

PROJECT JANUS
QUARTERLY REPORT: JULY-SEPTEMBER, 1969

GENERAL

In the last Quarterly Report, it was pointed out that the animal supplier, ASL, Ltd., had experienced an outbreak of Salmonella montevideo in his mouse colony. Dr. Kiendl and Dr. Kramer visited ASL, Ltd. and were satisfied that the disease had been eradicated and, consequently, Battelle were able to accept further shipments of mice. It was necessary, however, to delay the start of Experiment B8 until mid-October, to allow sufficient time for ASL, Ltd. to build up their breeding stock and deliver a sufficient number of mice in a single shipment.

During this quarter, Hr Militzer was awarded his doctorate for a thesis entitled "Histometrische Untersuchungen an der Epidermis der Maus nach Applikation von Zigarettenrauchkondensat im Kurzzeitversuch."

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LONG-TERM TESTS

Experiments B1-B3

The latest results are summarised in Table 1 and the conclusions which are indicated by the results remain unchanged from the last quarter, i.e.

- (a) The specific activity of condensate is decreased as the volume of the puff is increased (Experiment B1).
- (b) The activities of the condensates are in the order:

Cigarette B3 < Cigarette B2 < Cigarette B0
(100% PCL) (U.S. blend) (CN102 lamina)

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TABLE 1
LONG-TERM TESTS: EXPERIMENTS B0-B3

	N_H	$\Sigma \Delta P$	$\frac{\Sigma \Delta P}{\Sigma N_H}$	PTI
<u>156 WEEKS</u>				
Control: unpainted	0	0	0	0
solvent painted	0	0	0	0
<u>Experiment B0 (CN102 lamina)</u>				
25 mg	0	110	228	228
50 mg	0	178	495	495
75 mg	0	127	476	476
<u>136 WEEKS</u>				
<u>Experiment B1 (puff volume)</u>				
10 ml	0	131	581	581
25 ml	0	172	524	524
35 ml (ex B0)	0	178	495	495
50 ml	0	168	475	475
<u>140 WEEKS</u>				
<u>Experiment B2 (U.S. blend)</u>				
25 mg	4 (2%)	89	161	164
50 mg	0	183	444	444
75 mg	0	179	480	480
<u>128 WEEKS</u>				
<u>Experiment B3 (100% PCL)</u>				
25 mg	26 (10%)	39	68	76
50 mg	2 (1%)	142	284	286
75 mg	2 (1%)	171	370	373

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Experiment B4 (50% CRS)

The cigarette for this experiment was made from equal portions of CRS and CN102 lamina.

The results after 92 weeks are compared with those obtained after the same time interval in Experiments B0 and B3 (Table 2). It would appear that:

- (a) The incorporation of CRS into a cigarette leads to a reduction in the specific activity of the condensate.
- (b) The activity of the condensate from PCL is less than that of the condensate from a blend of the "equivalent starting tobaccos."

TABLE 2

LONG-TERM TEST: EXPERIMENT B4 (92 WEEKS)

	N_H	$\Sigma\Delta P$	$\frac{\Sigma\Delta P}{\Sigma N_H}$	PTI
<u>Experiment B4 (50% CRS)</u>				
25 mg	119 (47%)	37	74	140
50 mg	49 (19%)	137	326	404
75 mg	25 (10%)	158	411	456
<u>Experiment B0 (CN102 lamina)</u>				
25 mg	82 (32%)	78	174	258
50 mg	20 (8%)	163	459	498
75 mg	8 (3%)	121	455	470
<u>Experiment B3 (100% PCL)</u>				
25 mg	154 (61%)	9	18	(46)
50 mg)	84 (33%)	106	222	333
75 mg	63 (25%)	128	289	385

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Experiment B5

Comparison with the earlier experiments, all at 92 weeks, is shown in Table 3, and it would appear that:

- (a) The results from an examination of Cigarettes B0 and B3 are in good agreement with those from the earlier experiments.
- (b) There is poor agreement between the current and previous results obtained from the examination of Cigarette B2 (U.S. blend). At present, an estimate has not been made of whether the predicted 20% divergence is likely to be significant.
- (c) There is no indication that changing from one puff per minute to three puffs per minute has a significant effect on the activity of the condensate.

