

JUNE - DEC 88 ✓
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DENSITY
ADDITIVE.
DEFROT 113

RECORD TYPE : P
SUB TYPE : S
SECURITY CODE :
FUNDING BODY :
ORGANIZATION : ITC CANADA
GROUP NUMBER : 578
LOCAL PROJECT NUMBER(S) : T-7711
PROJECT TITLE : Cigarettes Designed for Low Mutagenic Activity
PERSON RESPONSIBLE : PORTER, A.; BILIMORIA, M.
EFFORT : 1988
PROJECT DESCRIPTION : Various options for the design of low mutagenicity cigarettes will be considered and prototype products made. As well as testing for Ames activity, consideration will be given to the smoking qualities of the product and the smoke deliveries.

SCOPE : GROUP
DEPTH : FUNDAMENTAL
FUNCTION : GENERAL
OBJECTIVE : REGULATORY
CLUSTER : BIOLOGY

DATE REVIEW WRITTEN : January 1989
REVIEW TITLE : Cigarettes Designed for Low Mutagenic Activity.
REVIEW TEXT : Further experiments have been carried out using the John Payne Tar Predictor (JPTP) as a means of generating condensate for Ames testing. The effect of tobacco packing density in the combustion tube was investigated using 1 g samples of Senior Service tobacco. With increasing packing density, condensate weight decreases and specific mutagenic activity increases as follows:

Density (g/cc)	Condensate Wt. (mg)	Spec. Mutagenicity
0.157	255	0.52
0.225	219	0.78
0.393	198	0.85

The effect of various salts added to tobacco on condensate activity was also investigated with the JPTP. Solutions of sodium nitrate, sodium chlorate, sodium chloride, potassium chloride, magnesium chloride, potassium carbonate and silver oxide (slurry) were sprayed onto Senior Service tobacco to achieve a 5% (w/w) level of addition after conditioning. Condensates were generated from

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1 g samples of treated tobacco. Compared with a water treated control, the oxidizing agents, sodium nitrate and sodium chlorate, reduced specific Ames activity by about 30%. Potassium and magnesium chlorides reduced specific activity by about 15-20% while sodium chloride and silver oxide caused no significant change in activity. Potassium carbonate, which is thought to inhibit combustion, increased activity by about 35%.

Cellulose, pectin and wood chips have been combusted in the JPTP. They yielded condensate weights of 387, 352 and 620 mg/g material, respectively. For these non-nitrogenous materials there was no indication of Ames activity of the condensate for levels of up to 400 µg/plate.

Various amino acids have been injected into cigarettes and their effects on condensate activity examined. The l-amino acids: proline, alanine, lysine-HCl, glycine, cysteine and tryptophan, were dissolved in water and individually injected into de Maurier K.S. cigarettes. At addition levels of 1 mg/cigarette none of these amino acids had a significant effect on condensate mutagenicity. Lysine HCl, glycine and threonine were separately injected at a level of 16 mg/cigarette (1.9% w/w) and 60-70% increases in the mutagenic activities of the condensate were noted. This increase in activity was unaffected by heating the cigarettes at 100°C for 5 min. prior to lighting them.

It has been well established that the protein nitrogen content of tobacco is highly correlated with its Ames mutagenic activity.

A few years ago a study at B.A.T., Southampton showed that Streptomyces protease treatment of tobacco resulted in reduced mutagenic activity. We have employed the same protease (Type XIV) and shown that protein removal is associated with reduced mutagenic activity, and that buffer extraction does not result in protein removal or reduction in mutagenicity. We have also employed another Streptomyces preparation (Type XXI) which has so far shown the highest removal of protein if treatment is

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carried out in a buffered suspension of tobacco (70% removal). However, when tobacco is extracted at 70% moisture and at the pH of tobacco, the Bacillus protease (Type VII) and the fungal proteases (XIX and XXIII) have been found superior to the Streptomyces preparation (Type XXI). The success in these initial attempts has been encouraging and we plan to screen many more commercially available proteases. We also plan to use other polymerases, such as pectinase, in conjunction with the proteases, to see if this enhances protein removal. Solvent extracted tobaccos are also being treated with enzymes to overcome the problem of removal of peptides and amino acids, which also are precursors of mutagenic compounds.

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