

TO: Mr. E.B. Wilkes

FROM: M.H. Bilimoria

SUBJECT: Statistical analysis of Ames
test data.

Mr. R.S. Wade has shown me your memo entitled "Ames Test", in which you commented on the statistical procedures employed by me. He also told me that you had kindly agreed to spare some time and analyse the raw data (B11 and Agriculture Canada Phase 1 cigarettes), which I am enclosing with this memo. However, in this memo I also wish to outline why I employed the procedures I did, and to point out that the write-up on the Agriculture Canada cigarettes was intentionally non-technical and brief, and a continuation of an earlier short report on cigarettes belonging to the Janus B11 series. Perhaps, if you had the opportunity of reading my report on the Janus B11 series, the reasons for the statistical approaches used in the second report would have been clearer. Besides, the latter report was prepared in such a way that the information contained in it could be disseminated quickly before the February group research conference in Southampton, England. These brief reports, prepared for the benefit of upper management, will be followed up with a regular research report in the usual manner.

Working with cigarette smoke condensates, I have repeatedly found that at the concentrations which I use to determine mutagenicity, there is an excellent straight line relationship between mutagenicity and concentration of condensate. A visual illustration of this linearity was presented in the report on the B11 series. Thus, with such straight line relationships, and correlation coefficients of over 0.90 suggesting excellent goodness of fit, the employment of the quadratic or any other model could improve the curve fitting very little, if at all. Consequently, I felt justified in using slopes of these straight lines to express the mutagenicity of the condensates. Thus, it was only after I was convinced about the linearity of the data that I stated that the high correlation coefficients indicated good linearity, a statement that is justified, unless one takes a very conservative approach.

105384115

Nevertheless, in order to make the variances independent of the means, I did attempt to use logarithmic transformations for both variables as done in the B11 series, and did consult a qualified statistician at McGill University in doing the ANOVA test for parallelism of the regression lines obtained from the transformed data. These lines, however, were not parallel, intercepts could not be used to express mutagenicity, and this approach was abandoned. It is the opinion of this statistician that the ANOVA shown in Table 3 does indeed test for the parallelism of the lines obtained from the transformed data, and not merely whether there is any significant regression, as suggested by you.

Finally, concerning your remarks relating to Tables 8 and 9, in which are presented a comparison of various short-term tests, it should be pointed out that the way Dr. Basrur treated her data, or expressed mutagenicity of the samples, should have no bearing on the comparisons drawn in the tables. The low correlation for the results from the two laboratories is merely a reflection of the differences in the experimental procedures followed. Dr. Basrur in her study used fresh whole smoke, and has expressed mutagenicity as the number of colony-forming units per ml smoke, obtained from the slope of the initial portion of the dose response curve. Dr. Basrur, I believe, no longer employs fresh whole smoke for the Ames test, and some time ago expressed an interest in attempting to organize a meeting to prepare a standard protocol to be followed by all laboratories testing smoke condensate in this bacterial test. However, to the best of my knowledge, nothing further has transpired with respect to drawing up such a protocol.

I do appreciate your interest in offering to examine the data from the two experiments, and look forward to receiving the analyses in the near future.

c.c. Mr. L.C.F. Blackman
Mr. N.E. Willis
Dr. S. R. Evelyn
Mr. R. Gibb
Mr. R. S. Wade

M. Bilimoria
(M.H. Bilimoria)

105384116