TABLE 3

LONG-TERM TESTS

	Current				Previous			
	N_H	$\Sigma\Delta P$	$\frac{\Sigma\Delta P}{\Sigma N_H}$	PTI	N_H	$\Sigma\Delta P$	$\frac{\Sigma\Delta P}{\Sigma N_H}$	PTI
B0	13	156	479	505	20	163	459	498
B2	19	182	509	550	41	154	384	457
B3	67	101	224	305	84	106	222	333
B0 triple	7	168	529	545	-	-	-	-

Experiment B6/B7

This experiment has two aims:

- (a) To determine the effect of a yeast-treatment of CN102 lamina;
- (b) To determine the effect of a change in c.p.i.

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Six samples of cigarettes are involved and in each case the condensate is applied at three dose levels (25, 50 and 75 mg) to groups of 84 mice.

- (i) B6-1 Yeast treated 30 c.p.i.
- (ii) B6-2 Yeast treated 60 c.p.i.
- (iii) B6-3 Yeast treated 120 c.p.i.
- (iv) B7-1 CN102 lamina 30 c.p.i.
- (v) B7-2 CN102 lamina 60 c.p.i.
- (vi) B7-3 CN102 lamina 120 c.p.i.

In effect, Cigarette B7-2 is a repeat of Cigarette B0. The experiment has been running for 48 weeks, and the results from the two cigarettes are in reasonable agreement (Table 4).

TABLE 4
LONG-TERM TESTS: CIGARETTE B7-2 vs. CIGARETTE B0
(48 weeks)

	Cigarette B7-2 84 mice per group			Cigarette B0 252 mice per group		
	N_H	$\Sigma\Delta P$	$\frac{\Sigma\Delta P}{\Sigma N_H}$	N_H	$\Sigma\Delta P$	$\frac{\Sigma\Delta P}{\Sigma N_H}$
25 mg	77	1	10	230	8	28
50 mg	54	12	146	184	46	170
75 mg	45	5	68	136	22	102

By combining the "cuts per inch" groups it is possible to compare the yeast-treated and control results (Table 5). There is a slight indication that yeast-treatment leads to a reduction in the specific activity of the condensate. It must be emphasised, however, that the experiment is still in the early stages.

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TABLE 5

LONG-TERM TESTS: YEAST-TREATED vs. CONTROL

(48 weeks)

	Yeast-treated		Control	
	$\Sigma\Delta P$	$\frac{\Sigma\Delta P}{\Sigma N_H}$	$\Sigma\Delta P$	$\frac{\Sigma\Delta P}{\Sigma N_H}$
25 mg	12	43	8	28
50 mg	19	91	37	163
75 mg	9	62	28	135

The effects of changes in c.p.i. are shown in Table 6. In view of the small numbers of tumour-bearing animals, the dose-levels have been combined. At the present time, there is an indication of a non-linear trend in that the lowest activity is associated with the 60 c.p.i. strand-width.

TABLE 6

LONG-TERM TESTS: EFFECT OF c.p.i.

	30 c.p.i.		60 c.p.i.		120 c.p.i.	
	$\Sigma\Delta P$	$\frac{\Sigma\Delta P}{\Sigma N_H}$	$\Sigma\Delta P$	$\frac{\Sigma\Delta P}{\Sigma N_H}$	$\Sigma\Delta P$	$\frac{\Sigma\Delta P}{\Sigma N_H}$
Yeast-treated	14	72	7	33	19	83
CN102 blend	33	152	18	71	22	89
Yeast-treated + CN102	47	114	25	54	41	87

Calibration Groups

To monitor the response of the mice to known skin carcinogens, in each experiment four groups of 27 mice are painted with:

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- (i) Benzpyrene for 13 weeks
- (ii) Benzpyrene for 26 weeks
- (iii) Dibenzanthracene for 13 weeks
- (iv) Dibenzanthracene for 26 weeks

The results from Experiments B0-B3 are very nearly complete and whereas similar results were obtained with the B0 and B1 mice, there is an indication that the response of subsequent groups is decreasing (Table 7).

TABLE 7
CALIBRATION GROUPS: EXPERIMENTS B0-B3

	Tumorigenic Index $\frac{(\Sigma \Delta P)}{\Sigma N_H}$				
	Benzpyrene		Dibenzanthracene		Combined
	13 weeks	26 weeks	13 weeks	26 weeks	
B0	590	979	438	782	658
B1	757	1038	363	721	679
B2	377	864	458	511	525
B3	288	597	282	496	394

Experiments B4 and B5 were started at the same time on the same batch of mice, i.e. there is only a single set of calibration results. The experiments have been running for 92 weeks and there is an indication that the trend of a decreasing response is continuing into the B4/B5 batch of mice (Table 8).

