found oryzalin to be a rat oncogen, having caused thyroid follicular cell and mammary tumors, for example (memo of 1/7/81, this PP, p. 6). Although Petitioner noted increased skin tumor incidence in treated rats, he did not analyze this for statistical significance.

Per suggestion of Dr. L. Kasza, TB/HED Pathologist, we asked Petitioner for numbers of skin and thyroid tumors at 15, 18, 20, 22, and 24 months on test (corresponding to survivor figures in original report). Dr. Kasza pointed out that lesser survival of test rats (which is dose-related) might affect tumor occurrence.

Inspection of Petitioner's submitted tables indicates that time-distribution of skin tumors is roughly similar for control and test rats. Also, groups of skin tumors which are biologically similar show dose-related increase in incidence, frequently, in males and/or females.

Tumors of skin were grouped as suggested by Dr. Kasza. Recently, the International Agency for Research on Cancer (IARC) stated that....The experienced pathologist uses his knowledge of histogenesis and pathogenesis....in grouping lesions for statistical analysis....In general, it is accepted practice to group together tumours of the same general histological type that arise in the same type of tissue.... [Long-term and short-term screening assays for carcinogens: a critical appraisal. IARC Monographs, Supplement 2, Lyon, France, 1980, p. 69.]

Although oryzalin is a rat oncogen, only analysis of appropriate data can provide basis for judgment as to the degree and biological importance of its oncogenicity in the rat - and to the degree realistically possible - to the human being.

EPA, including Toxicology Branch, Hazard Evaluation Division, is continually trying to improve its mode of statistical and "risk" analysis of oncogenicity data. Computer-based analyses are now - and since only recently - provided by Mr. Bert Litt, TB/HED Statistician, according to priorities set at Division level or higher.

We gave Mr. Litt (4/28/81) detailed tables of incidence of various lesions in individual rats, including time of each on test. Categories included were chosen with the kind assistance of Dr. Kasza. They are: a. Fibroma and/or fibrosarcoma, skin; b. Keratoacanthoma and/or squamous cell carcinoma, skin; c. Papilloma, skin; d. Basal cell adenoma, preputial gland adenoma, sebacious gland adenoma, Zymbal's gland adenoma, and/or tricoepithelioma, skin; e. Hepatic adenoma; f. Nodular hyperplasia, liver; g. Fibroadenoma, mammary gland; h. Adenoma, mammary gland; i. Adenocarcinoma, mammary gland; j. Follicular cell adenoma and/or carcinoma, thyroid gland; k. C-cell adenoma and/or carcinoma, thyroid; l. Cystic follicles in thyroid (non-tumor lesion described, p. 24 of original report, as "markedly enlarged follicles that closely resembled normal follicles except for their increased size and flattened epithelium "); m. Follicular hyperplasia, focal, thyroid gland; Malignant lymphoma, hemic-lymphatic system; Adenoma, pituitary gland; and Interstitial cell tumor tumor, testicle. Further comments on general oncogenetic implications of oryzalin are deferred pending receipt of Mr. Litt's findings.

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A somewhat separate issue (cf. Petitioner's discussion, pp. 25-6, Acc. No. 099517) requires evaluation and HED response.

Petitioner seems to imply that the thyroid follicular tumors produced in oryzalin-fed rats do not have consequence for human beings because of (1) a "known mechanism" operating, (2) which is reversible before tumor formation, and (3) to which man is either unsusceptible or less susceptible than the rat.

We read all of Petitioner's citations which bear on the implied claim (Refs. 4-14, inc., copies of which were provided us) and a considerable number of other pertinent references at hand (Nos. 15-25, inc., and No. 26).

(1) Rat thyroid tumors may result from indirect effect on the thyroid, via the pituitary gland. So-called "antithyroid" substances, e.g., thioamides; aniline derivatives - especially, para-substituted ones; and polyhydric phenols, inhibit formation of the thyroid hormone, thyroxin. To compensate, the pituitary gland pours out more "thyroid-stimulating hormone" (TSH; thyrotropin), which causes the thyroid gland to make more thyroxin. The overstimulated thyroid may show hyperplasia and, on prolonged exposure, may develop tumors, e.g., follicular adenomas and/or carcinomas (8, 10, 11, 12, 13, 14, 15, 16, and 17).

We agree with Petitioner that this may be the mechanism by which the rat thyroid follicular tumors were produced in the present study by oryzalin, which is a para-substituted aniline derivative (as well as being a sulfonamide).

[It might explain the production of thyroid tumors (adenomas and/or carcinomas) produced in rats by ethylenethiourea (a breakdown product of certain bis-dithiocarbamate pesticides as zineb and maneb) (15, 16) and in mice by 3-amino-1,2,4-triazole (aminotriazole; amitrole) (19). Both are well-known antithyroid substances (11, 17).

[In the rat, such process seems to progress from hyperplasia to adenoma to carcinoma, depending on degree and length of exposure to the antithyroid. Rats fed ethylenethiourea (ETU) for two years showed some hyperplasia at 5 and 25 ppm, but not tumors; chiefly, adenomas at 125 ppm; and, chiefly, carcinomas at 250 and 500 pppm (15, 16).

[Somewhat parallel effects on thyroid function were shown by rats fed ETU in the diet (0, 1, 5, 25, 125, or 625 ppm) for 30 or 60 or 90 days. At 125 and/or 625 ppm, rats had lower serum thyroxin and triiodothyronine, increased serum TSH, and decreased uptake of I^{125}. At 60 days on 25 ppm, rats had increased serum thyroxin. [At 625 ppm, clinical evidence of poisoning occurred, and red thyroids and adenoma were seen. At 125 ppm, there were red thyroids and thyroid hyperplasia. At both levels, thyroid-weight to body-weight ratios were significantly increased. There were no effects at 25 ppm, except for thyroid hyperplasia, seen only at 60 days.] There were no effects at 5 ppm (20).]-

Petitioner has not determined levels of oryzalin, specifically, which affect (and those which do not affect) thyroid function in the rat.

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Determining which levels of oryzalin did or did not cause significant increase in adverse microscopic findings or tumors in thyroids of rats fed it for two years awaits Mr. Litt's statistical evaluation.

A factor in making that judgment might be two instances of recorded low occurrence of some spontaneous thyroid lesions in the Fischer 344 rat, which was used in Petitioner's present study:

Among 144 aged, male Fischer 344 rats, incidence of thyroid spontaneous lesions consisted of: 0.7%, thyroid adenoma, and 0.7%, diffuse thyroid hypertrophy, and 2.1%, medullary carcinoma of thyroid (4).

"....Follicular cell neoplasms are uncommon in untreated Fischer 344 rats..., 1.1% or less each....in male and female Fischer 344 rats (1,800 per sex) in the (National Cancer Institute) Carcinogenesis Testing Program.... (therefore, any)....follicular cell tumors found in (test) rats assume greater importance," (7).

(2) Referring to the rat, Petitioner finds that, "The thyroid hyperplasia induced by sulfonamides has been shown to be reversible," (13).

True, we believe, but thyroid tumors do not regress. E.g., "Thyroid hyperplasia was not noticeably reversible in those rats in each group (at 5 to 500 ppm) that were changed to control diet after 66 weeks on ETU diets," (16).

(3) According to Petitioner, a. certain sulfonamides (para-aniline derivatives) which are antithyroid in rats are not clinically goitrogenic in man (9, 10, and 11) or in other animals (no reference cited), and, b. sulfamethoxazole (a para-aniline derivative) caused thyroid hyperplasia and neoplasia in rats but not in monkeys (12).

We note, a. It is true, in doses used clinically, sulfonamides are said to not be detectably antithyroid in man; however, the antituberculous drug, para-aminosalicylic acid (PAS), which like oryzalin is a para-substituted aniline, when given in doses of many grams daily for months, has caused hypothyroidism and goiter in man (9, 11), and antithyroid effects occurred in dog and mouse (and rat) in response to sulfonamides and thioureas (10), and, b. the sulfamethoxazole was given for a relatively much smaller fraction of the monkey's lifespan than the rat's (52 and 60 weeks, respectively) (12), a fact which, in itself, might preclude appearance of neoplasia in the monkey.

[Comparative potencies of the antithyroid compound, ETU, fed to monkeys for five to six months and to rats for three months, are indicated in the table:

PROPRIETARY: NO-OBSERVED-EFFECT DOSE LEVELS FOR ETU IN THE DIET BASED ON THYROID AND PITUITARY PARAMETERS, ppm.

Study	T-3(↓)[a]	T-4(↓)	^{125}I Uptake	Thyroid Weights(↑)	Thyroid Hyperplasia	Pituitary	TSH(↑)
Phase I (Rhesus)	250	50	10(↓)	50	<50	50	50
Phase II (Rhesus)	50(M) 150(F)	50	<50(↑)	50 <50(F)	<50	50	50

(table cont'd., next page)

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(table continued from previous page)

Study	T-3(↓)[a]	T-4(↓)	^{125}I Uptake	Thyroid Weights(↑)	Thyroid Hyperplasia	Pituitary	TSH(↑)
Rats	125	25	125(↓)	25	5(60 d) 25(30 & 90d)	Not examined	25

(a) The arrows represent the trend of the parameter as higher doses of ETU are given.

Note. Data come from references 20 (rat) and 21 (monkey) - this table is Table 18 (21, p. 52).

[Quoting from p. 51 of the monkey ETU study (21), "The question as to whether the rat and rhesus monkey are similarly affected by the antithyroid agent, ETU, (has) not (been) answered in terms of its carcinogenic activity as judged by histopathological examination of thyroid tissue....Nor has (a no-effect level) been established for primates. Therefore, the relative sensitivity to the carcinogenic action of ETU in the two species is unknown.

["Similar sensitivities are observed in the two species to ETU for T-3, T-4, and TSH and thyroid weights. T-4 and TSH are both sensitive biochemical indicators of ETU toxicity, and could be monitored in intact animals. Thyroid hyperplasia occurs at dose levels below the no-effect levels for TSH and T-4 alterations. However, the hormonal alterations may still be accurate predictors of a neoplastic condition of the thyroid in experimental animals and perhaps human (beings)."]

We do not imply that relative antithyroid potencies of oryzalin in rat and monkey are similar (or dissimilar). We point out, however, that Petitioner has failed to determine these for oryzalin, specifically, with regard to either thyroid function or thyroid oncogenic activity.

In sum, regarding Petitioner's implied claim (Para. 2, p. 3 of this memo), we agree that oryzalin probably shows antithyroid activity in the rat by way of the proposed mechanism and that it is probably reversible at some early (but as yet undetermined) stage. However, in no way, do we find Petitioner's cited, mostly indirect evidence (Refs. 5-14) sufficient to establish the relative antithyroid or oncogenic potencies of oryzalin in rat and monkey (or man).

[The following brief discussion concerns choice of procedures for extrapolating thyroid oncogenic activity of an antithyroid substance, ETU, from rat to man.

ETU-induced rat thyroid neoplasms are said to result from excessive pharmacological stimulation, i.e., they occur because of excessive endocrine-organ stimulation and not because of the reaction of one molecule of carcinogen with one cell to initiate neoplasia (16).

The Midwest Research Institute (MRI) agrees (22) and adds that the "one hit model" is irrelevant to these thyroid tumors. As reasons, MRI notes (given in abbreviated form) (22, p. IV-14):

a. The thyroid gland in man has a large store of hormone and slow rate of normal turnover, and functioning of the rat thyroid is not comparable to that of man.

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b. In the rat thyroid, there is the "Wolff-Chaikoff effect," a phenomenon which is qualitatively different in the rat and man.

c. A sex difference in rat response (in thyroid tumor production) to ETU is shown (23). This indicates that such a tumor could be related to the rodent endocrine system, requiring consideration of its many variables before human extrapolation of the data is valid.

Thus, use of the one-hit model for human risk assessment in the ETU-induced thyroid tumor (based on rat data) is a "poor choice;" even though other (linear or one-hit?) mathematical model of risk determination may be adopted for most regulatory determinations (of liver tumor risk, for example) (MRI, 22).]

