

RESTRICTED

Mouse skin-painting
Preliminary assessment of the toxicity
of B14 cigarette smoke condensate to
mice.

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Preliminary assessment of B14 cigarette smoke condensates

14 groups composed of 18 six week old, female CF1P mice from Anglia Laboratory Animals were treated with 0.3ml of cigarette smoke condensate applied to the shaved dorsum three times a week (Monday, Wednesday and Friday) for a total period of six weeks as follows:

<u>Treatment</u>	<u>Level</u> (mg/application)	<u>No. mice</u>
B14/1	28.3	18
	40	18
	56.6	18
B14/2	28.3	18
	40	18
	56.6	18
B14/3	28.3	18
	40	18
	56.6	18
B14/4	20	18
	28.3	18
	40	18
	56.6	18
	80	18

Mice were examined at hourly intervals after each treatment for signs of nicotine toxicity, a maximum observation period of 6 hours being possible in a working day. A summary of the signs of nicotine toxicity for the various treatments is given in Table 1.

No mice died during the six week treatment period.

Mice were weighed initially and at weekly intervals throughout the study. Group mean bodyweights are given in Table 2 and showed no evidence of any inter-treatment effect.

At the end of the treatment period all mice were killed and subjected to a detailed macroscopic examination which revealed pathology commonly seen in mice of this age and strain.

Based on these findings the dose levels for the main experiment were agreed as 28.3, 40 & 56.6 mg/application.

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TABLE 1

Summary of signs of nicotine toxicity.

<u>Treatment</u>	<u>Level mg/application</u>	<u>No. mice effected.</u>	<u>No. occasions</u>	<u>Duration/ Recovery</u>
B14/1	28.3) 40) 56.6)	None		
B14/2	28.3) 40) 56.6)	None		
B14/3	28.3) 40) 56.6)	None 1-3	3	For up to 5 hours after dosing
B14/4	20) 28.3) 40) 56.6) 80)	1-5 8-15 18 18	4 8 All All All	Up to 5-6 hours after dosing. Definite signs of improvement within 6 hours after dosing. Most animals showed some signs of im- provement within 6 hours, recovery complete within 24 hrs. Frequently little ⁺ sign of improve- ment within 6 hours recovery complete within 24 hours.

+ Mice in this group tended not to clean themselves very well between treatments and in some animals there was evidence of a non-progressive local irritation probably related to the non-cleaning.

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TABLE 2
Group mean body weights (g)

Week	B14/1 (mg application)			B14/2 (mg application)			B14/3 (mg application)			B14/4 (mg application)				
	28	40	56	28	40	56	28	40	56	20	28	40	56	30
0	28	28	28	28	27	28	26	29	28	28	28	27	26	30
1	30	30	29	30	28	29	29	30	29	29	28	28	26	30
2	32	32	32	31	31	34	31	33	32	31	31	30	29	32
3	33	33	32	32	30	33	32	33	32	32	31	31	29	33
4	33	33	34	35	33	36	34	36	34	34	33	32	32	35
5	35	35	35	35	33	37	36	36	35	35	34	34	32	35
6	36	37	36	36	35	37	36	38	37	36	36	35	34	38
Mean gain	8	9	8	8	8	9	10	9	9	8	8	8	8	8

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BAT Skin-tumour code for tumours within the printed area

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Grade and general description

Sub-category.

<u>0</u> - Benign skin tumour	(1) squamous cell papilloma (2) kerato-acanthoma (3) sebaceous adenoma (4) mast cell tumour
<u>1</u> - Carcinoma <u>not</u> infiltrating the panniculus muscle	(1) squamous cell carcinoma (2) tricho-epithelioma (3) basal cell carcinoma
<u>2</u> - Carcinoma infiltrating the panniculus muscle	(1) squamous cell carcinoma (2) tricho-epithelioma (3) basal cell carcinoma
<u>3</u> - Sarcoma arising in the connective tissue below the epidermis	(1) fibrosarcoma (2) haemangiosarcoma (3) liposarcoma

Optional additional description

Anaplastic/poorly differentiated
Well differentiated/keratinising

Suggest keep a couple of free codes in each sub-category incase any
unforeseen tumours are identified.

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SRE/PSB/46D-2

26th May, 1977

REVISED PROPOSAL FOR EXPERIMENT ON VENTILATED CIGARETTES

Following various comments on the proposal circulated as document 296/8 the experimental design has been revised as set out below.