It is too early to draw conclusions from the B6/B7 calibration groups.

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TABLE 8

CALIBRATION GROUPS: B3 vs. B4/5 AFTER 92 WEEKS

	Tumorigenic Index $\frac{(\Sigma \Delta P)}{\Sigma N_H}$				
	Benzpyrene		Dibenzanthracene		Combined
	13 weeks	26 weeks	13 weeks	26 weeks	
B3	245	599	256	484	376
B4/B5	212	571	188	431	327

The trend towards a decreasing response is disturbing but two points must be emphasised:

- (a) As each group consists of only 27 mice, it is probably advisable to await the histologically confirmed results. If one or two mice have been mis-classified, this can have a marked effect on the tumorigenic index.
- (b) Before firm conclusions are drawn, it is also advisable to await a more sophisticated statistical analysis of the results.

Assuming that subsequent work confirms the trend, the interpretation remains an open question. For example, it would appear that the response of the B4/B5 mice towards benzpyrene and dibenzanthracene is less than that of the earlier shipments of mice. From Table 3, however, it would appear that the response to smoke condensate is either unchanged or has perhaps even increased for the B5 mice.

Coding Scheme

The final stages have been reached in the development of a Coding Scheme/Computer Retrieval Programme for the detailed information from the long-term tests. In essence, the information is transcribed at Battelle.

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onto Data Coding Forms for subsequent storage on the B-A.T. computer at Millbank. Management Services Department are writing the programmes which will enable the data to be retrieved in a form suitable for detailed statistical analysis at R. & D.E.

HYPERPLASIA TEST

The results from three sets of Hyperplasia experiments were received this quarter.

March Experiment

In these experiments, Battelle examined a series of cigarettes from R. & D.E. (Table 9).

TABLE 9

HYPERPLASIA TEST: MARCH EXPERIMENT

	Area Activity Rating (micron-days)	
	Observed	Normalised on S41
S41 100% CN102 lamina	108	100
S30 100% Burley PCL	79	73
S31 100% Flue PCL	101	93
S32 100% Stem PCL	71	65
S33 100% Flue/Burley PCL	89	83
S34 50% CN102 lamina:50% Burley PCL	88	82
S35 50% CN102 lamina:50% Burley BATEX	116	107
S36 50% CN102 lamina:30% Burley:20% CRS	103	95
S37 50% CN102 lamina:50% Flue PCL	105	97
S38 50% CN102 lamina:Flue BATEX	102	94
S39 50% Flue PCL:50% Burley	99	91
S40 50% Flue PCL:50% Flue BATEX	104	96

It would appear that:

1. The type of tobacco used in the manufacture of the PCL has an effect on the specific activity of the condensate (S30-S33). The results

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are in the order:

Flue PCL > Burley PCL > Stem PCL

and the results are self-consistent since the value for Flue/Burley PCL is midway between those for Flue PCL and Burley PCL.

2. There are other examples of self-consistency, e.g. S34 and S37.
3. The difference between S41 (100% lamina) and S31 (100% Flue PCL) is not as large as that between Cigarette B0 and Cigarette B3.

Cigarette B0: 100 micron-days

Cigarette B3: 79 and 74 micron-days

This is a disappointing result since, although S41 and S31 are not identical with B0 and B3, they are very similar.

4. A comparison of S38 and S40 with the other samples containing flue-cured tobacco indicates that making flue-cured tobacco into BATEX has little effect on the specific activity.

On the other hand, a comparison of S35 and S34 indicates that Burley BATEX has a high activity. There is no cross-check on this result, and, if it is important, it may be worthwhile to get confirmation.

5. Sample S36 contains a mixture of CN102 lamina, Burley and CRS. As an approximation, the "30% Burley/20% CRS" is the starting material used to make Burley PCL. A comparison of results from S34 and S36 indicates that the PCL "process" leads to a reduction in activity.

April Experiments

The samples for these experiments were supplied by T.R.C., Harrogate. Following the examination of cigars by a long-term experiment at Harrogate, it was decided to examine the effect of changes in the physical parameters of a cigarette, i.e. strand width, paper porosity, and circumference. It

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was agreed that the samples of cigarettes would be examined by three different tests, i.e.

