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MONTREAL 101, CANADA

FILE 467
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Dr. S.R. Evelyn,
British American Tobacco Company,
38 Regent's Park Road,
Millbrook, Southampton SO9 1PE,
England.

March 26, 1975

Dear Sam,

Thank you for your letter of Feb. 27, accompanying the proposal for an experiment to examine the possible effect on tumorigenicity of nicotine added to tobacco.

The required series of cigarettes is very reminiscent of a series we prepared based on our *Matinée* Regular cigarette, which we reinforced with nicotine. Details of the *Matinée* control cigarette and the two reinforced samples, coded 2N and 3N, are shown in the table.

The control cigarette had a nicotine delivery of 0.45 mg/cigt. We reinforced the cigarettes by adding nicotine as the citrate salt to PCL. Although the level of incorporation was very high, we had showed in earlier experiments that virtually no nicotine was lost during the PCL drying process. Unfortunately, the smoke TPM from the reinforced cigarettes had a higher pH and higher % extractable nicotine.

The nicotine citrate for these samples was dark-coloured, obtained from British Nicotine Co. (I.T.L., Bristol). We had prepared an early series of cigarettes using different levels of another reinforced PCL sample (IN). For this sample, an equimolar mixture of nicotine (ex. Matheson, Coleman & Bell) and citric acid had been used, and the smoke from these samples did not have an increased TPM pH and % extractable nicotine. However, we later switched to the British Nicotine material because of the quantities required.

Hence, while we have used the reinforced *Matinée* cigarettes in studies here (e.g. of human smoking behaviour), we believe it should be possible to make a very good series for the proposed biological studies by a similar approach of adding nicotine to PCL, but using redistilled nicotine, and adding it with equivalent amounts of citric acid or mixed acids. This should give smoke of matching pH, without upsetting the good features we achieved in our series, namely suitably elevated nicotine deliveries, but matched cigarette construction, and tobacco blend and virtually unchanged TPM deliveries and puff number.

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Cigarettes Series with Increasing Nicotine Deliveries

Cigarette Type	Matinée	2N	3N
Code	097	013	402
Length (mm)	72	72	72
Paper Porosity (Greiner/sec.)	13	13	13
Filter type	← CA →		
Filter P.D. (ins.)	2.8	2.8	2.8
Total P.D. (ins.)	5.5	5.5	5.5
<u>Blend</u>			
PCL-% nicotine	1.07	11.64	23.24
% PCL	7.8	7.8	7.8
% CRS	18	18	18
% Total Alkaloids	1.17	1.82	2.67
<u>Smoke</u>			
Butt Length (mm)	30	30	30
Puff Number	7.0	7.1	7.1
TPM mg/cig.	11	12	12
Nicotine mg/cig.	0.45	0.68	0.88
Extr. Nicotine mg/cig.	0.08	0.16	0.22
% Extr. Nicotine	17.2	23.9	25.3
pH of TPM	5.61	6.00	6.10

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Possibly our elevated TPM pH arose from the presence of other tobacco alkaloids or oxidation impurities in the commercial sample, or through an imbalance of nicotine and citric acid. However, there is a chance that it was due to the much higher levels of nicotine incorporated in the PCL samples.

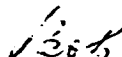
I am sure a lot of thought has been put into the means of achieving the different nicotine levels. The ITL Bristol work on nicotine pectinates comes to mind, and the Bw process for reducing the nicotine content of a blend, although this probably brings about other changes in the smoke.

I believe you would also be interested in a study recently started here. Stemming from the concern for the possible biological activity of nicotine, Dr. Bilimoria is using his microbial mutant systems to see whether nicotine shows mutagenic activity. With the E. coli system at neutral pH, nicotine did not inhibit either the parent or mutant culture (i.e. showed no mutagenic activity). However when nicotine was completely un-ionised, at pH9, it was mutagenic in that it inhibited the mutant culture more than that of the parent. We are now checking its effect at physiological pH. A Salmonella study of nicotine with activation by induced liver and lung enzymes is also planned.

I hope the information will be of help. If more detailed information is required, please do not hesitate to ask.

With kind regards,

Yours sincerely,



R.S. Wade
Manager
Research & Development Dept.

RSW/cp

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