[We note, MRI (22) holds that, since thyroid effects (hyperplasia) are seen at lower doses than the tumors (in the rat, caused by ETU), thyroid toxicity is sufficiently important to be considered, itself, as a trigger area for RPAR.]

We believe that consideration of other possible target organs than the thyroid gland - below - is very germane to assessing the rat oncogenic potential of oryzalin.

Liver.

Antithyroid substances have caused liver tumors in mice, i.e., ETU and aminotriazole (19).

ETU caused biochemical effects on hamster liver, including increased hepatic glutamic-pyruvic transaminase and alkaline phosphatase (Gak (23), as quoted by MRI (22), p. II-23).

Although liver tumors (adenomas) occurred in approximately one per cent of rats (at FDA) in the authors' experience, yet the antithyroid compound, "thicurea, administered orally to (those) rats for a prolonged period of time, induce(d) liver tumors, without liver cirrhosis, in a large percentage of cases at concentrations which may be below those producing hyperplasia of the thyroid gland," (18).

Liver adenomas and hyperplastic nodules are not common spontaneous lesions in most rat strains (personal communication from Dr. L. Kasza, EPA Pathologist).

Following examples are taken from the NCI study on 2,4-diaminoanisole sulfate, a para-substituted aniline derivative, in the Fischer 344 rat (and mouse).

Skin and related glands (and thyroid).

Dietary administration of a para-substituted aniline derivative, 2,4-diaminoanisole sulfate, to the Fischer 344 rat increased the incidences of malignant tumors of the skin and its glands and malignant thyroid tumors in each sex (24).

The former include(malignant) squamous-cell carcinomas, basal-cell carcinomas, or sebaceous adenocarcinomas of the skin and associated glands (Zymbal's gland/ear canal and preputial/clitoral gland).

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The latter consist of various malignant follicular-cell, thyroid tumors (adenocarcinoma NOS, papillary adenocarcinoma, papillary cystadenocarcinoma, or follicular-cell carcinoma).

Male test rats had increased incidence of either a C-cell adenoma or a C-cell carcinoma, as well.

The incidence of integumentary neoplasms was increased, particularly in males, and the incidence of thyroid neoplasms was increased in rats of both sexes.

Mammary gland.

The response of the mammary gland to 2,4-diaminoanisole sulfate appears equivocal; since there was wide variation in the numbers of mammary tumors found in low-dose control females compared with high-dose control females.

Although the incidence of mammary fibroadenomas in low-dose females (35%) was significantly ($p = 0.011$) greater than in low-dose controls (10%), yet that for high-dose females (8%) was inversely related to that for high-dose controls (38%). Historical control incidence of spontaneous mammary fibroadenomas in untreated female rats, numbering 585, of this strain, Fischer 344, is 18%.

[We note that Handler et al. (25, p. 948) find that, "The status of thyroid functioning in man markedly influences the rates of metabolism" of estrogens, e.g., with respect to hydroxylation and conversion of hydroxy to keto groups. We do not know whether such a relation obtains in the rat.]

Other organs or tissues.

Incidence of testicular interstitial cell tumors in males was high (80 to 98%) and not different in control and test groups (24).

Incidence of pituitary tumors, adenomas or carcinomas, in test or control rats of either sex did not differ (24).

Incidence of malignant lymphoma was low in male and female test rats and not higher than in respective controls (24).

Other species.

In this study, dietary administration of 2,4-diaminoanisole sulfate, also, induced thyroid tumors in B6C3F1 mice of each sex (24). They were follicular adenomas in males; in females (45 in number) there were six rats with follicular-cell adenomas and two with follicular-cell carcinomas.

These female mice (24) showed possibly increased incidence of malignant lymphoma; males did not.

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Discussion.

This study (24), then, seems to give a coherent picture of tumors produced in Fischer 344 rats (the strain used in the oryzalin oncogenicity study) by dietary administration of a para-substituted aniline derivative, 2,4-diaminoanisole sulfate (a chemical class to which oryzalin belongs), which is, demonstrably, an antithyroid substance.

Note that thyroid follicular tumors and tumors of skin and related glands (Zymbal's/ear canal, preputial/clitoral) were significantly increased in rats of both sexes and were malignant.

Without prejudicing evaluation of the oryzalin study (in the Fischer 344 rat), we note that numerical increases in thyroid follicular tumors and tumors of skin and related glands occurred in test rats. There were, also, dose-related increases in benign mammary fibroadenomas in females and some neoplastic lesions of liver in males at the high dose (only).

We suggest that tumors of any organ or tissue found in oryzalin-treated rats (which exceed in incidence that in the controls) are worthy of consideration in extrapolating oryzalin's oncogenic activity from rat to man.

Further, we suggest extrapolating the non-neoplastic, antithyroid activity of oryzalin from rat to man as a factor in making safety judgments of its use in agriculture or occurrence in the human diet.

To date (as noted), insofar as we are aware, Petitioner has (a) provided neither determination of activity of oryzalin on thyroid function in rat or monkey, yet (b) seemed to imply that man will be relatively insensitive to such effect.

Petitioner has cited lack of hyperplasia or neoplasia of the thyroid of monkeys - in contrast to presence of these in rats - fed sulfamethoxazole (a sulfonamide as oryzalin is) (12) [implying thereby that man is either less sensitive or insensitive to antithyroid effects of sulfonamides than the rat]. These are structural effects on the thyroid gland.

Therefore, it is of great interest to note that this same compound (sulfamethoxazole), when given to human, healthy adults for only 10 days, "Lowered serum T-3, T-4, and free T-4 indexes in both sexes....Thus, 10-day periods of drug administration do not cause hypothyroidism, but may reduce conversion of iodine to the organic form," (26).* These are functional effects on the human thyroid gland.

Conclusion:

To this reviewer, the desirable toxicologic characterization of oryzalin, for the purpose of extrapolating safety of proposed uses(s) to man, remains incomplete without determining its potency in affecting function of the thyroid gland - at least, in the rat and, preferably, in the monkey, too, in comparative studies.

This seems especially urgent, in case a clear cut "no-effect level" is found not to have been established in this two-year feeding study in the rat, with respect to adverse effects of oryzalin on thyroid gland structure.

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* To man, sulfamethoxazole was given in 5 to 1 combination with the drug, trimethoprim (26). To monkeys and rats, sulfamethoxazole was given either alone, or among various combinations, 5 to 1 with trimethoprim; antithyroid effects, when manifested, were unaffected by presence of trimethoprim (12) and, thus, must be ascribed only to the sulfamethoxazole. [Doses of sulfamethoxazole given are: To man, 13 mg/kg BW/day (if the adult weighed 60 kg), for 10 days (26). To rat, 25 to 600 mg/kg BW/day, for 60 weeks; to monkey, 50 to 300 mg/kg BW/day for 52 weeks (12).]

We thank Mr. R. Ceder of OPP User Service Center for locating (5/29/81) Ref. 26 for us.

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6/3/81

010612

Two-year Feeding Study, 2., b. and c.

In our memo of 1/7/81, this PP, we noted (under Summary) apparent discrepancies in composition of lots of test chemical (oryzalin) used in various toxicologic studies, including reproduction and chronic/oncogenic studies.

So-called Lots X28607 and 9SY47 were variously expressed as being 96.5 and 96.0% pure; 97.4 and 98.9 mole %, respectively, and Lot X28607 was also given as 99.0% pure.

In addition, [redacted] were found present on analysis of oryzalin used in this 2-year feeding/oncogenicity study. We asked for their identity.

We wanted information as to whether the oryzalin which was tested in reproduction and chronic feeding/oncogenic studies is entirely typical of that produced commercially for uses in connection with this PP.

Because one impurity is acknowledged by Petitioner to be mutagenic in Ames-type testing (cf. our 1/23/81 memo, this PP, Summary, point 5) and because of the acknowledged presence in technical oryzalin of di-n-propylnitrosamine, a known carcinogen and mutagen (personal communication of Dr. I. Mauer, TB/HED, 5/29/81), the question is important.

In reply of 2/10/81, Petitioner notes that, "Our chemists advise us using three different analytical methods on the same lot of material over a three-year period, one could expect a 2% variation in assay results." Chemical identity of the impurities is provided.

In reply of 3/5/81, it is stated that results were determined by both differential scanning colorimeter, a method used to determine small amounts of impurities in a highly purified material, i.e., an analytical standard, and by high pressure liquid chromatography, which is specific for oryzalin.

[redacted] impurities not of consequence in trace amounts.

Since RCB (memo of M. Nelson, 9/7/78, PP 8E2075) finds less than 1 ppm of di-n-propylnitrosamine to be present in 75% W Surflan (oryzalin tech., also) and based on EPA's proposed choice of 1 ppm as a practical limit of detection for analytical methods for nitrosamine contaminants in pesticides, as noted in the Federal Register, V. 45, No. 124, p. 42856, 6/25/80, concern as to presence of this impurity seems to be alleviated in accord with Agency policy.

Since the oryzalin used in diets of experimental animals in oncogenic and reproduction studies on it was found by chemical analysis to not have more than 1 ppm di-n-propylnitrosamine, then that test material corresponds to commercially marketed oryzalin (Surflan 75W, e.g) with respect to content of di-n-propylnitrosamine (cf. TB memos, this PP, of 1/7/81, p. 2, and 1/23/81, p. 5).

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

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[redacted]

The Nelson memo (of 9/7/78, PP 8E2075) notes, also, that purity of technical grade oryzalin may vary, typically, from 96.9 to 98.8%(purc). Therefore, we defer to RCB that this degree of variation is acceptable; since we regard known impurities acceptable, as noted in preceding paragraphs.

Conclusion: Our questions as to apparent discrepancies in composition of lots of technical oryzalin used in TOX studies are answered satisfactorily and our concern is satisfied. This "deficiency" is resolved.

Three-generation rat reproduction study, 5.

Because of the fact that oryzalin is a dinitro compound (and, thus, theoretically, possibly related to known cataract-causing chemicals), we asked for details of adverse eye effects noted in course of the rat reproduction study.

Petitioner has provided the information asked for.

Inspection of these tables provides assurance that the effects found were not related to exposure to the test chemical, oryzalin. Petitioner says (in 3/2/81 response), "The majority of the eye conditions noted in the multi-generation study were considered to be infectious in origin and were of short duration. The infection was not considered related to oryzalin treatment," and, "....findings at termination showed no evidence that the conditions were either dose or treatment-related....if result of in utero exposure, F_2 generation rats would have been similarly affected....(Instead) in the F_2 generation, the only eye opacity occurred in the control group."

Conclusion: Petitioner's response relieves the (point 5) deficiency with respect to the reproduction study. We now rate it as CORE-MINIMUM. Conservatively, we judge the NOEL to be 250 ppm. (There was borderline effect on pre- and post-weanling growth of rats in the 750-ppm group.)

Rat dominant-lethal study, points 1, 2, and 3.

We stated that this study is supplementary and cannot be upgraded to CORE (minimum or guideline).

Petitioner's defense of the conduct of the study is judged inadequate to change its status. In this view of it, we are joined by prior TB/HED reviewers, Dr. L. Anderson And Dr. M. Adrian Gross.

Conclusion:

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Petitioner's response to points 1, 2, and 3 on the rat dominant-lethal study is judged insufficient to allow upgraling of the study to acceptable status. The dominant-lethal study (rat) on oryzalin remains CORE-SUPPLEMENTARY.

We note that Petitioner promises to run a DNA Repair Study, starting in mid-April and ending (final report to EPA) by June 15, 1981. [TB/HED did not request this study, and our opinion of the worth of carrying it out was not sought by Petitioner.]

12

MANUFACTURING PROCESS INFORMATION IS NOT INCLUDED

Final conclusion, No. 4, p. 4, of the 2/4/81 EPA ltr. to Eli Lilly.