Harrogate:	Long-term skin-painting test
Battelle, Frankfurt:	Hyperplasia test
Battelle, Geneva:	"Hairless mouse" test

The experiments at Harrogate have been running for approximately 20 weeks, and it is too early to have any indication of the probable outcome of the results. It is not known when the Geneva experiments will be run.

The samples (70 mm plain cigarettes) involved are:

T44 - Control. A blend of flue-cured tobaccos with a "normal" (approximately 15%?) stem content.

50 c.p.i.

Fletcher Imperial paper, 65-100 ml/min porosity.

Circumference 25.3 mm.

T45 - Blend, paper and circumference as in T44.

Shattered tobacco.

T46 - Blend, paper and circumference as in T44.

25 c.p.i.

T47 - Blend, paper and circumference as in T44.

100 c.p.i.

T48 - Blend, circumference and c.p.i. as in T44.

Bollore 252, 10-16 ml/min porosity.

T49 - Blend, circumference and c.p.i. as in T44.

Bollore C556, 187-297 ml/min porosity.

T50 - Blend, paper and c.p.i. as in T44.

Circumference 31.5 mm.

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T51 - Blend, paper and c.p.i. as in T44.

Circumference 19 mm.

The results from the Hyperplasia Test are given in Table 10.

TABLE 10

HYPERPLASIA TEST: APRIL EXPERIMENT

	Area Activity Rating (micron-days)	
	Observed	Normalised on Cigarette B0 = 100
Cigarette B0	104	100
T44 Control	100	96
T45 Shattered tobacco	122	117
T46 25 c.p.i.	128	123
T47 100 c.p.i.	121	116
T48 Low porosity	112	108
T49 High porosity	127	122
T50 Large circumference	122	117
T51 Small circumference	95	91

The interpretation of these results is not easy.

- (a) Several of the results are in the region of 120 micron-days. Compared with the results from earlier tests, these results are unusually high. However, the results from the next set of experiments (July experiments) also contain "high" activity ratings and it is possible that the response of the mice has increased recently.

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- (b) From a detailed inspection of the deliveries of smoke condensate, pressure drop and puff number of the cigarettes, there is some minor evidence to indicate that the T44 cigarette is "out of step". Certainly the Hyperplasia result from this cigarette appears to be low and tends to destroy the emergence of any simple pattern in the results, i.e. in the samples involving changes in c.p.i. or porosity, a minimum activity is associated with the T44 cigarette.
- (c) Although the current results from a long-term test (Experiment B7, Table 6) indicated a "minimum activity" associated with tobacco cut at 60 c.p.i., two points must be remembered:
- (i) Experiment B7 is in the early stages and it is very feasible that the trend will disappear as the experiment progresses.
 - (ii) The cigarettes from Experiment B7 were examined by the Hyperplasia test in November, 1968, and a "minimum activity" for the 60 c.p.i. sample was not detected.
- (d) There is no indication from the Hyperplasia results that any of the cigarettes will have a low activity in the long-term test. If the T44 result is ignored, then the indications are that changes in c.p.i. will have no effect, whilst a small reduction in activity might follow from a reduction in the porosity or circumference of a cigarette.

July Experiments

In these experiments, Battelle examined three samples from B-A.T.

(Hamburg).

Code 27: Control, filter cigarettes with about 20 mg of condensate.

Code 28: The same tobacco rod with AL filter reducing condensate to about 10 mg.

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Cofis 29: The same tobacco rod with a filter with high pressure drop also reducing condensate to about 10 mg.

The result from the Hyperplasia Test are given in Table 11.

TABLE 11
HYPERPLASIA TEST: JULY EXPERIMENTS

	Area Activity Rating (micron-days)	
	Observed	Normalised on Cigarette B0 = 100
Cigarette B0	116	100
27 Control filter	112	96
28 AL filter	132	113
29 High PD filter	122	104

The specific activity of the condensate from the cigarette with the AL filter is higher than that from the cigarette with the control filter. A difference of 10 micron-days is normally regarded as significant and on this basis the difference between the AL filter and the High PD filter, or between the High PD filter and the control filter, just fails to be significant. The increase in activity associated with the AL filter is consistent with the earlier observations from the examination of the effects of reducing the puff volume. It must be remembered, however, that a similar effect was not found from an examination of the VISCOUNT cigarette.

FURTHER EXPERIMENTS

In later tests, cigarettes from Southampton, (water filter), Sydney and Louisville are being examined. The cigarettes have been smoked and the condensate applied to the mice but the evaluation of the skin-sections is not yet completed.

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