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SUMMARY OF AMES TESTS
FOR MUTAGENICITY OF SMOKE CONDENSATES
CONDUCTED BY ITL, MONTREAL

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SUMMARY OF AMES TESTS FOR MUTAGENICITY OF SMOKE
CONDENSATES CONDUCTED BY ITL, MONTREAL

1. Mutagenicity of Janus experimental cigarettes in the Ames test and comparison with carcinogenic activity.

Introduction

Traditional methods employing small rodents for predicting carcinogenicity of chemicals and complex mixtures such as tobacco smoke are both expensive and time consuming, and neither resources nor manpower are available to test all the compounds that man is introducing into his environment. For these reasons considerable interest has existed in finding short-term tests that could reliably predict the carcinogenicity of chemicals. Of the several short-term tests that have recently been proposed for detecting the mutagenic/carcinogenic potential of chemicals, the one developed by Professor Bruce N. Ames appears to be the most reliable, and consequently has gained world-wide acceptance. On account of the excellent correlation obtained between Ames bacterial mutagenicity and rodent carcinogenicity of pure compounds, considerable interest has also developed in studying the correlation between mutagenicity and carcinogenicity of complex mixtures such as tobacco smoke condensates.

Thus, we developed the capability of conducting the Ames test on smoke condensates in a quantitative manner, and decided to measure the mutagenicities of condensates from several series of experimental cigarettes (Janus series) produced by B.-A.T., Southampton. These cigarettes were designed to study several parameters thought to be important in determining the carcinogenic activity of cigarette smoke. Since B.-A.T. has undertaken carcinogenic studies on most of these cigarettes, they were ideal candidates for a comparative study of mutagenicity and carcinogenicity of cigarette smoke. GR & DC have followed our studies closely and are planning a meeting in July '81 to review these data as well as those obtained on some of the same samples by B.-A.T. Hamburg using the Ames test and another predictive short-term

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test utilising mammalian cell cultures. The following is a summary of data obtained at ITL, Montreal.

Summary of Results

Janus B9

This series was designed to study the effect of extracting tobacco with ethanol as well as incorporating into PCL the additives employed in the Gerlach process of making reconstituted tobacco. Gerlach reconstituted tobacco is made commercially in Germany and has been demonstrated to have an uniquely low biological activity. B.-A.T., some years ago carried out these studies to try to determine the reasons for the low biological activity. The following conclusions were drawn from the mutagenicity and carcinogenicity studies:

- (a) Both tests show that ethanol extraction reduces activity significantly, and returning the extract restores original activity.
- (b) Both tests show that the sheet materials are much lower than tobacco, and Gerlach is significantly lower than PCL made using Gerlach additives.
- (c) The carcinogenicity test shows that Gerlach made from ethanol-extracted tobacco is significantly lower than Gerlach made using unextracted tobacco. In contrast, the mutagenicity test shows the former to be slightly higher in activity.

Janus B11

Janus B11 cigarettes were produced to compare sheet materials made by the PCL and Schweitzer paper process. The following conclusions were drawn:

- (a) Both mutagenicity and carcinogenicity tests showed that the sheet materials were significantly lower in activity than flue-cured tobacco.

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- (b) The mutagenicity showed the two sheets to possess equal activity, while the carcinogenicity test showed that the Schweitzer sheet had significantly lower activity than PCL.

Janus B14

This Janus series was designed to study the effect of added nicotine to tobacco on the long-term skin painting activity on account of reports indicating that nicotine may be a co-carcinogen. Conclusions drawn from the experiments are as follows:

- (a) A dose-related increase in Ames mutagenicity with increasing total nicotine alkaloid content has been observed. A similar result is being obtained in the long-term carcinogenicity test by B.-A.T., Southampton (Binns, personal communication).
- (b) Both mutagenicity and carcinogenicity tests show that only at the highest concentration of nicotine is there statistical significance in difference from control.

Janus B15

This series, consisting of 12 samples, was designed to study the effect of ventilation on biological activity. Increasing use of ventilation in commercial cigarettes has rendered such a study of great importance to the tobacco industry. Ventilation was achieved by using papers of different porosities and by using a ventilated tip. A study of pressure drop was also built into the design of the experiment. Final carcinogenic data are not as yet available, and the following conclusions have been drawn from mutagenic data only:

- (a) Higher pressure drop appears to be associated with higher mutagenicity, while higher paper porosities resulted in lowering mutagenic activities. The differences were statistically significant.

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- (b) There was no difference between cigarettes made using non-ventilated and ventilated tipping (30 percent ventilation).

