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The Mutagenicity by the Ames Bacterial Test of Smoke Condensates  
from Agriculture Canada Phase 1 Cigarettes

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Background

Early last year it was decided to examine by the Ames test two series of cigarettes for which biochemical and biological data were available. The results obtained with three samples selected from the Janus B11 series have already been reported (memo dated 18 October, 1978). Presented in this report are the results obtained with cigarettes made for Agriculture Canada Tobacco and Health Study (Experiment No. 40, 1973).

The mutagenesis assay was carried out according to the procedures described by Ames et al., Mutation Res., 31, 347, 1973. The cigarettes were tested for mutagenicity in groups of five, which always included Players Check 20 for intercomparison of samples, and each group was tested six times. Dose levels of 50, 100, 150, 200 and 400 ug condensate per plate were tested in triplicate. Regression lines were computed for the relationship between the number of revertant colonies and amount of smoke condensate (dose response). Correlation coefficients and slopes were calculated from these regression lines and the slopes were used to express mutagenicities of the condensates.

Results

A brief description of the cigarettes employed in this study is presented in Table 1. As will be seen from the table the cigarettes were made from a variety of tobacco materials which were produced under controlled conditions. The mutagenicities of these cigarettes, expressed in terms of these regression lines, are presented in Table 2. From the correlation coefficients presented in Table 2, it will be seen that the dose responses are linear. Thus, slopes of these dose response curves can be used to calculate the mutagenicities of the condensates. Logarithmic transformations of the data were also carried out, but the slopes of these regression lines were not parallel as will be seen from the results of the analysis of

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variance (ANOVA) presented in Table 3 ( $p < 0.001$ ). Because the slopes of these regression lines were not parallel, the intercepts could not be used to express the mutagenicities of the condensates, as was done in the case of the Janus 811 cigarettes.

Since these sixteen cigarettes were tested in four groups, it was necessary to find out if the data from these groups could be pooled for statistical evaluation. Consequently, an ANOVA test was performed to determine whether there were any differences in the mutagenicities of the Player Check cigarettes tested with each of the four groups. The results of this ANOVA test, presented in Table 4, show that there was no significant difference between these Players cigarettes tested at four different periods in time ( $p < 0.5$ ).

Having shown that the results of the Players cigarettes were similar, the data from these four groups of experiments were pooled, and an ANOVA test was performed to find out if there were significant differences between the mutagenicities of these sixteen cigarettes. From Table 5 it will be seen that the mutagenicities (slopes) were indeed significantly different ( $p < 0.001$ ). A Student-Newman-Keul test was performed to detect differences between cigarettes, and these results are summarized in Table 6. From this table it can readily be determined whether the difference in mutagenicity between any two pairs of cigarettes is significant or not.

From Table 7 it will be seen that homogenization of tobacco to make PCL leads to a decrease in mutagenic activity, which, with the exception of PCL made from flue-cured whole plant leaf blend, is significant at the 5% level. The latter PCL, however, is significantly lower only at the 10% level. In this table are also given the differences between the flue-cured tobaccos and burley.

In Table 8 a comparison is drawn between the mutagenicities as determined at Imperial Tobacco and Guelph University (Dr. Basrur), Sebaceous gland as done at B.-A.T. Hamburg and NMFI, as done at I.T.L.,

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Montreal. The data in each test have been ranked and the rankings are shown in brackets in the table. Correlation coefficients have been obtained for pairs of data from regression lines as well as by the Spearman rank test. These results are presented in Table 9. That the highest correlation was obtained between the mutagenicity tests as done at I.T.L. and Guelph, should not be surprising. The correlation is, however, rather low probably because different procedures were employed in the mutagenicity testing in these two laboratories. Dr. Basrur in her study employed whole smoke, whereas at Imperial, condensate was used, and whole smoke is likely to be more cytotoxic because of the presence of vapour phase components. The mutagenicity test correlated significantly with the NMFI and not at all with the Sebaceous gland. Since both mutagenicity and NMFI tests are expected to measure the reactive compounds in the condensate, it is possible that incorporating an activation system in the NMFI test and employing DNA instead of albumin, would increase the correlation between these two tests. A detailed report on the mutagenic activities of cigarettes belonging to the B11 and Agriculture Canada series will be issued within the next few weeks.

