

SRE/PSD/46D-2 (BR Papers)

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THE UTILIZATION OF CRS, WASTE AND LAMINA TOBACCO:
A REVIEW OF THE TUMORIGENICITY OF CRS AND SOME
RECONSTITUTED TOBACCO SHEETS

SUMMARY AND CONCLUSIONS

By (a) ignoring the evidence from the calibration groups, and (b) eliminating the high dose level groups, it is possible to determine the tumorigenic ratio of Janus sample B4 (50% CRS) with respect to B0 (100% FC lamina). These results indicate that the inclusion of 50% CRS leads to a 30% reduction in tumorigenic activity.

Examination of the provisional results from Experiment B11 shows that when a FC blend (60% lamina/40% stem) is manufactured into PCL the activity is reduced by 20%, while manufacturing into SRT reduces condensate activity by 60%.

The TWG experiment shows that Schweitzer stem sheet, CRS and AMF stem (plus scrap and strips, ? "fines") sheet all have significantly lower activity than the standard experimental blend (SEB). Nevertheless, the TWG results are based on a ranking of blends using a likelihood ratio and it is not possible on the information available to assess any possible differences between the CRS and/or the sheet samples. Further, the RTS samples produced from the SEB included a variety of additives or no additive and were manufactured at various densities and it is not possible to rationalise these results at the present time.

The information clearly shows that significant reductions in tumorigenicity can be achieved by the incorporation of CRS and even greater reductions achieved by the use of a paper reconstitution process. This information does not indicate, however, whether stems should be utilized as CRS or to produce PRT and no information is available to indicate the relative effect of the PRT process on stem and/or fines compared with lamina.

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In the commercial situation it is obvious that waste tobaccos will be utilized in the manufacture of PRT even when PRT is manufactured at well above waste utilization levels.

The questions to be answered may be stated as follows:

- (1) should stem be utilized in the blend as CRS (WTS) or should all or part of the stem be incorporated into PRT along with the waste;
- (2) should some of the lamina be incorporated into PRT; and
- (3) should some of the lamina plus some or all of the stem be incorporated?

It is proposed that (1) above should be examined in a promotion skin-painting experiment. Since an important principle is involved in (2) and (3) above and the materials can be defined more exactly, it is considered that the relative contributions of lamina and stem in PRT should be examined in a single long-term experiment. Proposals for both experiments will be circulated.

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A REVIEW OF THE TUMORIGENICITY OF CRS AND SOME
RECONSTITUTED TOBACCO SHEETS

The optimum method of using stem (i.e. as CRS or incorporated into a reconstituted tobacco) has been discussed on a number of occasions and at a previous BR meeting a request was made that the position should be reviewed.

JANUS RESULTS

A long-term mouse skin painting experiment (34) has been undertaken to examine the effect of incorporating CRS at the 50% level into the all-lamina blend used for experiment B0.

In the report on experiment B4 (Report No. B32) the response of the B4 calibration groups was compared with that found in B0. This comparison showed that there were significant differences in response for the benzpyrene and dibenzanthracene groups used in these experiments. On this basis, the results of experiment B4 cannot be compared directly with those found in experiment B0.

Some further analysis of these experiments has now been undertaken. An initial attempt to fit common Weibull parameters to the data from B0 and B4 was not successful and reinforced the conclusion based on the calibration groups. It was considered, however, that the lack of fit could be due to differences in the "high dose anomaly" between the two experiments. On this basis, common k and w factors were fitted to the two lower dose groups from experiments B0 and B4 and to the 50 mg dose level B0 condensate group included in the B5 experiment. A satisfactory fit was obtained and it was also shown that for the 50 mg B0 groups there was not a significant difference between experiments B0 and the repeat experiment B5 (Report No. B33).

The tumorigenic ratio calculated on this basis for condensate B4 (50% FC lamina:50% CRS) with respect to B0 condensate was 0.7

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with 95% confidence limits of 0.63 and 0.76. Thus the tumorigenic response from 1 g B4 condensate is equivalent to that from 0.7 g BO (100% FC lamina) condensate.

These results may be summarised together with the final provisional values from experiment B11 as set out below with the proviso that the "controls" (BO and B11/1) which are both put equal to 1.0 cannot be equated.

		<u>Assumed Ranking</u>
BO (flue-cured lamina)	= 1.0	1.0
B4 (50% FC lamina/50% CRS)	= 0.7	0.7
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B11/1 (60% FC lamina/40% CRS)	= 1.0	(say) 0.80
B11/2 (PCL made from B11/1)	= 0.81	0.65
B11/3 (SRT made from B11/1)	= 0.41	0.33

From this summary it is obvious that the specific activity of SRT is much lower than that of a blend containing 50% CRS. If gross assumptions are made to equate the two experiments then the activity ratios for PCL and particularly SRT, manufactured from a blend containing 40% CRS, are reduced even further. Nevertheless the results, which indicate the probable advantages of a paper reconstituted sheet process, do not give any indication as to whether the reduction is achieved by processing the lamina portion of the blend or the stem.

