

SURVEY OF JANUS LONG-TERM EXPERIMENTS

The following is based on an analysis of the current situation undertaken by E.B. Wilkes.

The prime aim was to examine the dose-response curves and, if necessary, to attempt to extrapolate the observed results to lower dose levels. Consideration is given to Experiments B0-B7, except for B1 and B5, which are single dose experiments. B6 and B7 are considered as single condensate experiments by pooling the results at the three strand-widths.

High Dose Anomaly

The problem is clearly seen by plotting the observed tumour response against dose levels (Fig. 1). In each case, except B3, the response for the 75 mg dose is either less than or only marginally greater than that at the 50 mg dose; in the extreme case, B6, the response decreases with dose level at both the 50 mg and the 75 mg dose levels.

The age-standardised values (PSR) are shown in Fig. 2. Although the trends noted above are reduced, it is clear that the tumour response at dose levels above 50 mg still decreases in both B6 and B7. The observed (Obs) and standardised (Std) tumour response values are given in Table 1.

Table 1
Percent Tumour Response

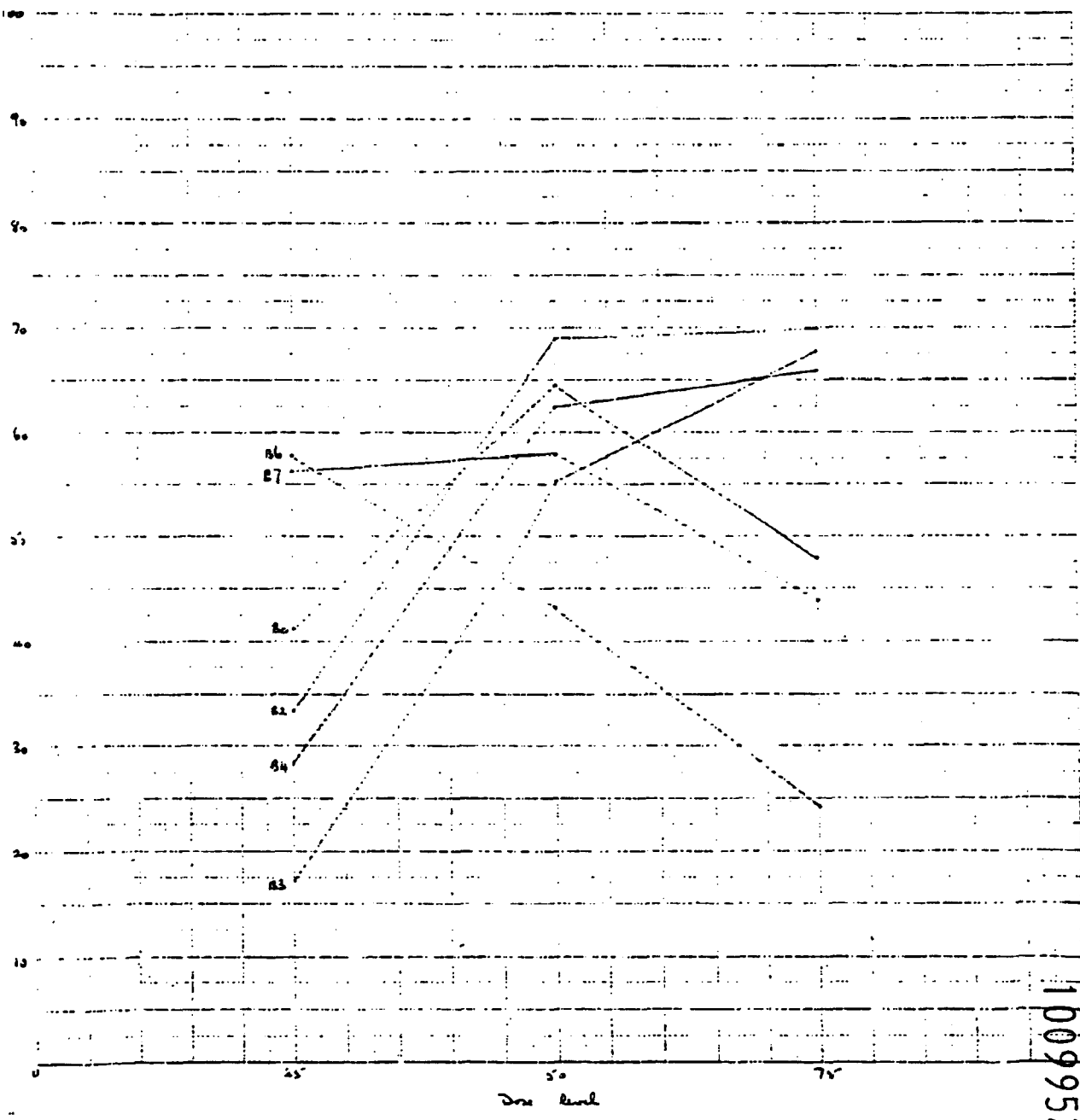
Expt. No.	Dose Levels (mg)					
	25		50		75	
	Obs	Std	Obs	Std	Obs	Std
B0	41	48	65	80	48	84
B2	33	34	69	76	70	80
B3	17	17	55	55	68	65
B4	28	31	63	68	66	76
B6	58	59	43	81	24	81
B7	56	62	58	85	44	84

