

3-12-82

MEMORANDUM

Date March 12, 1982

Subject Carcinogenicity / Oncogenicity Assessment of Oryzalin 'Sungha'

Re: Lilly 9 year Rod Feeding Study R107 + R177;

Permanent Tolerance Action 6F1859

Registration # 1471-96 + 1471-12

Caswell 623 A

From Berttram D. Litt - Statistical Toxicology Branch (TS-769)

To Bruce Jaeger, Head Section I, Toxicology Branch (TS-769)

Toxicological review of the subject study identified large numbers of tumor bearing animals. Dr Kagan, Branch pathologist, advised that a statistical analysis be performed to determine the level of significance for the following groups of tumors (Fibroma and/or Fibrosarcoma; Keratinocanthoma and/or squamous-cell carcinoma; Papilloma; Basal Cell and related tumors); Hepatic Adenoma; Mammary gland Fibroma; Adenoma or Adenocarcinoma as separate categories; Thyroid Follicular cell adenoma and/or carcinoma; Thyroid C-cell Adenoma and/or carcinoma; Parathyroid; Lymphoma; Pituitary Adenoma; Testicular interstitial cell tumors. The Toxicologist, Dr Anaise, has prepared the stat data for analysis by abstracting and tabulating the tumors for each animal by dose and sex, age, day and type of death, after noting the small number of tumors (especially) in the report itself for these animals have been recorded and some of them were found to have pancreatic and/or adrenal tumor and these findings are considered in the following analysis of tumor bearing animals.

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(see Table 1.)

The initial consideration is the relative survival reported. It should be clear that there is a dose related reduction in survival of rats treated with Oryzalin ($P < .01$) in both sexes. Moreover, it is shown that there is a 20 percent reduction among the high dose (2700 ppm or 135mg/kg/day) rats. This statistically significant reduction in high dose survival suggests that the maximum tolerated dose has been exceeded and that tumor finding may be biased among the high dosed groups.

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Table 1. Orizulin Study 167+177 Rats: Survival

Survival of Male Rats

Dose (PPM)	n	Number of Survivors				Percent Survival			
		12m	15m	21m	24m	12m	15m	21m	24m
0	60	58	54	52	47	96.7	90.0	86.7	78.3
300	60	60	59	53	41	100.0	98.3	88.3	68.3
900	60	59	53	50	36	98.3	88.3	83.3	60.0
2700	60	59	49	43*	34**	98.3	81.7	71.7	53.3

Survival of Female Rats

Dose (PPM)	n	Number of Survivors					Percent Survival			
		0m	12m	18m	21m	24m	12m	18m	21m	24m
0	60	60	60	55	49	44	100	91.7	81.7	73.3
300	60	60	60	58	51	40	100	96.7	85.0	66.7
900	60	60	60	56	47	37	100	93.3	78.3	61.7
2700	60	59	53	42 ^B	31*		98.3	88.3	70.0	51.7

B: one tail test P < .1

* " " " P < .05

** " " " P < .01

When the total number of tumor bearing animals was examined we found almost all males and the majority of all study rats had at least one tumor:

Dose Group	Tumor Free Proportion of Rats	
	Males	Females
Control	4/60	16/60
Low Dose	0/60	12/60
Mid Dose	1/60	3/60
High Dose	2/60	9/60

When, as suggested by the survival data, one deleted the high dose group a statistically significant increase in tumor bearing animals is observed to be associated with increased Oryzalin exposure (or dose.) At the suggestion of Dr. Tox Brant Pathologist we reexamined the total tumor picture treating animal with a single tumor diagnosed as interstitial cell tumor in males or a mammary tumor in females or a Pituitary adenoma in either sex. The proportion of tumor bearing rats was:

Dose Group	Males	Females
Control	33/60	25/60
Low-Dose	43/60 *	30/60
Mid-Dose	43/60 *	39/60 *
High-Dose	53/60 ***	37/60 *

(* One Tail Test $P < .05$; *** One Tail Test $P < .001$)

The increased proportion of tumor bearing rats is clear evidence of the need to look more closely at individual tumor types and particularly among the males.

Table 2. Orizalin Study 167+177 Rat Thyroid Findings

Thyroid: Follicular Cell Adenoma

Females 167+177	Interstitial	Terminal	Total	
0 ppm	1/12	0/43	1/55	
300 ppm	0/20	1/40	1/60	
900 ppm	0/23	3/37	3/60	None
2700	3/26	6/29	9/55	

Males 167+177	Interstitial	Terminal	Total	
0 ppm	—	—	4/59	
300 ppm	—	—	10/59	x
900 ppm	—	—	5/57	
2700 ppm	—	—	6/56	

C-Cell Adenoma

Females 167+177	Interstitial	Terminal	Total
0 ppm	1/12	4/43	5/55
300 ppm	0/20	2/40	2/60
900 ppm	2/23	3/37	5/60
2700 ppm	5/26	0/29	5/55

Males 167+177	Interstitial	Terminal	Total
0 ppm	—	—	6/59
300 ppm	—	—	8/60
900 ppm	—	—	1/60
2700	—	—	4/60

9) Thyroid tumors and more especially Follicular cell Adenomas were identified by the toxicologist as a target tissue response. However, when the data displayed as in table 2 we find that the principle response, or high tumor rate, is observed among high dose females. This may easily be a spurious finding if the preceding observation concerning bias in the high dose group and higher sensitivity for tumors among males.