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TABLE 1

Description of Agriculture Canada Phase 1 Cigarettes

Code	Description
205	Flue-cured, whole plant leaf blend (75% lamina, 25% flue-cured stem CRS).
385	205 made into PCL.
454	Flue-cured, whole plant stem blend (100% CRS).
514	454 made into PCL.
694	Flue-cured, upper leaves (75% lamina, 25% flue-cured stem CRS).
938	694 made into PCL.
365	Flue-cured, middle leaves (75% lamina, 25% flue-cured stem CRS).
282	365 made into PCL.
862	Flue-cured, lower leaves (75% lamina, 25% flue-cured stem CRS).
081	862 made into PCL.
848	Flue-cured, close-planted, chopped plant - homogenized.
176	50-50 blend of 205 and 848.
803	176 with high efficiency acetate filter.
891	Burley, whole plant leaf blend.
726	891 made into PCL.
PLCK 20	Laboratory control (73% lamina, 8% PCL and 19% CRS).

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TABLE 2

Mutagenicity of Smoke Condensates from  
Agriculture Canada Phase 1 Cigarettes

Cigarette Code	Experiment No.	Regressions from Data	
		Correlation Coefficient	Slope (Mutagenicity)
PLCK 20	1	0.9882	1.4640
	2	0.9959	1.3000
	3	0.9748	1.1250
	4	0.9814	1.6970
	5	0.9884	1.4210
	6	0.9827	1.5190
205	1	0.9579	1.2360
	2	0.9845	1.2060
	3	0.9685	1.0640
	4	0.9942	1.2130
	5	0.9662	1.0770
	6	0.9861	1.3590
385	1	0.9514	0.9100
	2	0.9784	0.8040
	3	0.9914	0.9200
	4	0.9739	0.7800
	5	0.9812	0.9460
	6	0.9642	0.8920
454	1	0.9930	1.1030
	2	0.9869	1.1570
	3	0.9882	0.9078
	4	0.9922	1.2170
	5	0.9915	1.2620
	6	0.9901	1.3660
514	1	0.8588	0.4607
	2	0.9918	0.6358
	3	0.9637	0.6200
	4	0.9396	0.7414
	5	0.9891	0.8478
	6	0.9755	0.6498

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TABLE 2 - (Cont'd.)

PLCK 20	1	0.9780	1.1525
	2	0.9929	1.3839
	3	0.9660	1.1470
	4	0.9949	1.3992
	5	0.9921	1.4417
	6	0.9762	1.0460
694	1	0.9892	1.4102
	2	0.9915	1.2732
	3	0.9796	1.3415
	4	0.9917	1.4156
	5	0.9824	1.4233
	6	0.9840	1.0817
938	1	0.9835	0.9530
	2	0.9884	0.8835
	3	0.9802	0.8693
	4	0.9803	1.0137
	5	0.9844	0.9405
	6	0.9884	0.8609
891	1	0.9546	3.2502
	2	0.9776	3.3364
	3	0.9815	4.1171
	4	0.9767	3.1809
	5	0.9818	2.5666
	6	0.9862	2.9166
726	1	0.9314	1.7359
	2	0.9854	1.5960
	3	0.9891	1.4611
	4	0.9786	1.8426
	5	0.9716	1.6032
	6	0.9882	1.2395

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TABLE 2 (Cont'd.)

PLCK 20	1	0.9823	1.0553
	2	0.9914	1.3865
	3	0.9871	1.3516
	4	0.9857	1.6011
	5	0.9913	1.4307
	6	0.9810	1.5326
862	1	0.9899	1.2002
	2	0.9915	1.5349
	3	0.9934	1.6609
	4	0.9874	1.6482
	5	0.9807	1.5579
	6	0.9905	1.5116
081	1	0.9882	0.9596
	2	0.9760	1.1189
	3	0.9823	1.1919
	4	0.9811	1.2735
	5	0.9759	1.1669
	6	0.9839	1.3238
365	1	0.9829	1.0951
	2	0.9845	1.3447
	3	0.9653	1.3591
	4	0.9721	1.2067
	5	0.9933	1.3004
	6	0.9906	1.5569
282	1	0.9688	0.5419
	2	0.9775	0.6104
	3	0.9640	0.7684
	4	0.9696	0.6645
	5	0.9628	0.5908
	6	0.9744	0.8405

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TABLE 2 (Cont'd.)