RESULTS OBTAINED IN OTHER CENTRES

(a) T.R.C. (Harrogate)

In a single experiment condensates from FC lamina, CRS and RTS were compared with a FC control blend. The lamina was slightly more active (1.12) than the control while the reduction in activity for CRS (0.53) was slightly greater than that for the RTS sample (0.62), the latter being only just significant. These results must, however, be treated with some caution since the experiment had to be terminated at week 88.

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(b) T.W.G. Experiment

The provisional results of the first large TWG experiment provide some guide to the activity of both CRS and reconstituted sheets. This assessment of the results is limited, however, to the "Ranking of Blends on Likelihood Ratio Chi-Squared Tests of Difference from the Combined Standard Experimental Blend". It will also be remembered that this ranking is made much more difficult in some cases because of the differences found for the low and high dose groups.

The TWG results clearly show that condensate from CRS is very much less active than that from the blend and that from the lamina. The relative position of the lamina with respect to the blend cannot be interpreted; it is ranked more active at the low dose level and less active at the higher dose.

The CRS processed into RTS without additives by Schweitzer produced condensate with the lowest activity in the series. The same CRS was also processed by AMF but the ingredients included 27% scrap and strip tobacco, invert sugar (5.3%) and glycerine; the results for this sample were also significantly less active than the standard blend (SEB) but were not ranked as high as CRS or the Schweitzer sheet (Table 1).

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TABLE 1

T.W.G. RANKINGS

SAMPLE	RANKING	
	LOW DOSE	HIGH DOSE
Schweitzer sheet	1	1
CRS	3	2
AMF sheet	6	11
SEB	18	22
Lamina	19	12

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DARK TOBACCO CIGARETTES

Summary

Although a number of studies have been undertaken, there is relatively little information on dark tobacco cigarettes. Apart from the interest in cigar tobaccos, this may be partly due to the local 'native' nature of dark tobaccos, which are often produced from local, or locally naturalised, seed lines.

Mouse-skin studies show that condensate from dark tobaccos, including cigar tobacco manufactured into cigarettes, are at least as tumorigenic and often more tumorigenic than that from flue-cured Burley and oriental tobaccos.

Following a long-term inhalation experiment, assessed on the basis of the severity of laryngeal lesions in hamsters, Dontenwill concluded that smoke from a "black" cigarette was biologically very much less active than smoke from the reference cigarette; the latter being a 'standard' German blend. It should be noted, however, that the reduction in biological activity could be accounted for almost entirely by a lower accumulated dose from the "black" cigarette due to a shorter exposure time and the lower delivery of smoke. A similar reason may also be advanced for the marked reductions found for filter tip versions of the reference blend.

Because of the publicity which has surrounded the work of Passey and his colleagues, this must also be included in a consideration of dark tobacco cigarettes. Nevertheless, the studies, which were undertaken under poor experimental conditions, have been severely criticised and do not add materially to our knowledge of dark tobacco cigarettes.

In conclusion, the hypothesis that smoke from dark cigarettes is "safer" than that from flue-cured or blended cigarettes is certainly not supported by the results of the mouse-skin painting experiments and the Dontenwill inhalation study appears to provide only shaky supporting evidence. On the present evidence, it is suggested that any concept of a lower risk attached to dark cigarettes can, in a given population, only be associated with a reduced level of inhalation (accumulated dose) from such products

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DARK TOBACCO CIGARETTES

a) Study of Cigar Tobacco Condensate

Following reports from Croninger (1) and Homburger (2), which suggested, but did not prove statistically, that cigar smoke condensate was more carcinogenic than that from cigarette smoke, Davies and Day (3) re-examined the position.

The results obtained after 116 weeks showed that the condensate from the cigar was more carcinogenic than that of the cigarette manufactured from cigar tobacco or from flue-cured tobacco. There was no significant difference in carcinogenicity to mouse-skin of the condensates from the two cigarettes. The differences in mortality rates were small and the results were not affected by age-standardisation.

b) Comparison of Tobacco Types

In a further TRC experiment, a number of different types of tobacco, including an air-cured fermented (Paraguay) tobacco, were examined. The tumorigenic ratios and 95% confidence limits for the condensates from the various cigarettes compared with the flue-cured "control" (T4) were:

Sample T16	Air-cured fermented	1.38	(1.14 - 1.65)
T17	Burley	1.07	(0.89 - 1.29)
T18	Oriental	1.11	(0.92 - 1.33)
T19	Indian sun-cured	1.55	(1.29 - 1.90)
T20	Indian flue-cured	1.22	(1.02 - 1.47)

These results show that condensate from air-cured fermented tobacco is more tumorigenic than the flue-cured control. Condensate from Indian sun-cured is also more tumorigenic, while that from Indian flue-cured tobacco is just significantly more tumorigenic than the control. The differences for Burley and Oriental tobaccos are not significant.