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Graph 2.

The observed results of experiment B1, B2, B3, B4, B6, B7.

Tumour Response
%



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Graph 2

The PSR Results of experiments 30-37 inclusive (31 and 35 omitted)

PSR %

100

70

50

30

10

0

20

40

60

80

100

25

50

75

Dom Level

37

36

30

32

34

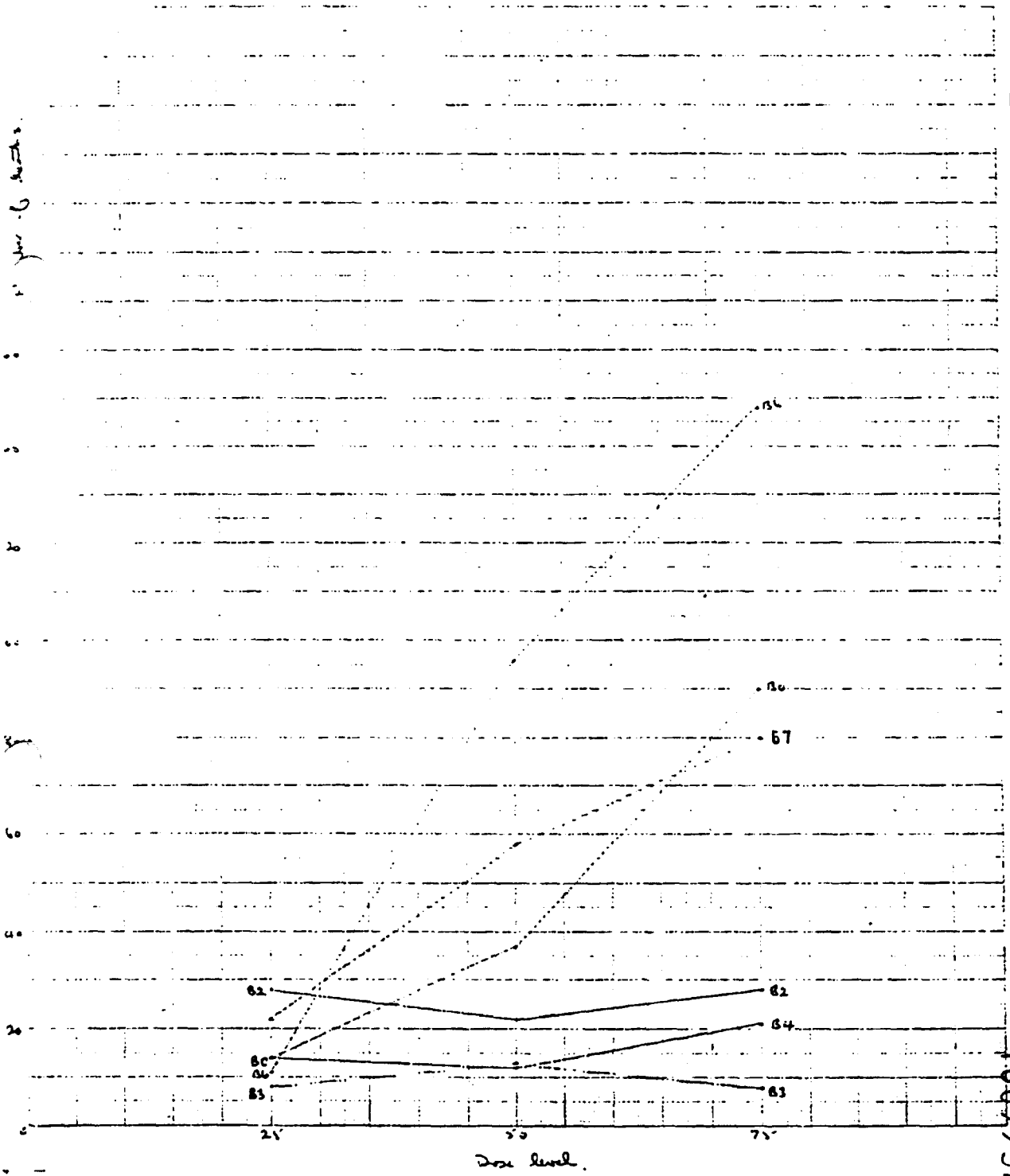
33

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Graph 3.