Petitioner concludes that, "A general metablism study on oryzalin is needed," (bottom of p. 3, 3/5/81 ltr., Lilly - R. Taylor), while stating that three previous metabolism studies have been submitted.

We are agreeable with Petitioner's intent to do a new general metabolism study (in accord with proposed guideline requirements, F. R., 8/22/75). We ask Petitioner to send TB/HED a proposed protocol before the study is done.

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Case file

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

MAR 12 1985

MEMORANDUM

OFFICE OF PESTICIDES AND TOXIC SUBSTANCES

SUBJECT: Oryzalin Registration Standard - Oncogenic Studies on Oryzalin in Rodents [623A]

TO: Robert Taylor/James Yowell
Product Managers (25)
Herbicides-Fungicides Branch
Registration Division (TS-767)

THRU: Robert B. Jaeger
Head, Review Section I
Toxicology Branch
Hazard Evaluation Division (TS-769)

FROM: John H.S. Chen, D.V.M.
Review Section I
Toxicology Branch
Hazard Evaluation Division (TS-769)

Toxicology Branch is asked to comment on the oncogenic potential of oryzalin to humans from the final evaluation of long term oncogenic studies in mice and rats.

Following our previous review of the 2-year feeding/oncogenic study in the rat (TB Memo 1/7/81 M.L. Quaife), the 2-year feeding/oncogenic study in the mouse (TB Memo 10/7/81 M.L. Quaife), and the carcinogenicity/oncogenicity assessment of the 2-year feeding/oncogenic study in the rat (TB Memo 3/17/82 B.D. Litt), we have reached the following conclusions:

1. In the 2-year mouse oncogenic study, oryzalin was negative for oncogenicity in the mouse at up to 3650 ppm in the diet (highest level fed).

2. In the 2-year rat oncogenic study, large numbers of tumor bearing animals were identified in both control and treated animals. Statistical evaluation of the 2-year rat chronic feeding/oncogenicity study indicated that the incidence of thyroid tumors (follicular cell adenoma and C-cell adenoma) was not considered a significant finding. However, the skin tumors (keratoacanthoma and/or squamous cell carcinoma and basal cell and related tumors) were significantly increased in the treated group as compared to the control group. Therefore, TB considers oryzalin to be a rat oncogen.

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3. According to the Guidance for Analysis and Evaluation of Long Term Rodent Studies Recommended by EPA-OPTS (Paynter 12/1/84), the most persuasive evidence of potential oncogenicity in man should come from competently designed and conducted human epidemiology studies supported by appropriate animal studies. Since no human epidemiology study for oryzalin is available to the Toxicology Branch, and since the results of oncogenicity experiments provide positive response in only one specie with no decrease time to tumor incidence, the availabe data provide only limited evidence that oryzalin is oncogenic to experimental animals. The weight of evidence from these long term rodent studies may be classified in the category of Group C- Possible Human Carcinogen (See also the Guidance recommended by Paynter). Tox Branch has been regulating oryzalin on the basis of this rat oncogenicity study and the subsequent risk assessment (Litt, 3/12/82) since 1982 and therefore, special review of these studies appears unnecessary at the present time.

Reference:

Paynter, O.E. Evaluation Procedures for Oncogenic Potential: Guidance for Analysis and Evaluation of Long Term Rodent Studies, EPA - OPTS, Revised December 1, 1984

Reviewed by: Virginia A. Dobozy, V.M.D., M.P.H. Virginia A Dobozy 7/21/93
Section I, Toxicology Branch II (H7509C)
Secondary Reviewer: Yiannakis M. Ioannou, Ph.D. [signature] 7/26/93
Section I, Toxicology Branch II (H7509C)

ADDENDUM TO DATA EVALUATION REPORT FOR CARCINOGENICITY STUDY IN MICE[1]

I. RESULTS

The study report indicates that there were no toxicologically important differences between the two replicate studies, therefore the results were presented together.

A. Diet Analyses

Every month, a standard ration was mixed with appropriate quantities of oryzalin and then pelleted. Analyses of the fresh mash (ration plus oryzalin), fresh pellets and aged pellets were done at various times during the study. The concentration of the chemical in the fresh mash approximated the theoretical levels. The average percent loss in the fresh and aged pellets was less than 10% for the diets containing 0.05% and 0.135% of the chemical and less than 21% for the diet containing 0.365%.

B. Estimated Compound Consumption

It was not possible to measure food consumption due to the propensity of the animals to spill the pellets. The testing laboratory had no food consumption data on the B6C3F$_1$ mouse so they extrapolated data from the ICR mouse and estimated 3 g/mouse for daily intake. Using this estimate and mean body weights, the following intake of the chemical was determined.

% Oryzalin in Diet	Sex	Mean Daily Intake (mg/kg)*	Nominal (ppm/7)
0.05	M	38.5	71.4
	F	42.25	71.4
0.135	M	104.25	192.9
	F	112.25	192.9
0.365	M	287.0	521.4
	F	333.75	521.4

* Calculated by the reviewer from page 18 of the study report.

The study report states that there was less than 10% loss of oryzalin during the pelleting process in the diets containing levels of 0.05 and 0.135% and less than 21% in the diets containing 0.365%. Therefore, it is reasonable to assume that the mice were exposed to levels of oryzalin ≥ 35, 100 and 245 mg/kg/day during the study. The calculations for these levels are not included in the study report.

C. Survival and Clinical Observations

According to the statistical analysis (Appendix I, page 956), the twelve-month

[1] Caswell number for the original DER was 623A; Accession Numbers were 244746, 244747 and 244748.

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mortality was decreased in treated males and increased in treated females but at the end of the study, survival was comparable between the treated and control groups. The number (percentage) of animals which survived to the end of the study is presented below.

% Oryzalin in Diet	Replicate #	Males	Females
0	M-9087	26 (43)	43 (72)
	M-9097	38 (63)	38 (63)
0.05	M-9087	28 (70)	29 (72)
	M-9097	21 (52)	30 (75)
0.135	M-9087	24 (60)	26 (65)
	M-9097	24 (60)	26 (65)
0.365	M-9087	19 (48)	30 (75)
	M-9097	29 (72)	31 (78)

Extracted from Tables 2 and 3 (pages 34-38 of the study report).

Clinical Observations

The study report states that there was no evidence of a treatment-related response, however no data on daily observations were submitted.

D. Body Weight and Body Weight Gain

There were no statistically significant differences in body weight between the treated and control groups.

At three months, body weight gain was decreased in the 0.365% males and females by 13 and 10%, respectively, in comparison to the control weight gain. It was also decreased in the 0.365% group males during the last six months of the study; the differences from the control group were statistically significant at 21 and 24 months in one replicate. In the 0.365% group females, there were statistically significant decreases during the last 18 months of the study. Table 1 summarizes the body weight gain data at selected time periods.

Table 1
Mean Body Weight Gain (G) in Mice Treated with Oryzalin in the Diet for Two Years[a]

	Dosage Levels (% Oryzalin in Diet)							
	Males				Females			
	0	0.05	0.135	0.365	0	0.05	0.135	0.365
Month 3								
Study M-9087	8.3	9.8*	9.0	7.2	7.1	6.6	6.7	6.4
% Control	--	118	108	87	--	93	94	90
Study M-9097	9.0	8.3	8.1	8.8	7.5	7.5	8.1	7.8
% Control	--	92	90	98	--	100	108	104
Month 12								
Study M-9087	15.2	18.5	16.6	14.7	20.2	21.7	20.7	16.7*
% Control	--	114	102	91	--	107	102	83
Study M-9097	16.5	15.7	15.7	16.6	21.7	19.8	21.3	15.8*
% Control	--	95	95	101	--	91	98	73
Month 18								
Study M-9087	17.6	20.7*	18.2	16.9	26.0	24.8	23.6	19.8*
% Control	--	118	103	96	--	95	91	76
Study M-9097	16.8	16.5	15.6	16.5	26.2	22.4*	23.9	18.1*
% Control	--	98	93	98	--	85	91	69
Month 24								
Study M-9087	14.5	16.0	14.8	13.4	20.9	18.9	16.8*	14.2*
% Control	--	110	102	92	--	90	80	68
Study M-9097	16.3	13.7	13.8	13.3*	23.0	19.6*	18.7*	14.9*
% Control	--	84	85	82	--	85	81	65

[a] Extracted from Tables 4 and 5 (page 38-39 of the study report); percentages calculated by the reviewer.

E. Food Consumption

As stated previously, food consumption data were not collected.

F. Clinical Pathology

Hematology

There were statistically significant decreases in the HCT (1 replicate), MCV and MCHC in the 0.365% group females No reference values were submitted for these parameters so no determination can be made as to whether the values were within

the normal range for this species.

Clinical Chemistry

Blood glucose levels were significantly decreased in the 0.365% group females in one replicate and in all the treated females in the other replicate. Again, no reference values have been submitted so no judgment can be made about these changes.

G. Post-mortem Examinations

Organ Weights

The study report states that variation in organ weights was not treatment-related except for two instances. Treated females had a dose-dependent decrease in relative and absolute uterine weights due to a decreased incidence of cystic endometrial hyperplasia in these animals. The treated females also had an increase in relative liver weights; no morphological changes were present on histopathology. On review of the uterine weight data, only the two highest dose groups were affected and the changes were not consistent between the two replicates. The relative liver weight was increased in the 0.365% group females in one replicate. The data are summarized in Table 2.

Table 2
Absolute and Relative Weights of the Uterus and Liver in Mice Treated with Oryzalin in the Diet for Two Years[a]

		Dosage Levels (% Oryzalin in Diet)							
		Males				Females			
		0	0.05	0.135	0.365	0	0.05	0.135	0.365
Terminal Body Weight									
Study M-9087		41	41	40	39	40	39	37*	34*
Study M-9097		41	37*	39	38*	43	39*	38*	34*
Liver									
Study M-9087	A	1.58	1.67	1.58	1.73	1.68	1.83	1.66	1.72
Study M-9097	A	1.76	1.66	1.64	1.47	1.69	1.64	1.68	1.55
Study M-9087	R	3.92	4.17	4.05	4.56	4.23	4.80	4.55	5.04
Study M-9097	R	4.40	4.65	4.32	3.84	4.02	4.27	4.52	4.62*
Uterus									
Study M-9087	A					1.04	.83	.61*	.31*
Study M-9097	A					.77	.95	.38*	.39*
Study M-9087	R					2.59	2.18	1.69	.92*
Study M-9097	R					1.84	2.50	1.02	1.17

[a] Extracted from Tables 16.01 - 19.02 (pages 129-136 of the study report).

* Significantly different from control, $p \leq 0.05$

Non-neoplastic Post-mortem Findings

A variety of non-neoplastic lesions was seen in both the treated and control groups. The incidences were comparable between the groups, except for cystic endometrial hyperplasia which was decreased in the 0.365% group females. In Study M-9087, the lesion was observed in 23/60 (38%) control females vs. 9/40 (22.5%) 0.365% group females. In Study M-9097, the incidence was 18/60 (30%) in the control vs. 7/40 (17.5%) in the 0.365% group females.

There was no increased incidence of three thyroid lesions (cystic follicles, C-cell hyperplasia and focal follicular hyperplasia) in the treated animals as was seen in the combined chronic toxicity/carcinogenicity study in rats (replicate studies R-167 and R-177).

Neoplastic Post-mortem Findings

The incidence of benign and malignant neoplasms was comparable between the treated and control groups, except for pituitary adenomas which were reduced in incidence in the 0.365% group females. In the combined chronic toxicity/carcinogenicity study in rats (replicate studies R-167 and R-177), there was an increased incidence of thyroid, skin and mammary gland neoplasms. In these replicate studies, only in M-9097 was there a slight increase in skin fibrosarcomas [3/60 (5%) in the control vs. 5/40 (12.5%) in the 0.365% group males]; no statistically significant difference was found.