Accordingly we next examined the proportion of animals with skin tumors. The total number of skin tumor bearing animals is displayed in table 3. The males are definitely more sensitive with respect to skin tumors although a higher level of statistical significance is seen among ^{multiple} females ^{relative to} _{control} (.006 for females vs. .024 for males). The sensitivity of the males is demonstrated by the highly significant dose response observed among the animals dying during the study - i.e. earlier onset, while females do not show an effect until the terminal kill.

When the individual or specific types of skin tumors are examined one at a time we find two Tumortypes of special interest: The keratoacanthoma and for squamous cell carcinoma shows an increase in females and in males at the high dose but not ~~define~~ significantly at mid or low doses; the basal cell and related adenomas or viceptithoma group show a highly statistically significant increased rate at low and mid-doses and a significant effect at the highest dose with the same patterns discussed for total skin tumors.

As the result of the preceding we conclude that all statistical weight indicates that due to the increased and earlier incidence of Basal cell type tumors in both sexes in males that this tumor be 5.

Table 3 Cryzoline Study 167 + 177 Rat Skin Tumors

Proportion of Animals with Skin Tumors				
Sex	Dose	Interim # (%)	Terminal # (%)	Total # (%)
Males	0 ppm	3/13 (23.1)	15/47 (31.9)	18/60 (30.0)
	300 ppm	6/19 (31.6)	15/41 (36.6)	21/60 (35.0)
	900 ppm	15/22 (68.2)	15/36 (41.7)	30/58 (51.7)
	2700 ppm	18/25 (72.0)	25/34 (73.5)	43/59 (72.9)
	Females	0 ppm	2/16 (12.5)	3/44 (6.8)
300 ppm	4/20 (20.0)	6/40 (15.0)	10/60 (16.7)	
900 ppm	4/23 (17.4)	14/37 (37.8)	18/60 (30.0)	
2700 ppm	9/29 (31.0)	21/31 (67.7)	30/60 (50.0)	

Table 4 Oryzalin Study 167+177 Rat Skin Tumors

Skin: Keratoacanthoma and/or Squamous Cell Carcinoma

<u>Females 167+177</u>	<u>Interim</u>	<u>Terminal</u>	<u>total</u>	<u>Statistical Sig</u>
0 ppm	0/16	1/44	1/60	
300 ppm	1/20	0/40	1/60	
900 ppm	1/23	3/37	4/60	None
<u>2700</u>	<u>2/29</u>	<u>5/31</u>	<u>8/60</u>	**

<u>Males 167+177</u>	<u>Interim</u>	<u>Terminal</u>	<u>Total</u>	
0 ppm	1/3	4/47	5/60	
300 ppm	0/19	3/41	3/60	
900 ppm	4/24	1/36	5/60	None
<u>2700</u>	<u>7/25</u>	<u>11/34</u>	<u>18/60</u>	**

Skin: Basal Cell, Preputial, Sebaceous or Zymbal's gland Adenom or Tricripteli

<u>Females 167+177</u>	<u>Interim</u>	<u>Terminal</u>	<u>Total</u>	
0 ppm	0/16	2/44	2/60	
300 "	1/20	2/40	3/60	
900 "	3/23	10/37	13/60	**
<u>2700</u>	<u>4/29</u>	<u>6/31</u>	<u>10/60</u>	*

<u>Males 167+177</u>	<u>Interim</u>	<u>Terminal</u>	<u>Total</u>	
0 ppm	2/13	7/47	9/60	
300 "	5/19	8/41	13/60	
900 "	9/24	10/36	19/60	**
<u>2700</u>	<u>10/25</u>	<u>17/34</u>	<u>27/60</u>	*

used as the basis for risk assessment. The data were used as the basis for risk assessment after the dosage levels were adjusted to mg/kg/d based on the constant shown in Lehman's tables, 1ppm ~ .05 mg/kg/day, and adjusted to human exposure level equivalents using the surface area approximation of (human wt / animal wt)^{2/3} i.e. divide animal dose by 5 at (60000g / 500g)^{2/3} thus the rat doses of 300 and 600 ppm were adjusted to 3 and 9 mg/kg/day respectively.

Models tested for goodness of fit for low dose extrapolation included

Multi-stage model males	P > .9
" " Both sexes	P > .9
Multi-hit model	P < .01
One hit model	P > .05
Weibull with additive background	P < .01
Weibull with Independent Background	P < .01
Probit model	P < .01

This leads to the conclusion that the one hit and multihit models are the most logical but that the multi-hit is a better fit. Both of the multi-models lead to approximately the same level of potency as estimated by the 95% ^{upper} confidence Bound on the slope or dose-response as indicated by Q₁^{*} of 3.375 x 10⁻² for the males and female data and Q₁^{*} = 4.406 x 10⁻² for male data only. The estimate of potency Q₁^{*} when multiplied by individual exposures will provide an upper 95% confidence Bound on the expected risk of human cancer based on the findings of 4/130 probit all tumors in control rats 14/130 at 3 mg/kg/day human equivalent in the food at 32/130 at 9 mg/kg/day human equivalent in the food of study rats.