

COMMENTARY RELATING TO FE REPORT NO. 37TITLE: THE CILIASTATIC EFFECT OF SMOKE ON TETRAHYMENAINTRODUCTION

The reason given for this work was that the coefficient of variation (c.v.) for results obtained with Paramecium was too high. Although they were able to reduce the c.v. from ca 20% to 12-15% with Paramecium by changing techniques, they reckoned to get down to a c.v. of 5% using Tetrahymena. The reason is partly due to the fact that Tetrahymena can be grown in sterile culture.

The initials CAMT are used throughout for the standard test. The results are given either as seconds to kill or as $\text{sec}^{-1} \times 10^3$, defined as ciliastatic effectiveness (c).

Pp 9-16 are devoted to a description of the test apparatus etc. A 50 ml puff of whole smoke or vapour phase - more usually the latter - is taken by syringe and is passed over a 5 μl drop of culture. The time to kill is recorded. Descriptions of culture solutions for T.vorax and T.pyriformis are given - the culture solution for the latter is particularly complex having 42 constituents.

Pp 16-17 Cover the details of carrying out the test. The pH of the culture solution used (5.5 - 5.8) is low (relative to both the human system and to the R. & D.E. system where it is close to 7).

Pp 17-19 Cover the details of reproducibility (for vapour phase) which is clearly very good (Table 1 and 2, pp 18 and 19).

Pp 20 Day to day variation is shown to be slight (Table 3, p.20).

Pp 20-38 Cover Results

- (1) The effect of drop size is shown (Table 4).
- (2) The effect of temperature is shown to be slight over the range 5-30°C (Table 5).

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- (3) Photosensitivity was not observed.
- (4) Increasing culture age is connected with a lower pH value and to high values of c.
- (5) The effect of increasing pH is to reduce c (Table 6). This result is in accord with R. & D.E. reported results for Paramecium and whole smoke.
- (6) Rather different results were obtained with T.vorax and T.pyriformis (Table 7). This may be due to differences in the culture solution.
- (7) The influence of tobacco moisture on (vapour phase) toxicity is given in Table 9. This results again is in accord with R. & D.E. reported results for Paramecium.
- (8) Cigarette pressure drop variation appears to have no effect (Table 10).
- (9) Paper porosity appears to have some effect on the toxicity of vapour phase, interacting with puff number (Table 11).
- (10) Acetate and paper filters have a slight effect, as they frequently do in R. & D.E. Paramecium tests (Table 12).
- (11) Perforated paper was tested against unperforated paper. Perforations reduced the toxicity (Table 13).
- (12) Tobacco filters had a slight effect (Tables 14 and 15); Gauloise and Player's tobaccos were compared as filters.
- (13) The influence of puff duration (at constant volume) was tested, and was found to be very slight. There is an optimum duration (Table 16).
- (14) The influence of various puff volumes (all of 12 seconds duration) was tested. A slight but definite effect was found (Table 17).
- (15) The influence of puff volume at constant speed was tested; there was no difference between puff volumes of 20-50 ml (Table 18).
- (16) There was only a very slight effect of varying the puff-flow speed in the smoke cell (Table 19).

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- (17) The effect of varying the concentration of gas phase on the c value is given in Table 20 and Figure 11. (There is scope for some interesting arithmetic to explain the relationship between puffs and time to kill in ciliastasis work.)

The results suggests two other points:

(i) Since the relationship between concentration and time to kill is exponential, it becomes inconvenient to measure the time to kill at low levels of toxicity - below say 20% of the normal concentration of the vapour phase for example. Measurement of puffs to kill is more convenient in this respect.

(ii) It would be more meaningful if Hamburg, using their dose-response relationship, were to give their results on the basis of % change in delivery of toxic material. For example, they could calculate the filtration efficiencies of the filters tested.

- (18) There was a slight increase (Table 21) in toxicity towards the butt end of the Player's cigarette, and a marked increase with the Gauloise. This result fits with results reported for some air-cured tobaccos in R. & D.E.

- (19) Table 22 gives the results obtained with several different cigarettes.

The most important points in the Discussion (pp 39-40) are:

- (1) They recognise that you cannot directly test the effect of smoke on cilia in Protozoa; the culture solution always intervenes. One should therefore try to minimise the effect of the culture solution.
- (2) They point out the difficulties of working with whole smoke - you cannot see the organisms through it. They say one 2 second puff is not enough for ciliastasis.

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- (3) They do not like the Australian and R. & D.E. system of using intermediate fresh air puffs - because of selectivity of some smoke constituents. This argument is scarcely valid since the smoker breathes fresh air between puffs of smoke.
- (4) They do not like the LD₁₀₀ methods (like R. & D.E. solution method) because the smoke is not fresh. This is true, but unstable compounds such as acrolein do influence the results, so presumably only less stable compounds than acrolein are lost. They also criticise the method because, they say, there is selective solution. This is in fact probably less true of the solution method than of drop method such as theirs and ours. They say the simplest system is to use the vapour phase alone. This has never been acceptable in the work in Australia, Battelle (Frankfurt) or R. & D.E., for the obvious reason that the smoker uses whole smoke.
- (5) They say that the examination of single puffs does not permit conclusions about whole cigarettes. This is accepted for some air-cured tobacco cigarettes, where they and we have reported a large increase in activity towards the butt end. It is less important for flue-cured tobacco cigarettes. There is a difference in the Hamburg and R. & D.E. findings in that they find an increase in toxicity per puff (of vapour phase) towards the butt end, but we have never found differences in toxicity of 1st and 2nd halves of flue-cured tobacco cigarettes (for whole smoke). This does not seem to be due to the lower sensitivity of our system.
- (6) They say that by comparing smoke analysis figures with CAMT values no substance can be indicated. They say that G.L.C. examination of the drop after ciliastasis

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points to HCN as being active. This argument seems quite
unsound since compounds such as acrolein will not, ^{necessarily} remain
unaltered after having exerted their toxic effect.

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