II. STUDY DEFICIENCIES

1. No data on clinical observations or food consumption were provided.

2. No accurate mg/kg/day dosages were provided for the treated animals.

III. DISCUSSION/CONCLUSIONS

In two replicate studies, technical oryzalin was administered in the diet to B6C3F$_1$ mice at concentrations of 0.05, 0.135 and 0.365% (equivalent to 500, 1350 and 3650, respectively) for two years. Dose selection was based on the results of a three-month study in B6C3F$_1$ mice using diets containing 0.045, 0.09, 0.18, 0.365 and 0.8% oryzalin. Slight increases were observed in the mean relative liver, kidney and heart weights of males in the 0.8% group. In the females, the mean relative weight of the liver was increased in the three higher dosage groups, the kidney weight was increased in all but the lowest dosage group and the spleen weight was increased at the highest dosage group. In this study, minimal treatment-related effects were observed. At three months, body weight gain was decreased in the 0.365% males and females by 13 and 10%, respectively, in comparison to the control weight gain. It was also decreased in the 0.365% group males during the last six months of the study; the differences from the control group were statistically significant at 21 and 24 months in one replicate. Minor alterations in the clinical pathology evaluations were not consistent between the replicates and were judged not to be of biological significance. The absolute weight of the uterus was decreased in the 0.135 and 0.365% group females; the relative weight was decreased in the 0.365% group females in one replicate. On histopathology, there was a decreased incidence of cystic endometrial hyperplasia in the 0.365% group females. No other neoplastic or non-neoplastic findings were considered to be treatment-related.

There is no evidence that technical oryzalin is carcinogenic in B6C3F$_1$ mice when given in the diet at concentrations of 0.05, 0.135 and 0.365%.

The No Observed Effect Level (NOEL) = 0.135% (1350 ppm) in males and females

The Lowest Observed Effect Level (LEL) = 0.365% (3650 ppm) in males and females based on decreased body weight gain in males and females.

The Maximum Tolerated Dose (MTD) = 0.365% based on decreased body weight gains in both sexes.

IV. CLASSIFICATION - Minimum (See STUDY DEFICIENCIES)

010612

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

OCT 22 1981

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

623A

MEMORANDUM:

DATE: October 7, 1981

SUBJECT: Oryzalin, mouse oncogenicity/chronic study on, TB/HED evaluation of.

FROM: Mary L. Quaife, Ph.D. MLQ. 10/7/81
Toxicology Branch/HED (TS-769)

TO: PM Mr. Robert Taylor
Registration Division (TS-767)

THROUGH: Mr. W. Burnam, Acting Chief
Toxicology Branch/HED (TS-769)

"Identifying Number" 1471-100
Caswell Number 623A
Accession Numbers 244746, 7, and 8

Eli Lilly and Company
Greenfield, Indiana 46140

This two-year mouse feeding study was submitted by Registrant in support of varied tolerance petitions and registration submissions for oryzalin technical (and formulations).

The study is reviewed in detail in the text of this memo.

CONCLUSIONS:

The study is judged acceptable for characterizing possible chronic and oncogenic effects of oryzalin technical in the mouse.

Oryzalin is shown to be negative for oncogenicity in the mouse at up to and including 3,650 ppm in the diet.

Oryzalin is judged to provide a "no-observed effect" level of 500 ppm in the diet with regard to chronic-feeding effects.

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NEW TOXICOLOGY:

The toxicological evaluation of oryzalin (compound 67019) given to B6C3F1 mice in the diet for 2 years, J. L. Carter, DVM, PhD, and N.V. Owen, DVM, PhD, toxicology division, Lilly research laboratories, division of E. Lilly and company, Greenfield, Indiana 46104, March, 1981, Acc. Nos 244746, 7, and 8.

Test material. Oryzalin, 3,5-dinitro-N^4,N^4-di-n-propylsulfanilamide,* lot X-28607 (Sodyeco lot 86343) was employed. Additional information is given in Appendix A of this submission. Since the latter corresponds exactly to Appendix C of Acc. No. 699518 (report of two-yr rat study on oryzalin) - except that the 1/22/80 "attachment," of Appendix C, which consists of tests to confirm the identity of oryzalin lots by D.S.C., L.O.D., T.L.C., T.G.A., D.T.A., I.R., and N.M.R. procedures and elemental analysis, is not included - characterization of this material may be found in greater detail in our memos of 6/3/81, pp. 11-12, and of 1/7/81, on oryzalin (chronic rat study).

Procedure. In two identical studies, carried out concurrently, B6C3F1 mice (cesarean-derived, barrier-sustained) were obtained from Charles River Laboratories; allowed to acclimate for one week; and groups of 60M and 60F controls and 40M and 40F test mice each started on zero, 500, 1,350, or 3,650 ppm oryzalin in the diet for a period to last two years. Mice weighed 25.5 ± 0.2 or 19.8 ± 0.1 g, M and F in first study, and, correspondingly, 24.6 ± 0.2 or 19.3 ± 0.1 (mean and S.E.) in second study.

Diets were assayed before and after pelleting and two weeks after pelleting at time intervals specified in the study procedure.

Twice a day, all cages (each containing 3 or 4 mice, by separate sex, presumably) were inspected for "dead, dying or moribund mice," dead ones being taken for necropsy, dying ones being disposed of as decided by project leader. When mouse was weighed, it was examined for clinical appearance and behavior and for presence of any external masses. Mice were weighed weekly during first three months and twice monthly thereafter.

All two-year survivors were necropsied, blood samples being taken for hematology and serum prepared for blood chemistry assays:

Hematologic tests: Erythrocyte and white blood cell counts (RBC and WBC); hemoglobin (HGB); leukocyte (WBC) differential count; hematocrit; mean corpuscular volume (MCV); mean corpuscular hemoglobin (MCH); mean corpuscular hemoglobin concentration (MCHC); and erythrocyte cell morphology.

Blood chemistry assays: Glucose; urea nitrogen (BUN); creatinine (CREAT); total bilirubin (TB); alkaline phosphatase (AP); and alanine aminotransferase (SGPT).

All mice, killed or died, were to be necropsied by qualified pathologists. Organs and tissues were collected and fixed in 10% buffered formalin and examined grossly and tissue sections (stained with hematoxylin and eosin), microscopically, by ACVP-certified pathologist, J. L. Carter (see above).

* 96.5% pure by high-pressure liquid chromatographic assay, with 0.2 to 0.7 ppm di-n-propylnitrosamine present.

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Mammary gland	ileum	kidneys and adrenals*
salivary gland	colon	urinary bladder
lungs (with nodules)	lymph node	prostate
heart*	liver (with masses)*	testes*
thyroid	bone marrow	uterus and ovaries*
(with parathyroids)	(sternebrae)	brain
stomach	thymus	pituitary
duodenum	pancreas	eyes
jejunum	spleen*	skeletal muscle

Organs starred (above) - or combined organs starred - were trimmed and weighed.

Pituitaries were examined grossly; only those from mice that "had lesions" were examined microscopically.

Organs of gastrointestinal tract (duodenum, jejunum, ileum, colon, and stomach) were not collected, in case the tract was autolyzed, for microscopic examination, only for gross, unless "there were lesions."

Statistical evaluation of differences between control and treated group means of body-weight-gain, hematologic values, blood chemical levels, final body weights, and organ weights was made by a non-parametric equivalent to the statistical method described by Dunnett (Miller, R. G. Simultaneous statistical inference. McGraw-Hill, New York, N.Y.; Dunnett, C. W. New tables for multiple comparison with a control. Biometrics 20, 482-91 (1964)). Tumor (neoplasm) data were analyzed statistically for a linear treatment effect for benign and for malignant tumors and for male and female mice. The arcsin transformation of the square root of the ratio of tumor-bearing mice to total number of mice at risk (alive when the first neoplasms were found) was multiplied by an orthogonal polynomial for a linear effect. The result is distributed normally with a mean of zero and a variance of one. These results are tested using normal distribution theory (Young, S. S. Analysis of tumor incidence in chronic toxicity tests. Presented at the American Statistical Association meeting, Boston, Massachusetts, August 23-7 (1976)).

Results.

No clinical effects, judged compound-related, were seen.

Assays of diet for oryzalin content showed loss of oryzalin of 10%, on the average, for 500- and 1,350-ppm diets, and 21%, on the average, for the 3,650-ppm diet during pelletting. Based on the foregoing and on assumed consumption of 3 g food per mouse, daily, mice may have been exposed to levels of oryzalin of at least 35, 100, and 245 g/kg BW/day in this study.

Mortality at 12 months was statistically significantly increased for females and significantly decreased for males (Appendix I):

12 Month	Male (%)	14.2	10.0	13.9	1.3, con. on up;	"Z" = -3.30
	Female (%)	0.0	3.8	2.5	7.6 " " " "	+3.47

(Z value is statistically significant if outside the range, -2.48 to +2.48.)

24 Month	Males (%)	47	39	40	40
	Females (%)	32	26	35	23

(24-Month values are approximate ones taken from Tables 3 and 2 of the report.)

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Survival was judged adequate in all groups.

Body weight at six-month intervals, means for each sex separately:

	6 mos.	12 mos.	18 mos.	24 mos.
MALE				
Control	38 g	42 g	43 g	41 g
500 ppm	38	42	43	39
1,350 ppm	37	41	42	40
3,650 ppm	37	41	42	39
FEMALE				
Control	33	41	46	42
500 ppm	32	41	44	39
1,350 ppm	33	41	43	38
3,650 ppm	31	36	39	34

Body-weight values, which are approximate, are averaged from Tables 4 and 5 of the report. Body-weight gains (not shown) were significantly less for females in the highest dose-level group from 6 months on.

There was no effect of oryzalin treatment on group hematology values, and, evidently, no toxicologically significant effect on blood chemical values. (Blood glucose in females at 3,650 ppm averaged significantly less than that of controls, 91 vs. 116 mg/100 ml.)

Organ weights among treatment and control groups varied in two instances. Mean weights of uteri (including ovaries) were decreased in dose-related fashion, and markedly so, in oryzalin-treated females, significantly so ($p < 0.05$) in the two highest dose-groups. Study report attributes this to the lower incidence of cystic endometrial hyperplasia in the oryzalin groups. Although oryzalin-treated female mice showed some increase in relative liver weights, absolute values were unaffected by treatment.

Incidence of benign and malignant neoplasms was similar between control and treated mice: Following data are from Appendix I.

Tumor Analysis.

	Sex	Dose: Control	500 ppm	1,350 ppm	3,650 ppm	Z Statistic
Benign	Male	50/108	25/75	23/71	34/78	-0.39
	(%)	(46.3)	(33.3)	(32.4)	(43.6)	
	Female	38/119	28/77	29/78	17/73	-1.19
	(%)	(31.9)	(36.4)	(37.2)	(23.3)	
Malignant	Male	23/108	14/75	19/71	23/78	+1.60
	(%)	(21.3)	(18.7)	(26.8)	(29.5)	
	Female	42/119	28/77	32/78	27/73	+0.42
	(%)	(35.3)	(36.4)	(41.0)	(37.0)	

As shown in above analysis, no significant effect is shown for benign or malignant tumors in either sex. Nor did tables of tumor incidence show any.

Inspection of tables of nonneoplastic lesions does not reveal any which can be related causally to oryzalin treatment of the mice, except for the uterine condition (see below).

Body weight at termination and weight of uterus in female mice (± S. E.)

	Dose (ppm)	Body weight (g)	Weight of uterus* Absolute weight (g)#	Relative weight (g/100 g BW)
First replicate study (M-9087):	0	40 ± 1	1.04 ± 0.15	2.59 ± 0.36
	500	39 ± 1	0.83 ± 0.08	2.18 ± 0.26
	1,350	37 ± 1**	0.61 ± 0.07**	1.69 ± 0.18
	3,650	34 ± 1**	0.31 ± 0.02**	0.92 ± 0.06**
Second replicate study (M-9097):	0	43 ± 1	0.77 ± 0.11	1.84 ± 0.27
	500	39 ± 1**	0.95 ± 0.14	2.50 ± 0.39
	1,350	38 ± 1**	0.38 ± 0.03**	1.02 ± 0.09
	3,650	34 ± 1**	0.39 ± 0.07**	1.17 ± 0.23

* Uterus with ovaries attached.
** Significantly less than control, $p \leq 0.05$, Dunnett's two-tailed "t."
# Original report tables (16.02 and 17.02) list these uterine weights as in "mg." However, the original individual weights, uterine (tables 20.5-20.8 and 22.5-22.8), are given in "g."

