UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
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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
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TO: PM Mr. Robert Taylor
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THROUGH: Mr. W. Burnam, Acting Chief
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"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.

NEW TOXICOLOGY:

The toxicological evaluation of oryzalin (compound 67019) given to $B_6C_3F_1$ 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, 1,250, 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: Erythocyte 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 [redacted]

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.)

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.