Subsequently, Imperial Tobacco Ltd. have compared condensates from Gauloises and other continental brands with Embassy cigarettes. Full details are not available, but at 92 weeks, it was clear that condensate from Gauloises Caporal plain

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was very much more tumorigenic than that of Embassy filter.
The ranking was :

Gauloises > Belga Rouge filter > Nazionali Esportazione >
Embassy

c) Inhalation Study Dontenwill et al. (4)

In this long-term experiment, smoke from a "black" cigarette manufactured from a blend of Burley (15%), Maryland (6%), and dark tobaccos was compared with a "standard" German blend and various other cigarettes.

Comparison of the various cigarettes was based on the severe laryngeal changes found following exposure of hamsters to 30 cigarettes twice a day for up to two years. On this basis, smoke from the "black" cigarette was found to have only 33% (stage 5) or 12% (stage 6) of the activity of the reference cigarette, ie. a 67-88% reduction in activity .

Apart from the absence of suitable methods for the analysis of such experiments, two main factors should be considered in attempting to put these results into perspective.

1. Cellulose acetate or paper filter versions of the reference cigarette also led to a considerable reduction in activity; 56-60% (stage 5) and 76-99% (stage 6) respectively.

2. The condensate delivery from the "black" cigarette was nearly 40% lower than that from the reference cigarette and the exposure time was also 20% lower. Similarly, condensate deliveries from the filter cigarettes were 30 and 33% lower than that of the reference cigarette.

Since the above reduction in condensate delivery and exposure time for the "black" cigarette compared with the reference cigarette obviously reduce the total accumulated dose to the respiratory system, it is considered that the marked reduction in biological response should be treated with considerable caution.

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Other Inhalation Studies Passey et al. (5)

In two experiments, groups of 12 rats were exposed to smoke under what can only be considered as poor experimental conditions. In one experiment, cigarette smoke was compared with smoke from cigarettes manufactured from cigar tobacco (TRC C2). A commercial cigar tobacco cigarette (Calypso) and a cigarette manufactured from Burley tobacco (TRC T17) were included in the second experiment. The rats exposed to smoke from flue-cured tobacco died early and with enlarged and severely diseased lungs.

The conclusions drawn were that smoke from flue-cured cigarettes is more dangerous to man and to animals than that from air-cured tobaccos and that this is related to the method of curing (and the sugar content) of the tobaccos.

These experiments have been criticised severely because the pathological findings of the rats which died suddenly could be related to an inflammatory condition not regularly encountered in man: Passey himself stated that chronic respiratory disease and bronchiectasis were endemic in the strain of rats used for their studies. Passey also undertook some limited mouse-skin painting experiments.

Passey's studies have become linked to lung cancer (because of a limited mouse-skin study) and also to a theory of lung cancer induction advanced by Braven and Fenner (7): the latter because of Passey's contention that the sugar content of the tobacco was an important factor. In the same review (6), it is argued that, although the studies of Passey may conceivably have some relevance to chronic inflammatory lung disease in man, there are no grounds for regarding them as relevant to lung cancer.

The link with sugar content and Braven and Fenner's work is also criticised, since there is no evidence that cystein offers protection from cancer inducing effects of chemical agents.

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 2. Homburger, F. et al J. Natn. Cancer Inst. 1963, 31, 1445
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 5. Passey, R.D. et al Br. med. J. 1971, IV, 198
 6. Editorial Lancet, 1973, 187
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APPENDIX

Details of Cigarettes and Cigars used in TRC Cigar Study

Table 1

Code	Length (mm)	Circum (mm)	Weight (g)	Butt Length (mm)	Puff No.	NVWSC ⁽⁴⁾ (mg)
C1 ⁽¹⁾	83	33.7	1.86	25	19.8	37.8
C2 ⁽²⁾	70	25.1	0.94	20	8.4	19.4
T4 ⁽³⁾	70	25.3	1.09	20	10.9	26.3

- (1) Composite blend cigar tobacco; granulated wrapper and binder of natural leaf.
- (2) Blend as C1 cut at 50 cpi for cigarette manufacture
- (3) Composite blend of flue-cured tobacco.
- (4) Non volatile whole smoke condensate.

Smoking conditions : puff volume 25 ml
duration 2 seconds
one puff per minute

Table 2

Summary of results from Dontenwill Inhalation Study

Sample	Condensate		Relative Exposure Factor	Laryngeal Changes Relative Potency	
	Dry (mg/cig)	Factor		Stage 5	Stage 6
Control ⁽¹⁾	33.7	1.0	1.0	1.0	1.0
"Black" ⁽²⁾	20.9	0.62	0.8	0.33	0.12
Control Acetate Filter	23.5	0.70	1.0	0.44	0.06
Control Paper Filter	22.7	0.67	1.1	0.40	0.24

- (1) German blend: 55% flue-cured, 35% oriental and 10% Burley
- (2) Black cigarette: 15% Burley, 6% Maryland, 6% Java, 19% cigar tobacco, 54% other dark tobacco

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