The design outline has been planned to meet the objective of the experiment which is "to compare the tumorigenicity of smoke condensate from cigarettes ventilated by various methods with that from an unventilated control cigarette".

At the last Biological Research Meeting, it was suggested that (a) the design should aim to reduce the delivery of TPM by approximately 50%, and (b) the experiment should include (1) Wattens 200 paper and (2) a comparison of two additives, i.e. citrate vs phosphate. Further, the methods of ventilation should include (a) naturally high porosity paper, (b) electrostatically perforated paper and (c) ventilated filter tipping coupled with porous plugwrap. Since it is usual commercial practice to use a filter of higher pressure drop with ventilated cigarettes this has been included in the design.

Considering the main objective and all the subsidiary factors set out above, the most efficient design is a basic 2 x 2 x 2 factorial experiment. This would examine natural porosity, standard and ventilated tipping and include the difference in pressure drop of the filter (samples 1-8, Table 1). To this is added:

Samples 9 & 10	Wattens paper Wattens paper + ventilated tipping
Sample 11	Effect of additive
Sample 12	Effect of electrostatic perforation

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Description of Samples to be Examined

	Paper		Filter p.d.	Type of Tipping
	Type	Porosity		
1	365C	95	Low	Std
2			Low	M1000
3			High	Std
4			High	M1000
5	DM95C	700	Low	Std
6			Low	M1000
7			High	Std
8			High	M1000
9	Wattens 200	1800	High	Std
10			High	M1000
11	95P	700	High	Std
12	365C ep	700	Low	Std

M1000 perforated tipping ex Malaucene coupled with highly porous plugwrap.

It should be noted that with this design samples 1-8 will allow us to examine interactions between porosity, tipping ventilation and filter pressure drop. Samples 3, 4, 7, 8, 9 and 10 form a 3 x 2 experiment in which paper porosity (linear and non-linear), plug ventilation, and their interactions can be examined.

The effects of (a) additive and (b) natural vs perforated porosity will be examined using samples 7 vs 11 and 5 vs 12 respectively. Since the comparisons within these two pairs are not integral in the factorial experiment, it is proposed that the number of animals associated with these groups are increased by 50%.

In redesigning this experiment the following additional factors have also been taken into account.

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- 1) De Mauduit paper has been chosen in preference to Fletchers so that all the papers with the exception of Wattens come from a single source.
- 2) The broad line electrostatically perforated paper used in the trial is no longer available from Fletchers.
- 3) Although there may be some shift in deliveries, it is believed that the new series will give comparable values and it is not proposed to undertake a further trial.
- 4) The design is a sub-set of a larger series which is being considered as a basis for a detailed study of ventilated cigarettes. Thus additional samples in a larger factorial design will be available for appropriate short-term tests.

Animal Numbers: With this orthogonal design it is possible to reduce the numbers of mice in each group. The numbers proposed are:

Samples 1, 2, 3, 4, 6, 8, 9, 10

81 mice at each of three dose levels (28.3, 40 and 56.6 mg)

$$8 \times 3 \times 81 = 1944$$

Samples 5, 7, 11, 12

126 mice at the same three dose levels

$$4 \times 3 \times 126 = 1512$$

Sample 1 Add additional mice to establish a dose response curve at HRC

162 mice at 20 mg

$$81 \text{ mice at } 80 \text{ mg} = 243$$

$$\text{Total number of mice} = \begin{array}{r} 1944 \\ 1512 \\ \hline 3699 \end{array}$$

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This number of mice may be compared with that suggested originally for the experiment with eight samples, each examined at three dose levels using 150 animals, i.e. 3,600 mice.

The cost estimated by HRC for the original experiment, 3,600 mice over 120 weeks, was £126,236. This quotation would be subject to inflation (since August 1976) and the 2.75% increase in the number of animals, say 20% overall, to give a revised estimated cost of £151,500.

The overall costs will, of course, include costs of manufacturing the samples and the cost of condensate production. The latter would amount to approximately £50,000 over 2+ years.

