

Re RSW → DGF 17/10/78
Reply 10/11/78



TO	Dr. D.G. Felton	FROM	E.B. Wilkes
REF	EBW/CAL/46D	DATE	1st February 1979

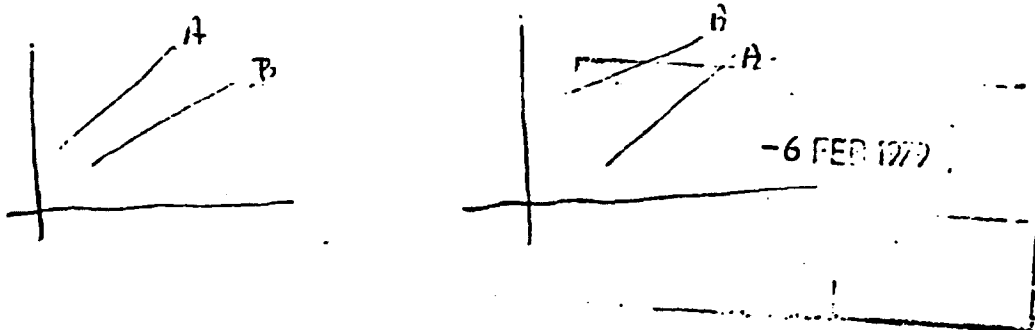
AMES TEST

I have looked at the paper by Mino Bilimoria which accompanied Bob Wade's letter of 16th January 1979 and would make the following observations.

1. Linearity of response is not tested by using the correlation coefficient. I will return to the question of a more proper test later. At this point I will just mention that if the correlation coefficient between the integers 1 to 10 and their squares 1 to 100 is calculated, the result is $r = 0.975$, but if the line is plotted it is very obviously not straight.
2. If a logarithmic transformation is to be used, then usually in biological work it is expected that the response is proportional to the logarithm of the dose, or alternatively that $\log(\text{response})$ is proportional to $\log(\text{dose})$. I'm not sure what Bilimoria did, but the text seems to suggest that he just took the log of the response.
3. Table 3 is not a test of the parallelism of the lines, as the 554 degrees of freedom in the within-slopes sum of squares clearly reveals (c.f. Table 5 for a similar test using un-transformed data). Table 3 would seem to be testing whether there is any significant regression at all, and so it is hardly surprising that a highly significant result is found.
4. The analyses of variance are based upon the slopes of the least squares regression lines i.e. six lines of the form

$$R = a + b \cdot (\text{dose})$$

were fitted to the data for each condensate (except PLCK20, where 24 lines were fitted). This does not seem to me to be a proper approach to the data. Consider the following two diagrams:



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According to the analysis, A would be regarded as less mutagenic than B in both cases, even though the mean level of response to A is much higher than B in (b), because of the slope of the dose/response line for A is less than that for B in both cases.

It is clear from the top of page 2 of his paper that Bilimoria was looking for some form of parallel line assay, and it is therefore somewhat surprising that he should be quite happy to adopt a model for the analysis which is founded upon non-parallel lines.

5. Assuming for the moment that the regression slopes do provide an adequate basis for the statistical analysis, the results seem fairly clear. Burley lamina yields condensate which is considerably more mutagenic than any of the other leaf types tested, and homogenisation of the tobacco during manufacture reduces the mutagenicity of the condensate for all the leaf types tested (although not significantly so for the flue-cured lamina - all positions). The lowest observed mutagenicity came from the homogenised flue-cured chopped plant leaf, this being over five times less active than Burley lamina before homogenisation.
6. Tables 8 and 9 show a comparison of the results of various tests of these 16 samples. It would appear that there is little agreement between them in terms of their ranking of these materials on a scale of biological activity. Whether this is reasonable from a biochemical viewpoint I am not able to judge. From a numerical point of view however the two results of mutagenicity testing present a strange picture. The mean level of response reported by Guelph is more than 12 times greater than the mean response from ITL. The range of responses from Guelph is from 3.9 to 28.0, compared with a range of 0.626 to 3.228 for ITL. Finally, on the ITL scale the Burley lamina (before homogenisation) was over twice as mutagenic as the flue-cured whole plant stem blend, whereas according to Guelph the ratio is 1.04 i.e. a marginal difference. What is happening of course is that it is the computed statistics that are being compared, and it would seem that the computational methods used by the two establishments are not the same. Moreover, it also seems that the ITL mode of analysis is somewhat suspect.
7. I do not know Mino Bilimoria, nor am I acquainted with the set-up at ITL. My overall impression is that Bilimoria is probably a competent biologist who has carried out the Ames test quite well, but he has then tried to become his own statistician, and, with the aid of a pre-programmed calculator, has attempted his own statistical analysis. In doing so he seems to have made several errors.

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8. It seems to me that the statistical analysis should be based upon a consideration of the mechanics of the test with a view to the correct selection of dose levels and regression models. In (1) Ames points out that the dose/response curves are, or tend to be, humped, showing a positive dose/response relationship at low doses, which becomes negative at high doses. For this reason he recommends a dose range wherein the highest dose is 1000 times the lowest dose. He claims that it should then be possible to identify a linear dose/response portion of the curve in order to carry out an effective assay.

Having found a suitable dose range and regression model, lack of fit may then be examined using the 3 replicates per point that Bilimoria provides; linearity may be tested by adding quadratic and, possibly, cubic terms to the model in the usual way, and parallelism can be tested via the residual sums of squares of the appropriate linear models.

Finally, if no suitable regression model can be found then, providing the dose range has been correctly selected, the data could be analysed using non-parametric methods.

REFERENCE

1. "Methods for detecting carcinogens and mutagens with the Salmonella/Mammalian - microsome mutagenicity test."
Bruce N. Ames, Joyce McCann and Edith Yamasaki Mutation Research 31 1975, 347-364.

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c.c. Dr. S.R. Evelyn

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