3-12-82

MEMORANDUM
Date March 12, 1982
Subject Carcinogenicity / Oncogenicity Assessment of Oryzalin 'Surflan'
Re: Lilly 2 year Rat Feeding Study R167 + R177;
Permanent Tolerance Action 6F1859
Registration # 1471-96 + 1471-112
Caswell 623 A

From Bertram D. Litt Statistician, 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 Kazu, Branch pathologist, advised that a statistical analysis be performed to determine the level of significance for the following groups tumors (Fibroma and/or Fibrosarcoma; KeratoAcanthoma 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 Malignant; Lymphoma; Pituitary Adenoma; Testicular interstitial cell tumors. The Toxicologist, Dr Quaife, has prepared the ~~stat~~ data for analysis by abstracting and tabulating the tumors for each animal by dose and sex, group, and day and type of death. After noting the small number of (tumor apparently) free the report states for these animals have been rechecked and none of them were found to have pancreatic and/or adrenal tumor and these findings are considered in the following analyses of tumor bearing animals.

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The initial consideration is the relative survival reported (see Table 1.). 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 135 mg/kg/day) rats. This statistically significant reduction in high dose survival suggests that the maximum tolerated dose has been exceeded and that tumor findings may be biased among the high dosed groups.

Table 1. Oryzalin Study 167+177 Rats: Survival

Survival of Male Rats

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

B: one Tail test $P \sim .1$

* " " " $P < .05$

** " " " $P < .01$

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 dosed 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. K. the Fox Branch Pathologist we examined 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. Cinnzalin Study/167+177 Rat Thyroid Findings

Thyroid: Follicular Cell Adenoma

Females 167+177	Interim	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	Interim	Terminal	Total	
0 ppm	—	—	1/59	
300 ppm	—	—	10/59	*
900 ppm	—	—	5/57	
2700 ppm	—	—	6/56	

C-Cell Adenoma

Females 167 & 177	Interim	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	Interim	Terminal	Total
0 ppm	—	—	6/59
300 ppm	—	—	8/60
900 ppm	—	—	1/60
2700	—	—	4/60

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 we said that the principle response or high tumor rate is observed among high dose females, this may easily be a spurious finding of the preceding observation or may 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 attaining a higher level of statistical significance is seen among females (mid-dose relative to control (.001) 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 - ie 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/or squamous cell carcinoma shows an increase in females and in males at the high dose but not ~~dose~~ significantly at mid or low doses; the basal cell and related adenomas or trichepthioma group show a highly statistically significant decrease rate at low and mid-doses and a significant effect at the highest dose with the same patterns observed 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

Table 3 Oxygalin Study 117 + 177 Rat Skin Tumors

Proportion of Animals with Skin Tumors

Sex / Dose	Interim II (%)	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	15/32 (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)	5/60 (8.3)
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 (38.7)	21/60 (35.0)

Table 4 Oryzalin Study 167+177 Rat Skin Tumors

Skin: Keratoacanthoma and/or Squamous Cell Carcinoma

Females 167+177	Interim	Terminal	Total	Statistical Sig
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
2700	3/29	5/31	5/60	**

Males 167+177	Interim	Terminal	Total	
0 ppm	1/13	4/47	5/60	
300 ppm	0/19	3/41	3/60	
900 ppm	4/24	1/36	5/60	None
2700	7/25	4/34	15/60	**

Skin: Basal Cell, Preputial, Sebaceous or Zymbal's Gland Adenoma or Trichoepithelioma

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

Males 167+177	Interim	Terminal	Total	
0 ppm	2/13	7/47	9/60	
300 "	5/19	8/41	13/60	
900 "	9/24	10/36	19/60	**
2700 "	10/25	7/34	17/59	*

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 stored in Lehman's tables, 1 ppm = .05 mg/kg/day, and adjusted to human exposure levels equivalents using the surface area approximation of (human wt ÷ animal wt)$^{1/3}$ i.e. - divide animal dose by 5 as (60 000g ÷ 500g)$^{1/3}$ thus the Rat doses of 300 and 600 ppm were adjusted to 3 and 6 mg/kg/day respectively.

Models tested for goodness of fit for low dose extrapolation includes

Multi Stage Model males	$P > .9$
" " Both Sexes	$P > .9$
Mult. 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 multi-stage 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 of dose-response as indicated by Q_1^* of 3.375×10^{-2} for the male and female data and $Q_1^* = 4.426 \times 10^{-2}$ for male data only. The estimate of potency Q_1^* when multiplied by individual exposures will provide an upper 95% confidence Bound on the expected risk of human cancer based on the findings of 11/120 Basal cell tumors in control rats 14/120 at 3 mg/kg/day human equivalents in the food and 32/120 at 6 mg/kg/day human equivalents in the food of study rats.