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SRE/PSB/46D-2

17th November, 1977

ADDITIVES IN PRT: AN OUTLINE PROPOSAL

The use of additives in PRT was reconsidered at the last Biological Research Meeting (Minute No. 287c). It was agreed that samples should be produced on the pilot plant in G.R. & D.C. for pre-screening by the NMFI and sebaceous gland tests. A considerable number of samples have been produced and examined using the NMFI test. Due to the slow progress at Life Science Research Limited and the subsequent switch of this type of experiment to Dr. Brune's laboratory, no results have been obtained using the sebaceous gland test. The results obtained for both insoluble fillers and soluble additives are set out in Table 1.

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TABLE 1

The Effects of Additives in PRT on the NMF Value, Puff Number and TPM Delivery of 100% PRT Cigarettes

Additive (% in Impregnated Product)	Product Soxhlet Sols(%)	NMF Value	Puff No.	TPM (mg/cig)
(a) <u>INSOLUBLE FILLERS</u>				
O (Control)	39	4.1	6.8	16.6
Chalk (16%)	38	3.9	6.6	17.0
Alumina Trihydrate (13%)	35	4.9	8.0	16.7
Calcium Sulphate Dihydrate (9%)	47	4.8	8.1	20.0
"Darco KB" Activated Carbon (7%)	42	5.8	6.5	14.7
Light, basic, Magnesium Carbonate (11%)	45	3.9	8.0	14.9
Calcium Phosphate (11%)	37	4.4	7.1	19.4
(b) <u>SOLUBLE ADDITIVES</u>				
O (Control)	43	4.6	7.1	26.0
DAP (di-Ammonium Hydrogen ortho-Phosphate) (5.1%)	36	7.0	10.1	27.0
DAP (3.3%)	41	6.4	7.9	26.9

(Control) (1.9% Potassium Potassium (added as Bicarbonate) to give a total Potassium content of 5.2%)	39	4.0	7.4	19
Ditto 5.3%	42	8.2	6.1	14
Ditto 5.3%	46	8.4	6.2	14
Ditto 6.0%	40	11.0	6.7	12
Ditto 6.9%	49	14.2	9.4	10

O (Control)	49	5.3	8.2	25.5
Ammonium Chloride (1.7%)	50	5.3	8.8	26.4
Ditto (2.5%)	50	6.7	9.5	30.9
Ditto (3.6%)	50	6.8	12.1	28.6
Ditto (3.7%)	52	7.5	11.8	30.4
(c) <u>WOODPULP</u>				
Unbleached Kraft Base Sheet impregnated with flue-cured lamina extract	48	Not Determined	6.8	28
Bleached Kraft Base Sheet impregnated with flue-cured lamina extract	45	Ditto	6.0	31

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From the compounds examined in Table 1, it is possible to list a number which it would be worthwhile to examine further: these are set out below.

<u>Compound</u>	<u>Level Proposed</u>	<u>Comment</u>
Chalk	20%	<NMFII = TPM
Magnesium Carbonate	10%	<NMFII <TPM
Woodpulp	25%	Commercial Interest
DAP	4%	Commercial Interest
Ammonium Chloride	3%	Possible Flavour Enhancer
Alumina	10%	Maintains Sheet Properties

At first sight the inclusion of diammonium phosphate (DAP) and ammonium chloride in this list may appear strange in view of the increased NMFII values. The NMFII values confirm previous observations that ammonia and ammonium salts generally lead to high values. At the risk of riding a 'hobby horse', it is suggested that we should establish whether this is confirmed in other tests since we need to (a) check on a possible anomaly in the NMFII test and (b) ensure that we can reach a definitive conclusion in respect of potentially useful flavour enhancing additives.

It is proposed that we aim to manufacture, at Glory Mill, samples of all the above hybrid PRT's in quantities at least sufficient for their submission to a promotion skin-painting test.

Further it is recommended that:

- (a) The lamina/stem ratio should be constant at 60/40. This level is comparable with that used in previous Janus experiments and should provide sufficient strength in the sheet to allow for some loss of strength which may be caused by incorporation of some additives.

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- (b) The level of tobacco solubles should be fixed to that in the tobacco base sheet except for (c).
- (c) The incorporation of woodpulp should be examined in two samples: (1) to have a solubles level related to the tobacco in the base sheet, i.e. comparable to all other samples and (2) have a solubles level related to the total base weight, i.e. including added woodpulp.

These proposals lead to a total of eight samples including a control. It is probable that the samples can be produced early in 1978 but timing will be dependent on ensuring sufficient stock levels and, possibly, negotiations with Customs.

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