Study ascribes these decreased uterine weights in oryzalin-treated mice to decrease in incidence of cystic endometrial hyperplasia (see below).

Incidence of cystic endometrial hyperplasia in female mice

	Dose (ppm)	Number of Animals total	Number with c.e.h.	(%)
First replicate study (M-9087):	0	60	23	38
	500	40	20	50
	1,350	40	14	35
	3,650	40	9	22
Second replicate study (M-9097)	0	60	18	30
	500	40	15	38
	1,350	40	10	25
	3,650	40	7	18

As uterine weight is often under influence of endocrine glands, one might suppose that oryzalin exerts a hormonal influence in these mice.

Conclusion.

In this study, oryzalin was negative for oncogenicity in the mouse at up to 3,650 ppm in the diet. For chronic effects, the NOEL is judged to be 500 ppm, with 1,350 ppm a minimum-effect level, - statistically significant (and dose-related) decrease in weight of the uterus (and attached ovaries).

CORE-Rating:

As oncogenicity study, Minimum.
As chronic study, Minimum.

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Primary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 7/28/93
Senior Pharmacologist, Review Section I, TB II/HED (H7509C)
Secondary Review by: Yiannakis M. Ioannou, Ph.D., D.A.B.T. 7/28/93
Section Head, Review Section I, TB II/HED (H7509C)

DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity - Teratology - Rabbit Guideline §83-3b)

EPA IDENTIFICATION NO.'s: MRID No: 00052557, 00073552, 00026785
EPA Pesticide Chemical Code 104201
Toxicology Chemical No. 623A

TEST MATERIAL: Oryzalin (purity of 97.4%, Lot# X28491).
SYNONYMS: 3,5-dinitro-N^4,N^4-di(n-propyl)sulfanilamide, EL-119, Compound 67019

TITLE OF REPORT: A Teratology Study on Lilly Compound 67019 (Oryzalin) in the Rabbit

SPONSOR: Lilly

TESTING FACILITY: Toxicology Division, Lilly Research Laboratories

STUDY NUMBER(S): B7366

AUTHOR(S): J.K. Markham, D.N. Kitchen, N.V. Owen, D.G. Hoffman, D.M. Morton

DATE REPORT ISSUED: September 1978 (Signed April 18, 1979)

CONCLUSIONS: Dutch Belted Rabbits from Nicely Rabbitry received either 0, 25, 55, or 125 mg/kg/day Oryzalin by oral gavage from gestation days 6 through 18, inclusive. There was a slight decrease in body weight gain in the mid and high dose groups during the dosing period, for the dosing plus post dosing period and for the entire gestation period. There was a slight decrease in food consumption during the treatment period and the post dosing period in the mid and high dose groups. Possible developmental toxicity was noted in the mid and high dose groups in the form of reduced mean litter size, increased resorptions and increased postimplantation loss (although the reduced number of corpora lutea might have affected these parameters).

Core Classification: Core Minimum Data.

Maternal Toxicity NOEL = 25 mg/kg/day
Maternal Toxicity LOEL = 55 mg/kg/day
Developmental Toxicity NOEL = 25 mg/kg/day
Developmental Toxicity LOEL = 55 mg/kg/day

This study satisfies the 1984 Pesticide Assessment Guideline requirement (40 CFR 158.340, § 83-3b) for a teratology study in rabbits.

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A. Materials and Methods A copy of the "materials and methods" section from the investigators report is appended.

Test Compound: Purity: 97.4% a.i.
Density: not provided
Description: not provided
Lot No.: X28491
Receipt date: not provided
Other provided information - none
Contaminants: list in CBI appendix - not provided

Vehicle(s): Aqueous acacia, 20% w/v

Test Animal(s): Species: Virgin female rabbits
Strain: Dutch Belted
Source: Nicely Rabbitry, Greenfield, Indiana
Age: not provided
Body Weight: 2.37 kg
any information of males used - same strain

B. Study Design

This study was designed to assess the developmental toxicity potential of Oryzalin when administered by oral gavage on gestation days 6 through 18, inclusive.

Mating Procedure

The investigators employed a modification of the method of Gibson (1966, reference citation provided) where each female was artificially inseminated with the semen from 4 male rabbits in the laboratory colony. The day considered to be gestation day 0 or 1 was not mentioned.

Animal Husbandry

It was not indicated if the animals were acclimated to the laboratory conditions or what animal care conditions they were kept under. However, it was stated that they received Purina Laboratory Rabbit Chow and tap water *ad libitum*.

Group Arrangement

The animals were randomly distributed (method not provided) to the following test groups:

Test Group	Dose Level (mg/kg/day)	Number Assigned
Control	vehicle	15
Low Dose	25	15
Mid Dose	55	15
High Dose	125	15

Dose Administration

All doses were administered in a volume of 5 ml/kg of body weight/day prepared daily during the dosing period. The dosing solutions were apparently not analyzed for concentration and stability. Doses were adjusted every third day. Doses were based on *A Pilot Reproduction Study on Lilly Compound 67019 (Oryzalin) in the Rabbit* (Study No. B-7326, October 1978, included) where pregnant rabbits received either 0, 25, 75, or 225 mg/kg/day by oral gavage from gestation day 6 through 18, inclusive. The high dose (225 mg/kg/day) was considered to be toxic, and the doses used for the primary study were lower.

Observations

The animals were checked *for changes in appearance or behavior...daily*. Dams were sacrificed on day 28 of gestation. Body weights were taken on gestation days 0, 6, 12, 18, 21, and 27. Food consumption was measured daily.

Examinations of the dam at sacrifice consisted of: an examination for *gross tissue changes. Ovaries were inspected for the number of corpora lutea, and the uterus for the number and location of fetuses and resorptions.*

The fetuses were examined in the following manner: *Progeny weights were recorded by litter. Each fetus was examined for sex, viability, and external abnormalities. The progeny were...placed in a sodium chloride (ca 1%). After 24 hours the viscera, including the brain, were examined* [procedure not described]; *special attention was directed to heart morphology; the carcasses were then prepared for skeletal examination* [reference citation provided].

Historical control data were provided for some parameters to allow comparison with concurrent controls.

Statistical analysis

The following statistical analysis methods were employed (from the investigators report):

> Mean values of the reproduction parameters were determined for each group. In the calculation of mean fetal weight, the litter was considered the independent sampling unit.[3] The following reproduction indices were reported:
>
> Fertility - the proportion of females that were pregnant.
>
> 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 the number of corpora lutea.
>
> The values of percentage change in maternal body weight and the litter values of live fetuses, resorptions, and implantations were subjected to statistical analysis by one-way analysis of variance.[4] Comparison of treatment means with that of the control was performed by Dunnett's test.[5]

Compliance

A signed Statement of (No?) Confidentiality Claims was not provided.

A signed Good Laboratories Practices statement was not provided.

A signed Quality Assurance Statement was not provided.

A Flagging Criteria Statement was not provided; however, it is the opinion of this reviewer that this study neither meets nor exceeds the applicable criteria.

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C. Results

1. Maternal Toxicity:

Mortality

One high dose animal died on gestation day 26; necropsy showed an *acute upper respiratory infection.* One mid dose animal was killed due to gavage error on gestation day 10.

Clinical Observations

One mid dose animal aborted on gestation day 28.

Body Weight

The investigators supplied the group mean and individual animal data. The following table presents body weight gain data:

Table I: Body Weight Gains (kg)[a]

Gestation Days:	0-6	6-18	18-27	6-27	0-27
Control	0.05	0.02	0.05	0.07	0.12 (5.2)
LDT	0.05	0.02	0.09	0.11	0.16 (6.6)
MDT	0.01	-0.10	0.03	-0.07	-0.06 (-0.2)
HDT	-0.03	-0.18	0.07	-0.11	-0.14 (-6.1) *

[1] = A = First replicate; B = Second replicate; Com = combined replicates
[2] = percent difference from control; * = $p < 0.05$, by one-way analysis of variance with Dunnett's test.
[a] = Calculated by reviewer from means, Study # B7366, Table 2.0.

There was a slight decrease in body weight gain in the mid and high dose groups during the dosing period (gd 6-18), for the dosing plus post dosing period (gd 6-27) and for the entire gestation period (gd 0-27); however, only the high dose was statistically significant for the entire period.

Food Consumption

The investigators supplied the group mean and individual animal data. The following table presents the food consumption data:

Table II: Food Consumption Data (unit)[a]

Gestation Days:	0-5	6-18	19-27
Control	112	93	76
LDT	110	90	84
MDT	96	55	69
HDT	82	31	59

[a] = Data extracted from Study # B7366, Table 1.

There was a slight decrease in food consumption during the treatment period (gd 6-18) and the post dosing period (gd 19-27) in the mid and high dose groups.

Gross Pathological Observations

No data on the dams were provided.

Cesarean Section Observations

Table III: Cesarean Section Observations[a]

Dose:	Control	LDT	MDT	HDT
#Animals Assigned	15	15	15	15
#Animals Inseminated	15	15	15	15
#Animals Pregnant	12	13	14	13
Pregnancy Rate (%)	80	87	93	87
Maternal Wastage				
#Died or killed	0	0	1	0
#Non pregnant[1]	3	2	0	2
#Aborted	0	0	1	0
Total Corpora Lutea	103	114	97	90
Corpora Lutea/dam	8.6	8.8	7.5	7.5
Total Implantations	88	83	81	79
Implantations/Dam	7.3	6.4	6.2	6.6
Total Live Fetuses	78	77	59	56
Live Fetuses/Dam	6.5	5.9	4.5	4.7
Total Resorptions[1]	10	5	20	15
Early	10	4	20	14
Late	0	1	0	1
Resorptions/Dam	0.8	0.4	1.5	1.4
Mean Fetal Weight(gm)	29.8	32.3	30.8	28.2
Preimplantation Loss(%)[1]	14.6	27.2	16.5	12.2
Postimplantation Loss(%)[1]	11.4	7.2	27.2	29.1
Sex Ratio(% Male)	63	57	54	45

[1] = Data calculated by reviewer

[a] = Data extracted from Study # B7366, Table 3.0.

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Possible developmental toxicity was noted in the mid and high dose groups in the form of reduced mean litter size (although the reduced number of corpora lutea could reduce the possible litter size, as seen from the decreased number of implantations), increased resorptions (not dose related) and increased postimplantation loss.

2. Developmental Toxicity

External, Visceral, and Skeletal Examinations

No treatment related effects were noted, see Table 7 from the investigators report attached as an appendix.

D. Discussion/Conclusions

a. Maternal Toxicity:

There was a slight decrease in body weight gain in the mid and high dose groups during the dosing period (gd 6-18), for the dosing plus post dosing period (gd 6-27) and for the entire gestation period (gd 0-27); however, only the high dose was statistically significant for the entire period. There was a slight decrease in food consumption during the treatment period (gd 6-18) and the post dosing period (gd 19-27) in the mid and high dose groups.

b. Developmental Toxicity:

i. Deaths/Resorptions:

Possible developmental toxicity was noted in the mid and high dose groups in the form of reduced mean litter size (although the reduced number of corpora lutea could reduce the possible litter size, as seen from the decreased number of implantations), increased resorptions (not dose related) and increased postimplantation loss.

ii. Altered Growth:

No treatment related observations were noted.

iii. Developmental Anomalies:

No treatment related observations were noted.

iv. Malformations:

No treatment related observations were noted.