PLCK 20	1	0.9865	1.4399
	2	0.9856	1.5704
	3	0.9853	1.2114
	4	0.9803	1.2392
	5	0.9889	1.4139
	6	0.9954	1.5051
176	1	0.9866	1.1765
	2	0.9876	0.9554
	3	0.9832	0.9505
	4	0.9920	0.9473
	5	0.9790	1.1271
	6	0.9624	0.7539
803	1	0.9875	1.0531
	2	0.9886	1.0393
	3	0.9833	0.9399
	4	0.9801	1.2023
	5	0.9831	0.8838
	6	0.9873	1.1755
848	1	0.9702	0.6743
	2	0.9718	0.7092
	3	0.9644	0.6514
	4	0.9786	0.5686
	5	0.9614	0.4988
	6	0.9773	0.6505

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TABLE 3

ANOVA Table - Transformed Data obtained from  
Agriculture Canada Phase I Cigarettes

Source of variation	SS	df	MS	F	p
Among slopes	1.0779	15	0.07186	2.61	<0.001
Within slopes	15.2402	554	0.02751		

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570 = 19

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TABLE 4

ANOVA Table - Players Check 20 Data

Source of Variation	SS	df	MS	F	p
Among slopes	0.0933	3	0.0311	1.017	<0.5
Within slopes	0.6113	20	0.0306		

TABLE 5

ANOVA Table - Agriculture Canada Phase 1 Cigarettes

Source of Variation	SS	df	MS	F	p
Among slopes	33.6547	15	2.2436	66.7937	<0.001
Within slopes	3.2919	98	0.03359		

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TABLE 6  
A Comparison of Agriculture Canada Phase 1 Cigarettes using the Student-Newman-Keul Test

LTL	Description	Code	Code No.														No. X		
			(1) 891	(2) 726	862	PLCK 20	694	365	205	081	454	803	176	938	385	282		514	848
(1)	891 Burley, whole plant leaf blend	(1)		SS ✓	SS ✓	SS ✓		SS ✓	SS ✓	SS ✓	SS ✓	SS ✓	SS ✓	SS ✓	SS ✓	SS ✓	SS ✓	SS ✓	3
(2)	726 891 made into PCL	(6)	SS ✓		<del>SS</del>	NS ✓	NS ✓	NS ✓	<del>SS</del>	SS ✓	SS ✓	SS ✓	SS ✓	SS ✓	SS ✓	SS ✓	SS ✓	SS ✓	2
(3)	862 Flue-cured, lower leaves	(2)	SS ✓	<del>SS</del>		NS ✓	NS ✓	NS ✓	S ✓	SS ✓	S ✓	SS ✓	SS ✓	SS ✓	SS ✓	SS ✓	SS ✓	SS ✓	3
(4)	PLCK 20 Laboratory control	(3)	SS ✓	NS ✓	NS ✓		NS ✓	NS ✓	NS ✓	<del>SS</del>	<del>SS</del>	<del>SS</del>	SS ✓	SS ✓	SS ✓	SS ✓	SS ✓	SS ✓	3
(5)	694 Flue-cured, upper leaves	(4)	SS ✓	NS ✓	NS ✓	NS ✓		NS ✓	NS ✓	<del>SS</del>	<del>SS</del>	<del>SS</del>	SS ✓	SS ✓	SS ✓	SS ✓	SS ✓	SS ✓	3
(6)	365 Flue-cured, middle leaves	(7)	SS ✓	NS ✓	<del>SS</del>	NS ✓	NS ✓		NS ✓	<del>SS</del>	<del>SS</del>	<del>SS</del>	SS ✓	SS ✓	SS ✓	SS ✓	SS ✓	SS ✓	3
(7)	205 Flue-cured, whole plant leaf blend	(5)	SS ✓	<del>SS</del>	S ✓	NS ✓	NS ✓	NS ✓		<del>SS</del>	<del>SS</del>	<del>SS</del>	<del>SS</del>	<del>SS</del>	<del>SS</del>	SS ✓	SS ✓	SS ✓	6
(8)	081 862 made into PCL	(9)	SS ✓	SS ✓	SS ✓	<del>SS</del>	<del>SS</del>	<del>SS</del>	<del>SS</del>		NS ✓	NS ✓	NS ✓	<del>SS</del>	<del>SS</del>	<del>SS</del>	SS ✓	SS ✓	6
(9)	454 Flue-cured, whole plant stem blend	(8)	SS ✓	<del>SS</del>	SS ✓	<del>SS</del>	<del>SS</del>	NS ✓	NS ✓	NS ✓		<del>SS</del>	<del>SS</del>	<del>SS</del>	<del>SS</del>	<del>SS</del>	SS ✓	SS ✓	7
(10)	803 176 with high efficiency acetate filter	(10)	SS	SS	SS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	S	S	S
(11)	176 50-50 blend of 205 and 848	(11)	SS	SS	SS	S	S	S	NS	NS	NS	NS	NS	NS	NS	NS	S	S	S
(12)	938 694 made into PCL	(12)	SS	SS	SS	SS	SS	SS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
(13)	385 205 made into PCL	(13)	SS	SS	SS	SS	SS	SS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
(14)	282 365 made into PCL	(14)	SS	SS	SS	SS	SS	SS	SS	SS	SS	SS	S	NS	NS	NS	NS	NS	NS
(15)	514 454 made into PCL	(15)	SS	SS	SS	SS	SS	SS	SS	SS	SS	SS	S	NS	NS	NS	NS	NS	NS
(16)	848 Flue-cured, close-planted, chopped plant made into PCL	(16)	SS	SS	SS	SS	SS	SS	SS	SS	SS	SS	S	NS	NS	NS	NS	NS	NS