Number of animals dying before the 1st lactation

Expts. B0 B2 B3 B4 B6 B7.



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Two reasons are put forward to explain the high dose anomaly:

- (a) The high initial mortality which is particularly pronounced at the 50 and 75 mg dose levels in B0, B6 and B7 (see Fig. 3). This reduces the population at risk, and if excessive is only partly corrected by the age-standardisation.
- (b) The time taken to produce tumours is significant; the first usually appear between weeks 28 and 32. Considering B0, 95% of the animals survive in the age-standardised experiment to week 28 and this would be the maximum tumour response. Since the final age-standardised result for the 50 mg dose level is 80%, the response to any dose greater than 50 mg is confined between 80% and 95%.

The only way to reduce these effects is to reduce the dose levels. The amount by which they must be reduced is judged from the tumour rates of the extrapolated low-dose experiments.

Approximations

Since satisfactory dose-response curves are not available in any of the above experiments, the results have been extrapolated to lower levels. The detailed analysis showed that the optimum dose levels were 20 mg, $20\sqrt{2}$ mg, and 40 mg. This extrapolation has been undertaken using data from B0, B2, B3 and B4, on the basic assumption that the tumour incidence is a log-normal distribution function. Using the pooled data from the above experiments a parabola was used to describe $\ln(\text{median age})$ vs. $\ln(\text{dose})$. It was assumed that this could be extrapolated linearly at the lower dose end. This general curve has been used to reconstruct the data on tumorigenic incidence from the individual experiments by adjusting the constants so that the curve passes through the 25 mg point in each case.

Reconstruction of the mortality rates, by a series of assumptions and approximations, was even more difficult as no convenient function analogous to the log-normal one used for tumour rates exists. (The curve was described by two parabolas and a geometric progression.)

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Simulated Experimental Results

The tumorigenicity of two condensates is best compared by estimating the dose required to produce a given response in each case. Obviously, this can only be done, and confidence limits applied, when the dose response curves (response vs. log dose) are approximately parallel; linearity is not essential but the curves must be a uniform distance apart.

Tumorigenic ratios are calculated from the 20, $20\sqrt{2}$ and 40 mg dose levels of the reconstructed data. Since there is no way of testing whether the slopes of these simulated experiments are significantly different or not, it is assumed that they are not significantly different. Thus the data, relating to the slope, was pooled for each pair of condensates compared. To obtain confidence regions for each comparison, it was assumed that the response rates are binomial variates. The results are presented in Table 2.

Table 2
Tumorigenic Ratios - Simulated Data

(The ratio is significantly different if the values between the upper and lower limits do not include 1.0)

	Lower 95% Limit	T	Upper 95% Limit
B0 - B2	1.001	1.229	1.536
B3	1.341	1.653	2.129
B4	1.063	1.302	1.634
B6	.632	.811	1.018
B7	.566	.741	.936
B2 - B3	1.100	1.335	1.659
B4	.870	1.059	1.296
B6	.506	.659	.821
B7	.451	.601	.757
B3 - B4	.644	.794	.962
B6	.355	.483	.613
B7	.312	.438	.564
B4 - B6	.474	.620	.774
B7	.421	.565	.714
B6 - B7	.702	.914	1.175

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The activity of the condensates can be ranked in the order:

← decreasing activity
B3 < B4, B2, B0, B6, B7

The activity of B3 is significantly lower than that of all other samples.

B4 is significantly lower than samples B0, B6 and B7.

B2 is significantly lower than samples (B0), B6 and B7.

B0 is significantly lower than sample B7, but there is no significant difference between B6 and B7.

THIS RANKING IS, OF COURSE, BASED SOLELY ON THE VALUES OF THE ACTIVITIES FOUND AT THE 25 mg DOSE LEVEL. THE RECONSTRUCTION PROCESS HAS ONLY MADE POSSIBLE THE CALCULATION OF APPROXIMATE CONFIDENCE LIMITS.

SRE/SEW/3.5/1113
4th November, 1970

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