E. Study Deficiencies:

The dosing solutions were not analyzed for homogeneity, concentration or stability.

F. Core Classification: Core Minimum Data.

Maternal Toxicity NOEL = 25 mg/kg/day
Maternal Toxicity LOEL = 55 mg/kg/day
Developmental Toxicity NOEL = 25 mg/kg/day
Developmental Toxicity LOEL = 55 mg/kg/day

This study satisfies the 1984 Pesticide Assessment Guideline requirement (40 CFR 158.340, § 83-3b) for a teratology study in rabbits.

C)

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Pages 70 through 74 are not included in this copy.

The material not included contains the following type of information:

_____ Identity of product inert ingredients.

_____ Identity of product impurities.

_____ Description of the product manufacturing process.

_____ Description of quality control procedures.

_____ Identity of the source of product ingredients.

_____ Sales or other commercial/financial information.

_____ A draft product label.

_____ The product confidential statement of formula.

_____ Information about a pending registration action.

X FIFRA registration data.

_____ The document is a duplicate of page(s) _____.

_____ The document is not responsive to the request.

The information not included is generally considered confidential by product registrants. If you have any questions, please contact the individual who prepared the response to your request.

CAS:

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

010612

DATE: January 2, 1980

SUBJECT: Teratology Studies on Oryzalin in the Rat and Rabbit - Addition of Data to Files. CASWELL#623A; EPA Reg.#1471-96, -100, 102, -11.

FROM: Larry Anderson
Toxicology Branch (TS-769)

TO: Robert Taylor
Product Manager#25

THRU: Dr. Adrian Gross, Chief
Toxicology Branch (TS-769)

Recommendations

The studies reviewed herein are adequate estimates of the fetotoxic/teratogenic potential of Oryzalin in the rat and rabbit. Findings in the rat study are unremarkable at dosages $\leq$ 225 mg/kg/day (highest dosage). A teratogenic effect is not evident in the rabbit study at dosages $\leq$ 155 mg/kg/day (highest dosage); however, the fetotoxic no-effect level is concluded to be 25 mg/kg/day due to adverse effects on reproduction observed in conjunction with maternal toxicity induced by higher dosages. Submission of a report on the pilot study in rabbits described herein for review by TOX is requested.

Review

I. Teratology Study with Lilly Compound 67019 (Oryzalin) in the Rat (Elanco Products Co., Study No. R-1186, 11/15/76, submitted by Elanco, 10/31/79, Acc. No. 241232).

A. Procedure

One hundred mated Wistar female rats, 252.0 ± 2.3 g, were divided into 4 groups of 25 animals each which received 0 (vehicle controls), 25, 75, or 225 mg/kg/day of Oryzalin (Lot X28491, Sodyeco Batch 243, 97.4% pure) by gavage on gestation days 6-15, inclusively. The dosing vehicle was a 20% acacia suspension. Dosages were based on body weight at gestation day 0 which corresponds to observation of an expelled copulatory plug.

Estimates of food consumption weekly and body weights on gestation days 0, 7, 14, and 20 were made on 12 dams/dosage group. These animals were subjected to necropsy at sacrifice on gestation day 20. The number of corpora lutea, number and distribution of fetuses in the uterus, and number of early and late resorptions were recorded. Fetuses were examined for external abnormalities, viability, and sex. Approximately 2/3 of the fetuses in each litter were evaluated viscerally, and the remainder were processed for skeletal examination.

Remaining dams in each dosage group were assigned to a delivery phase. Food intake on gestation days 0, 7, 14, and 21 and body weight on postcoital days 0, 7, 14, 21, 28, and 35 and on day 21 post partum were determined. Observations regarding reproductive performance included: number of live and dead pups at birth, duration of gestation, number of surviving pups at days 1, 7, 14, and 21 post partum, pup weights as litters on days 1, 7, and 14 and individuals on day 21 following birth, and pup sexes on day 21 post partum. All pups which died were examined externally, and the heart was evaluated grossly. All surviving progeny were subjected to necropsy with particular emphasis on the heart. The heart was further examined with a dye-infusion technique. Females failing to deliver were evaluated to determine pregnancy.

In this study the litter was used as the independent sampling unit for statistical analysis.

B. Results

1. Gestation Day 20 Component

a. Maternal Food Intake: Unremarkable
b. Maternal Body Weight: Unremarkable
c. Fertility: Two control, 4 low dose, and 2 high dose animals were not pregnant. One low dose dam was found dead on gestation day 15 with marked lung congestion; 15 implantation sites including 1 early resorption were found in utero.
d. Fetal Viability: Unremarkable
e. Resorptions (Early and Late): Unremarkable
f. Implantation Sites: Unremarkable
g. Corpora Lutea: Unremarkable
h. Fetal Sex Ratio: Unremarkable
i. Mean Fetal Weight: Unremarkable
j. Fetal Examination: Unremarkable. Findings unrelated to treatment include inguinal hernia and hydronephrosis. One high dose fetus was found to have encephalocele out of 81 examined.

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2. Delivery Component

a. Maternal Food Intake: Unremarkable
b. Maternal Body Weight: Unremarkable

c. Fertility: Two control, 3 low dose, 3 mid dose, and 3 high dose animals were not pregnant. One control dam with dystocia (1 fetus in cervix, 7 dead fetuses in utero) and 1 high dose female with 11 empty implantation sites were sacrificed before the end of the study. A mammary tumor and mammary tissue hyperplasia were found in 1 mid dose and 1 high dose dam , respectively.

d. Duration of Gestation: Unremarkable

e. Pup Viability: Unremarkable. Nine of 11 pups in 1 control litter and 1 high dose pup died.

f. Pup Survival through 21 Days: Treatment related effects were not evident, but the following survival indices at 21 days (proportion of newborn pups alive) should be noted: control, 0.72 (72/100); low dose, 0.91 (103/113); mid dose, 0.70 (76/108); high dose, 0.88 (98/111).

g. Pup Weights: Unremarkable

h. Pup Sex Ratio: Unremarkable

i. Pup Examination: Unremarkable. Findings include ringtail in 1 litter of 3 controls pups, hydronephrosis in 2 pups per dosage group except the low dose, and 1 high dose male pup with alopecia. No heart defects were reported.

C. Conclusions

1. Classification: Core-Minimum Data

   a. Although an adequate number of pregnant animals were used in this study, only 12 dams/dosage group were selected for evaluation of fetuses prior to term.

2. Under the conditions of this study, it is concluded that the test material is not fetotoxic/teratogenic in the rat at 225 mg/kg/day (highest level).

II. Teratology Study with Lilly Compound 67019 (Oryzalin) in the Rabbit (Elanco Products Co., Study No. B-7366, 9/14/76, submitted by Elanco, 10/31/79, Acc. No. 241232).

A. Procedure

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Sixty virgin Dutch Belted female rabbits. 2.37 ± 0.04 kg. were artificially inseminated with sperm from 4 males in the colony. Fifteen females/dosage group were given 0 (vehicle control), 25, 55, or 125 mg/kg/day of Oryzalin (Lot X2849) Sedvoss Batch 242, 97.49 pure) by gavage on

gestation days 6-18, inclusively. The dosing vehicle was a 20% aqueous acacia suspension. Every third day dosages were adjusted according to body weight. Food consumption was estimated daily, and body weight was recorded periodically. All decedents were subjected to necropsy. Survivors were examined grossly at sacrifice on gestation day 28. Number of corpora lutea, number and location of fetuses, and number of resorptions (early and late) were determined. Litters were weighed, and fetuses were examined for sex, viability, and external abnormalities. Visceral, with special emphasis on the heart, and skeletal evaluation of the fetuses was done. The litter is the sampling unit for statistical analysis in this study.

B. Results

1. Maternal Food Intake: Dose-related decrease in mid dose and high dose animals throughout gestation. During gestation days 6-18, food intake by mid dose and high dose animals approximated 59% and 33%, respectively, of the control group value.

2. Maternal Body Weight: Dose-related reduction in mid dose and high dose animals throughout gestation as shown by the following mean percentage change in weight ± S.E.: control, +5.2 ± 1.9; low dose, +6.6 ± 1.5; mid dose, -0.2 ± 3.1; high dose, -6.1 ± 2.9. Gain and loss are designated by (+) and (-), respectively.

3. Fertility: Three control, 2 low dose, 1 mid dose, and 2 high dose rabbits were not pregnant.

4. Mean Live Litter Size: Control, 6.5 ± 0.4 (S.E.); low dose, 5.9 ± 0.6; mid dose, 4.5 ± 0.6; high dose, 4.7 ± 0.7. Dead fetuses were not found.

5. Resorptions (Early and Late):

	Mean + SE	Total (Early:Late)	No. Dams Affected / No. of Dams Pregna
Control	0.8 ± 0.4	(10:0)	4/12
Low Dose	0.4 ± 0.1	(4:1)	5/12
Mid Dose	1.5 ± 0.4	(20:0)	9/13
High Dose	1.4 ± 0.4	(14:1)	8/11

6. Corpora Lutea: Mean values ± S.E. were: control, 8.6 ± 0.6; low dose, 8.8 ± 0.5; mid dose, 7.5 ± 0.7; high dose, 7.5 ± 0.4.

7. Sex Ratio: The percentage of male offspring decreased as follows: control, 63; low dose, 57; mid dose, 54; high dose, 45.

8. Mean Fetal Weight: Unremarkable

9. Fetal Examination: Findings related to treatment and dose were not evident. External examination revealed edema, hemorrhagic areas, pale skin, varus forepaws, missing tail, and eventration of intestines as incidental occurrences. Visceral abnormalities were restricted to 2 pups in the mid dose group, 1 with an enlarged heart and 1 with a displaced and misshapened kidney. Various skeletal anomalies, such as missing sternebrae, 13 ribs, fused ribs and fused vertebrae were found to be incidental findings. It should be noted that 3 fetuses from a single litter in the 55 mg/kg/day group exhibited multiple defects.

C. Conclusions

1. Classification: Core-Guidelines

2. It is concluded that the test compound is not teratogenic in this study at dosage levels $\leq$ 125 mg/kg/day (highest dosage); however, the fet oxic no-effect level is considered to be 25 mg/kg/day with respect to fetotoxicity observed in conjunction with maternal effects in the 55 and 125 mg/kg/day groups.

3. The registrant mentions a pilot study (Elanco Study No. B-7326) which was done with dosage levels of 25, 75, and 225 mg/kg/day of Oryzalin. However, since rabbits maintained at 225 mg/kg/day did not maintain normal pregnancy, the dosage levels used in the subsequent study described herein were selected. The registrant indicates that maternal toxicity was evident at all dosage levels in the pilot study. It is requested that a report on the pilot study be submitted to TOX for review to allow a more definitive estimate of the fetotoxic/teratogenic potential of Oryzalin in the rabbit.

Primary Review by: Stephen C. Dapson, Ph.D. Stephen C. Dapson 9/1/93
Senior Pharmacologist, Review Section I, TB II/HED (H7509C)
Secondary Review by: Yiannakis M. Ioannou, Ph.D., D.A.B.T. YMI 9/7/93
Section Head, Review Section I, TB II/HED (H7509C)

DATA EVALUATION RECORD

STUDY TYPE: Developmental Toxicity - Teratology - Rabbit Guideline §83-3b)

EPA IDENTIFICATION NO.'S: MRID No. 00094461
EPA Pesticide Chemical Code 104201
Toxicology Chemical No. 621A

TEST MATERIAL: Oryzalin (purity of 97.0%, Lot# 9SY47).
SYNONYMS: 3,5-dinitro-N^4,N^4-di(n-propyl)sulfanilamide, EL-119, Compound 67019

TITLE OF REPORT: A Replicated Teratology Study on Oryzalin (EL-119, Compound 67019) by the Oral Route in Dutch Belted Rabbits

SPONSOR: Lilly

TESTING FACILITY: Department DL76, Lilly Research Laboratories, Division of Eli Lilly and Company, Greenfield, IN 46140

STUDY NUMBER(S): B7281 & B7291

AUTHOR(S): William H. Jordan (Project Leader)

DATE REPORT ISSUED: March 31, 1982

CONCLUSIONS: Dutch Belted Rabbits from Langshaw Farms received either 0, 10, 25, 55, or 125 mg/kg/day Oryzalin by oral gavage from gestation days 6 through 18, inclusive. Minimal maternal toxicity was observed at the mid and high dose groups as reduced food consumption during the dosing period and since a previous study in rabbits showed toxicity at a similar dose level (Lilly Study B-7366) the mid dose may be considered a maternally toxic dose. There was no evidence of developmental toxicity in this study.
It should be noted that the pregnancy rates for both the replicate studies are very low; however, when the studies are combined, the data are marginally acceptable.