As a result of these findings it has been decided to conduct mutagenesis tests on commercial Canadian low-tar cigarettes in the near future.

Janus B16

The B16 series was designed to study the effects of application of "casing" to tobacco as well as the effects of other additives, viz., cocoa and the butterfat therefrom. These additives were studied because research at the National Cancer Institute, U.S.A., indicated that increased rodent carcinogenicity was associated with their use on tobacco.

Due to a policy decision to stop long-term mouse skin painting tests, B.-A.T., Southampton will not be doing the above long-term test on this series, and a comparison with mutagenicity will not be possible. However, on the basis of the Ames test, the following conclusions may be drawn:

- (a) Application of casing appears to lower mutagenicity, but the differences obtained, although significant, are small indeed.
- (b) The addition of cocoa to tobacco has no effect on mutagenicity, but the amount of cocoa butterfat added is significantly associated with lowering of mutagenicity.

II. Mutagenicity tests on other experimental and commercial cigarettes.

Introduction

Gradual acceptance of the Ames test as a good predictor of the carcinogenicity of pure chemicals and complex mixtures, such as tobacco smoke, was considered encouraging enough to test for mutagenic activity, a variety of condensates from experimental as well as commercial cigarettes, and the results have been summarized below.

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Summary of Results

Agriculture Canada Phase I Cigarettes

This series of 26 samples produced by the Delhi Research Station represents a carefully planned experiment to evaluate the chemistry of flue-cured and Burley tobaccos, as well as the effect of plant position, inclusion of stalk, conversion into sheet of the tobacco materials, etc., on the biological activity of smoke condensates. We selected 15 of these samples for mutagenicity testing and the following conclusions have been drawn:

- (a) Lamina from the lowest plant position showed the highest mutagenic activity, which was significantly different from the activities obtained for the middle plant position and whole plant laminas.
- (b) Sheet materials made from Burley and flue-cured laminas were significantly lower in activity than the original materials.
- (c) Burley lamina was significantly higher than flue-cured.
- (d) Mutagenicity is significantly correlated with the total and protein nitrogen contents of tobacco, confirming the results obtained in an earlier Japanese study.

French cigarettes

Evidence for the higher carcinogenicity of cigarettes made from dark highly fermented tobaccos, led us to evaluate three French cigarette brands, viz., Gauloise, Gallia and Celtique. The following conclusions were drawn:

- (a) All three brands produced condensates which were significantly higher in mutagenic activity than condensates from Players plain cigarettes.
- (b) Gallia, which is sold as a low biological activity cigarette, was significantly lower than the other two brands, which were similar in mutagenicity.

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Blue Mould infected tobacco

A considerable amount of blue mould damaged tobacco was purchased for cigarette making in 1979. Since concern was expressed about the use of this damaged tobacco, Ames tests were conducted on condensates from cigarettes made from this tobacco. Severely damaged "light" and "dark" tobaccos of both lug and cutter grades were employed in this study. This study led to the following conclusions:

- (a) Blue mould damage did not affect mutagenic activity of condensate.
- (b) Condensates from both blue mould infected and uninfected dark tobaccos were significantly higher than infected and uninfected light flue-cured tobaccos.

Ridomil and Ridomil-treated flue-cured tobacco

Severe damage by blue mould to a considerable portion of the Ontario tobacco crop in 1979 led to the use of Ridomil on almost the entire Ontario tobacco crop in 1980 when it was found to be very effective. In both these years the Delhi Research Station produced Ridomil treated tobacco on an experimental basis to study the effectiveness of the fungicide under Ontario growing conditions. Since nothing was known about the mutagenicity of Ridomil or the effect of Ridomil treatment of tobacco on the biological activity of cigarette smoke, it was decided to examine both Ridomil as well as experimental cigarettes made with the 1979 and 1980 Ridomil treated tobaccos in the Ames mutagenicity system. The results obtained are summarized below:

- (a) Ridomil was not mutagenic in the Ames system either with or without the addition of a rat liver activation system.
- (b) In 1979 when Ridomil was applied in late August and early September (too late in the growing season), a small but progressive increase in mutagenicity with increasing rate of application was observed for the 3 levels of treatment.

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- (c) In 1980 two rates of Ridomil were applied at the proper times in June and July, and no differences from the controls were found.

Conclusions

1. The Ames mutagenicity test is relatively inexpensive, easy to perform and permits quantitative comparisons of smoke condensates to be made.
2. Although correlations with the long-term carcinogenicity test are not perfect, any substance damaging the human genetic material should be of concern to man. Thus, tobacco smoke mutagenicity, should be studied for its own sake and not because it happens to correlate with its carcinogenicity.

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