NS = not significantly different; S = significantly different at the 5% level; SS = significantly different at the 1% level.

**TABLE 7**

**Tobacco Leaf Type, Homogenization and Mutagenicity**

Leaf Type	Effect of Homogenisation			
	Before		After	
Flue-cured lamina - upper	1.324	694	938	0.920***
Flue-cured lamina - middle	1.311	(1.217) 365	282	0.669***
Flue-cured lamina - lower	1.519	862	081	1.172**
Flue-cured lamina - all positions	1.193	205	385	0.877*
Flue-cured stem	1.169	484	514	0.659***
Flue-cured - chopped plant	-	-	848	0.609 / 0.626
Burley lamina - all positions	3.228	811	726	1.580***
Players Check 20	1.368	-	-	-
50/50 205 + 848	0.985	176	803	1.049

\*Significant at the 10% level; \*\*Significant at the 5% level;  
 \*\*\*Significant at the 1% level.

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**TABLE 8**

**A Comparison of the Agriculture Canada Phase I  
Cigarettes in Biochemical Tests**

Code No.	Description	NMFI (ITL)	Mutagenicity		Sebaceous Gland* (Hamburg)
			ITL	Guelph	
205	Flue-cured, whole plant leaf blend	5.6(11)**	1.193(10)	12.9(8)	22.1(13)
385	205 made into PCL	4.2(8)	0.877(4)	7.3(4)	26.0(9)
454	Flue-cured, whole plant stem blend	3.1(5)	1.169(8)	20.8(11)	31.5(4)
514	454 made into PCL	2.8(2.5)	0.659(2)	8.1(5)	35.6(1)
694	Flue-cured, upper leaves	9.1(15)	1.324(12)	28.0(15)	22.8(12)
938	694 made into PCL	2.9(4)	0.920(5)	10.7(7)	23.7(11)
365	Flue-cured, middle leaves	4.9(10)	1.311(11)	18.0(9)	18.6(14)
282	365 made into PCL	2.8(2.5)	0.669(3)	9.5(6)	24.6(10)
862	Flue-cured, lower leaves	7.9(14)	1.519(14)	20.0(10)	29.6(6)
081	862 made into PCL	2.7(1)	1.172(9)	27.4(14)	
848	Flue-cured, close-planted, chopped plant made into PCL	4.0(7)	0.626(1)	5.6(2)	30.2(5)
176	50-50 blend of 205 and 848	4.8(9)	0.985(6)	6.7(3)	27.5(8)
803	176 with high efficiency acetate filter		1.049(7)	3.9(1)	29.1(7)
891	Burley, whole plant lamina blend	7.3(13)	3.228(16)	21.7(13)	34.2(3)
726	891 made into PCL	3.9(6)	1.580(15)	21.0(12)	35.2(2)
PLCK 20	Typical Canadian cigarette	6.7(12)	1.368(13)		

\* In contrast to the other tests, the higher the Sebaceous Gland test value, the lower the biological activity.

\*\* Figures shown in brackets are the rankings.

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**TABLE 9**

Testing the significance of correlation coefficients from regression lines and Spearman rank tests, using Fisher's z-transformation and Spearman table of critical values, respectively.

Comparison between	Correlation coefficients from	
	Regression lines r	Spearman tests r <sub>s</sub>
Mutagenicity (ITL) versus Mutagenicity (Guelph)	0.54*	0.76**
Mutagenicity (ITL) versus NMFI (ITL)	0.55*	0.60*
Mutagenicity (ITL) versus Sebaceous gland (Hamburg)	0.30	0.00
Mutagenicity (Guelph) versus NMFI (ITL)	0.44	0.28
Mutagenicity (Guelph) versus Sebaceous gland (Hamburg)	0.00	0.00
NMFI (ITL) versus Sebaceous gland (Hamburg)	0.15	0.30

\* Significant at p<0.05  
\*\* Significant at p<0.01

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