Core Classification: Core Minimum Data

Maternal Toxicity NOEL = 25 mg/kg/day
Maternal Toxicity LOEL = 55 mg/kg/day
Developmental Toxicity NOEL > 125 mg/kg/day
Developmental Toxicity LOEL ≥ 125 mg/kg/day

This study satisfies the 1984 Pesticide Assessment Guideline requirement (40 CFR 158.340, § 83-3b) for a teratology study in rabbits and supports the previous study (MRID No.'s 00052557, 00073552, 00026785).

A. **Materials and Methods** A copy of the "materials and methods" section from the investigators report is appended.

Test Compound: Purity: 97.0% a.i.
Density: not provided
Description: not provided
Lot No.: 9SY47
Receipt date: not provided
Other provided information - none
Contaminants: list in CBI appendix - not provided

Vehicle(s): Aqueous acacia, 10% w/v

Test Animal(s): Species: Virgin female rabbits
Strain: Dutch Belted
Source: Langshaw Farms, Augusta, Michigan
Age: not provided
Body Weight: 2.20 kg - replicate 1; 2.45 kg - replicate 2
any information of males used - none provided

B. **Study Design**

This study was designed to assess the developmental toxicity potential of Oryzalin when administered by oral gavage on gestation days 6 through 18, inclusive.

Mating Procedure

The investigators employed a modification of the method of Gibson (1966, reference citation provided) where each female was artificially inseminated with the semen from a stock colony of males of the same strain. Following the insemination, each female received 10 U.S.P. units/kg of chorionic gonadotropin (A.P.L.®, Ayerst Laboratories, Inc., New York). The day considered to be gestation day 0 or 1 was not mentioned.

Animal Husbandry

Animals were acclimated to the laboratory conditions for a minimum of 2 weeks and kept under standard animal care conditions. They received Purina Laboratory Rabbit Chow HFS326® (Ralston-Purina Company, St. Louis, Missouri) and tap water *ad libitum*. The animals received, 2 weeks prior to shipment, a 3 day regimen of drinking water containing sulphaquinoxaline as prophylaxis for coccidiosis.

Group Arrangement

The animals were randomly distributed (method not provided) o the following test groups:

Test Group	Dose Level (mg/kg/day)	Number Assigned B7281	B7291
Control	vehicle	15	15
Low Dose	10	15	15
Low Mid Dose	25	15	15
High Mid Dose	55	15	15
High Dose	125	15	15

Dose Administration

All doses were administered in a volume of 5 ml/kg of body weight/day prepared daily during the dosing period. The dosing solutions were apparently not analyzed for concentration and stability. Doses were based on gestation day 6 body weights and adjusted on gestation day 13.

Observations

The animals were checked *daily for overt signs of toxicity.* Dams were sacrificed on day 28 of gestation. Body weights were taken on gestation days 0, 6, 13, 19, 24, and 28. Food consumption was measured daily.

Examinations of the dam at sacrifice consisted of: *A laparotomy was performed, and the uterus and ovaries of each animal was removed. A necropsy was performed on the carcass. Each uterus was opened along the antimesometrial border, and the number and distribution of implantations, live fetuses, and resorptions were recorded.*

The fetuses were examined in the following manner: *...fetuses were examined externally for anatomical anomalies, and individual fetal weights were recorded. Prior to visceral examination, all fetuses were stored overnight at ambient room temperature in aqueous sodium chloride (one percent, w/v). Following the visceral examination* [procedure not described], *the carcass was eviscerated and processed for skeletal examination by a modification of the Crary technique (Crary, 1962,* [reference citation provided]*).*

Historical control data were not provided to allow comparison with concurrent controls (although there was some mention of ranges in the report text) .

Statistical analysis

The following statistical analysis methods were employed (from the investigators report):

For the various maternal, reproductive, and fetal parameters, means and standard errors were calculated for individual and pooled replicates. Data from pregnant females that aborted were omitted from all calculations

For the purpose of statistical analyses, the litter was used as the independent sampling unit (Weil, 1970; Haseman and Hogan, 1975). Analysis of variance was used to test for "replicate", "treatment", and "replicate by treatment" effects on the following variables (Goodnight, 1979):

1. Percent change in body weight.
2. Food consumption on gestation days 0-5, 6-18, and 19-27.
3. Percent live fetuses.
4. Fetal weight.
5. Percent of fetuses with anomalies.
6. Percent of fetuses with vertebral, rib, or sternal anomalies.

There were no significant "replicate" or "replicate by treatment" effects that precluded analyzing the data by pooling over both replicates. Therefore, the data were pooled and the two replicates analyzed as one study. Arcsine square root transformations were used to stabilize variances on percent live fetuses, percent of fetuses with anomalies, and percent of fetuses with rib, sternal, or vertebral anomalies (Gaylor, 1978). Bartlett's test for homogeneity of variance was computed on each variable at the 0.001 level of probability (Anderson and McLean, 1974). Dunnett's procedure (1964) was used to analyze individual oryzalin dose group differences with the vehicle control group at the 0.05 level of probability.

Compliance

A signed Statement of [illegible] Confidentiality Claims **was not** provided.

A signed Good Laboratories Practices Quality Assurance Authorization statement (FDA GLP's) was provided.

A signed Quality Assurance Statement was provided (combined with above).

A Flagging Criteria Statement was not provided; however, it is the opinion of this reviewer that this study neither meets nor exceeds the applicable criteria.

C. Results

1. Maternal Toxicity

Mortality

No animals were reported to have died in either replicate.

Clinical Observations

Two animals aborted in the first replicate, one 25 mg/kg/day animal on gestation day 26 and one 125 mg/kg/day animal on gestation day 15.

Body Weight

The investigators supplied the group mean and individual animal data. The following table presents body weight gain data:

Table I: Body Weight Gains (kg)[a]

Gestation	Days:	0-6	6-19	19-28	6-28	0-28
Control	A[1]	0.08	0.24	0.16	0.40	0.48
	B	0.13	0.19	0.11	0.30	0.43
	Com	**0.10**	**0.23**	**0.14**	**0.37**	**0.47**
LDT	A	0.08	0.21	0.14	0.35	0.43
	B	0.07	0.16	0.13	0.29	0.36
	Com	**0.08**	**0.18**	**0.14**	**0.32**	**0.40**
LMDT	A	0.14	0.24	0.13	0.37	0.51
	B	0.07	0.18	0.11	0.29	0.36
	Com	**0.10**	**0.22**	**0.12**	**0.34**	**0.44**
HMDT	A	0.13	0.22	0.14	0.36	0.49
	B	0.06	0.19	0.12	0.31	0.37
	Com	**0.09**	**0.21**	**0.12**	**0.33**	**0.42**
HDT	A	0.15	0.23	0.16	0.39	0.54
	B	0.08	0.14	0.13	0.27	0.35
	Com	**0.11**	**0.18**	**0.15**	**0.33**	**0.44**

1 = A = First replicate; B = Second replicate; Com = combined replicates

a = Calculated by reviewer from means, Study #'s B7281 & B7291, Table 2.0.

There was no indication of an effect on body weight gain; however, a previous study in rabbits showed reduced body weight gains at 55 mg/kg/day and above (Lilly Study B-7366).

Food Consumption

The investigators supplied the group mean and individual animal data. The following table presents the food consumption data:

Body Weight

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Table I: Body Weight Gains (kg)[a]

Gestation	Days:	0-6	6-19	19-28	6-28	0-28
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	Com	**0.10**	**0.23**	**0.14**	**0.37**	**0.47**
LDT	A	0.08	0.21	0.14	0.35	0.43
	B	0.07	0.16	0.13	0.29	0.36
	Com	**0.08**	**0.18**	**0.14**	**0.32**	**0.40**
LMDT	A	0.14	0.24	0.13	0.37	0.51
	B	0.07	0.18	0.11	0.29	0.36
	Com	**0.10**	**0.22**	**0.12**	**0.34**	**0.44**
HMDT	A	0.13	0.22	0.14	0.36	0.49
	B	0.06	0.19	0.12	0.31	0.37
	Com	**0.09**	**0.21**	**0.12**	**0.33**	**0.42**
HDT	A	0.15	0.23	0.16	0.39	0.54
	B	0.08	0.14	0.13	0.27	0.35
	Com	**0.11**	**0.18**	**0.15**	**0.33**	**0.44**

[1] = A = First replicate; B = Second replicate; Com = combined replicates
[a] = Calculated by reviewer from means, Study #'s B7281 & B7291, Table 2.0.

There was not indication of an effect on body weight gain; however, a previous study in rabbits showed reduced body weight gains at 55 mg/kg/day and above (Lilly Study B-7366).

Food Consumption

The investigators supplied the group mean and individual animal data. The following table presents the food consumption data:

Table II: Food Consumption Data (unit)[a]

Gestation	Days:	0-5	6-18	19-27
Control	A[1]	148	153	144
	B	167	163	152
	Com	**153**	**156**	**146**
LDT	A	158	156	140
	B	173	151	128
	Com	**167**	**153**	**133**
LMDT	A	171	167	158
	B	154	147	139
	Com	**163**	**157**	**149**
HMDT	A	173	159	153
	B	154	139	138
	Com	**163**	**148**	**145**
HDT	A	170	154	171
	B	164	137	147
	Com	**167**	**145**	**158**

1 = A = First replicate; B = Second replicate; Com = combined replicates
a = Data extracted from Study #'s B7281 & B7291, Table 3.0.

There was a slight decrease in food consumption during the treatment period (gd 6-18) in the mid and high dose groups with a rebound in food consumption following the dosing period (gd 19-27) in the high dose group.

Gross Pathological Observations

No data on the dams were provided.

Cesarean Section Observations

Table III: Cesarean Section Observations[a]

Dose:	Control	LDT	LMDT	HMDT	HDT
#Animals Assigned	A 15	15	15	15	15
	B 15	15	15	15	15
#Animals Inseminated	A 15	15	15	15	15
	B 15	15	15	15	15
#Animals Pregnant	A 10	8	11	9	7
	B 4	11	9	10	7
Pregnancy Rate (%)	A 67	53	73	60	47
	B 27	73	60	67	47
Maternal Wastage					
#Died	none lost				
#Non pregnant[1]	A 5	7	3	6	7
	B 11	4	6	5	8
#Aborted	A 0	0	1	0	0
Total Corpora Lutea[1]	A 71	64	59	72	46
	B 27	87	76	65	55
Corpora Lutea/dam	A 7.1	9.1	5.9	8.0	7.7
	B 6.8	7.9	8.4	6.5	7.9
Total Implantations[1]	A 59	44	48	47	22
	B 21	83	55	56	37
Implantations/Dam	A 5.9	6.3	4.8	5.2	3.7
	B 5.3	7.5	6.1	5.6	5.3
Total Live Fetuses[1]	A 55	40	46	41	21
	B 19	79	50	53	36
Live Fetuses/Dam	A 5.5	5.7	4.6	4.6	3.5
	B 4.8	7.2	5.6	5.3	5.1
Total Resorptions[1]	A 4	4	2	6	1
	B 2	4	5	3	1
Early[1]	A 3	2	2	6	1
	B 1	4	4	3	1
Late[1]	A 1	2	0	0	0
	B 1	0	1	0	0
Resorptions/Dam	A 0.4	0.6	0.2	0.7	0.2
	B 0.5	0.4	0.6	0.3	0.1
Mean Fetal Weight(gm)	A 35.33	34.55	37.55	36.22	40.63
	B 35.35	32.57	34.07	37.23	37.23
Preimplantation Loss(%)[1]	A 16.9	31.3	18.6	34.7	52.2
	B 23.1	4.6	27.6	13.9	32.7
Postimplantation Loss(%)[1]	A 6.8	9.1	4.2	12.8	4.6
	B 9.5	4.8	9.1	5.4	2.7
Sex Ratio(% Male)	A 56.8	49.6	63.1	55.9	46.7
	B 64.6	50.0	43.4	51.5	58.7

[1] = Data calculated by reviewer

[a] = Data extracted from Study #'s B7281 & B7291, Table 1.0.

When the studies are viewed singly or combined, no apparent treatment related effects were noted. It is of note that the pregnancy rates for these studies are very low, the investigators stated that *The low number of gravid rabbits was due to atypically low fertility rates in both replicates.* No explanation was provided.

2. Developmental Toxicity

External Examinations

No treatment related effects were noted, see Table 5 from the investigators report attached as an appendix.

Visceral Examinations

No treatment related effects were noted, see Table 5 from the investigators report attached as an appendix.

Skeletal Examinations

No treatment related effects were noted, see Tables 5 and 6 from the investigators report attached as an appendix.

D. Discussion/Conclusions

a. Maternal Toxicity:

Very slight maternal toxicity was observed in the mid and high dose groups as reduced food consumption during the dosing period with a rebound in food consumption in the days following dosing for the high dose only. A previous study in rabbits showed toxicity at similar dose levels (*A Teratology Study on Lilly Compound 67019 (Oryzalin) in the Rabbit*, Lilly Research Laboratories. Lilly Study B-7366, September 1978, MRID No.'s 00052557, 00073552, 00026785), where there was a slight decrease in body weight gains in the mid and high dose groups and a slight decrease in food consumption. For that study the Maternal Toxicity NOEL was 25 mg/kg/day and the Maternal Toxicity LOEL = 55 mg/kg/day.

b. Developmental Toxicity:

i. Deaths/Resorptions:

No treatment related observations were noted.

ii. Altered Growth:

No treatment related observations were noted.

iii. Developmental Anomalies:

No treatment related observations were noted.

iv. Malformations:

No treatment related observations were noted.

The previously mentioned study (*A Teratology Study on Lilly Compound 67019 (Oryzalin) in the Rabbit*, Lilly Research Laboratories, Lilly Study B-7366, September 1978, MRID No.'s 00052557, 00073552, 00026785) noted possible developmental toxicity in the mid and high dose groups in the form of reduced mean litter size, increased resorptions and increased postimplantation loss (although the reduced number of corpora lutea might have affected these parameters). The Developmental Toxicity NOEL was 25 mg/kg/day and the Developmental Toxicity LOEL was 55 mg/kg/day.

D. Study Deficiencies:

The pregnancy rates for both the replicate studies are very low; however, when the studies are combined, the data are marginally acceptable.

The dosing solutions were not analyzed for homogeneity, concentration or stability.

E. Core Classification: Core Minimum Data.

Maternal Toxicity NOEL = 25 mg/kg/day
Maternal Toxicity LOEL = 55 mg/kg/day
Developmental Toxicity NOEL > 125 mg/kg/day
Developmental Toxicity LOEL ≥ 125 mg/kg/day

This study satisfies the 1984 Pesticide Assessment Guideline requirement (40 CFR 158.340, § 83-3b) for a teratology study in rabbits and supports the previous study (MRID No.'s 00052557, 00073552, 00026785).

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Pages 91 through 101 are not included in this copy.

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

010612

OFFICE OF
PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM AUG 4 1982

TO: Robert Taylor, PM #25
Registration Division (TS-767)

SUBJECT: Oryzalin; Replicated Rabbit Teratology Study;
Percutaneous Absorption of ^{14}C-oryzalin in Monkeys;
EPA Reg. Nos. 1471-96 and 1471-112. CASWELL #623 A

Submitted studies are reviewed and commented on as follows:

1. Study:

A Replicated Teratology Study on Oryzalin (EL-119, Compound 67019) By the Oral Route in Dutch Belted Rabbits. [Acc. #247229] Studies: B7281 and B7292 (Replicates). Date: March 1982. Lab: Lilly Research Laboratories.

Conclusion:

Oryzalin was not teratogenic to Dutch Belted Rabbits when administered, via gavage, at doses up to and including 125 mg/kg; NOEL = 125 mg/kg.

Method:

Two replicate studies were performed; each containing 75 virgin female Dutch Belted Rabbits which were randomly distributed among 5 experimental groups per study (15 rabbits per group). Animals were acclimated for a 2 week period and given physical/eye examinations prior to group assignment. Each female was artificially inseminated followed by an injection of chorionic gonadotropin. Environmentally controlled room conditions were: temp - 21±3°C; rel. hum. - 45%; 12 hr. light cycle; 12 air changes/hr. Animals were housed individually and received rabbit chow/tap water ad libiturm.

Oryzalin, (97% pure) was administered orally via gavage in doses of 0, 10, 25, 55 and 125 mg/kg/day on gestation days 6-18. Doses were based on gestation day 6 and adjusted on day 13 body weights.

Observations:

Daily for overt toxic signs
Body wt. - days 0, 6, 13, 19, 24 and 28
Food Consump. - daily
Gross necropsy - on all animals at day 28 and all animals that died prior to terminus.

Parents:

Uterus and ovaries
° # and distribution of implantations
° live fetuses
° dead fetuses
° # resorptions

Fetuses:

external anatomical anomalies
body wt.
skeletal/visceral exam.
sex

Analysis of Data:

Data pooled from both replicates.

Results:

Survival - No compound related effects
Body wt. - No compound related effects
Food Consumption - No compound related effects

Accumulated Data:
Replicate B7281/B7291

Dose (mg/kg)	0	10	25	55	125
#Animals	15/15	15/15	15/15	15/15	15/15
#Pregnant	10/4	8/11	11/9	9/10	7/7
#Aborted	0/0	0/0	1/0	0/0	1/0
%Fertility	66%/27%	53/73	73/60	60/66	46/46
(pooled)	(47)	(63)	(67)	(63)	(46)
Corpora Lutea	7.1/6.8	9.1/7.9	5.9/8.4	8.0/6.5	7.7/7.9
Implantations	5.9/5.3	6.3/7.5	4.8/6.1	5.2/5.6	3.7/5.3
Live Fetuses					
(%/Litter)	94/93.8	91.9/95.8	95.8/89.4	83.8/94	94.5/97.1
(#/Litter)	5.5/4.8	5.7/7.2	4.6/5.6	4.6/5.3	3.5/5.1
Resorptions (%/Litter)	6/6.3	8.1/4.2	4.2/10.6	16.2/6	5.6/2.9
%Males/Litter	56.5/64.6	49.6/50	63.1/43.4	55.9/51.5	46.7/58.7
Fetal Wt.	35/35	34.5/32.5	37.5/34	36.2/37.2	40.6/37.2
Fetuses Examined	55/19	45/79	46/50	41/53	21/36
# Fetuses w/ Anomalies	25/8	19/31	23/12	13/29	10/20
(%/Litter)	44/42	44/41	49/30	44/56	55/57
Exencephaly (# fetuses)	0/0	*1/0	0/0	0/0	0/0
Cleft Palate	0/0	*1/0	0/0	0/0	0/0
Hypoplastic kid.	0/0	*1/0	0/0	0/0	0/0
Displaced kid.	0/0	0/0	0/0	0/1	0/0
Malformed Sternebrae	1/0	*1/0	1/0	0/2	1/0
* Vertebrae	0/0	*1/0	1/0	0/1	0/0
Incompl. Closure of Vertebral centra	0/0	0/0	0/0	0/1	0/0

NOTE: *Same fetus (rabbit #6292, 1 of 4 fetuses)

Administration of oryzalin to rabbits during gestation days 6-18 did not adversely effect fetal viability, fertility rates, or prenatal mortality. Skeletal and visceral examinations did not reveal any treatment related effects. Oryzalin was not evidenced to be teratogenic to Dutch Belted Rabbits at doses up to and including 125 mg/kg.

It should be recognized that the fertility rates for both replicates were extremely low (e.g. 27 to 73%). However, there was no apparent dose-related trend in these fertility rates. Because of this, it was apparently necessary to combine the results from both studies in order to provide sufficient data on pups, and in fact may be the reason for a second or replicate study (B7291) started 2 weeks after the first study (B7281). In either event neither study provides adequate information when viewed as a separate study. However, when considered collectively, as submitted by the registrant, the replicate studies are considered CORE Minimum data and provide evidence that Oryzalin is not teratogenic or fetotoxic to Dutch Belted Rabbits under the conditions of the study.

2. Study:

Percutaneous Absorption of ^{14}C-Oryzalin in Monkeys. [Acc. #247229]. Studies: M-6231 and M-6012 Date: March 1982. Lab: Lilly Research Laboratories.

Conclusion:

Data presented are inconclusive with respect to absorption and excretion of dermally applied concentrations of ^{14}C-Oryzalin to the forearm of Rhesus Monkeys.

Method:

Two male and two female Rhesus Monkeys were given I.V. and percutaneous doses of ^{14}C-Oryzalin in ethanol and the rate of excretion via urine, blood and feces was measured for 96 hours after dosing. The same monkeys were used in both studies (M-6231 and M-6012). The studies were conducted 2 months apart.

Lab conditions temp = 22±1°c; Rel. humid = 45%; 12 hr. light/dark cycle.

Dose Administration:

a. Dermal - 250 ul of ^{14}C-oryzalin (ca 8.6 u Ci) were applied to 6 cm^2 area on the shaved ventral forearm (shaved 24 hrs. prior to administration). Solution applied slowly and evaporated with a hair dryer. After 24 hrs., application area was washed and the wash water saved and analyzed. During the test, monkeys were placed in metabolism chains for the first 24 hrs., arms restrained for the first 4 hrs. Afterwards they were placed in metabolism cages. Sampling: Blood- 0.5, 1, 2, 4, 6, 24, 48, 72 and 96 hrs. Urine/feces - 6, 24, 48, 72 and 96 hrs.

b. Intravenous - 1 ml (ca 9 mg oryzalin/animal or 9 u Ci/animal) injected into the saphenous vein. Animals placed in metabolism cages immediately after dosing. Sampling: Blood - 0.25, 0.5, 1, 2, 4, 6, 24 and 48 hrs. after dosing; urine/feces- 6, 24, 48, 72 and 96 hrs. [Bechman LS 9000 liquid scintillation spectrometer used for sample analysis.

Results:

After I.V. administration ^{14}C-oryzalin is apparently rapidly eliminated from the blood, with approx. 74% excreted in urine/feces within 96 hrs. The fate of oryzalin via dermal application is less accurately determined from the data presented. ^{14}C Oryzalin appears in the blood following application and attains a steady state for the duration of monitoring (96 hrs.), but shows no sign of decreasing (DPM/ML). The statement that "these data indicate that the rate of excretion was high compared to the rate of absorption", in my opinion, cannot be determined from the data (graphs) presented. The accum. % excretion (Figure 2) demonstrates an increase with time, as does the blood level DPM/ML (Figure 1). [Correction to report: Figure 3 does not contain excretion information for dermal dose, as stated on page 10, "RESULTS" of subject report.] Without actual data from which these figures were made it is difficult to determine how much of the dermal dose was absored. Furthermore, excretion via urine and feces may not be the only route of elimination, in which case the determination of % absorbed based upon such data may not be completely accurate.

It would appear that oryzalin is poorly absorbed via the forearm of Rhesus Monkeys, however, this can only be verified with submission of the data upon which these figures and general statements are based.

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8/4/82

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