THE MUTAGENICITY BY THE AMES BACTERIAL TEST OF SMOKE CONDENSATES FROM CIGARETTES BELONGING TO THE JANUS B11 SERIES

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Background:

It was decided earlier this year to conduct the Ames test on condensates from series of cigarettes for which data such as mouse skin carcinogenicity, sebaceous gland, promotion activity or NMFI are available. A comparison of the results of the Ames test with data obtained from the testing of the same tobacco products by these other tests, to see if any relationship exists, should provide interesting information to tobacco scientists involved in mutagenicity and carcinogenicity testing. It must be stated at the very outset, however, that even if no relationships exist, the result of these mutagenic studies can be of value by themselves in assessing the biological properties of tobacco products.

The Ames bacterial test:

While the mutagenesis assay on petri dishes was carried out essentially according to the procedures developed by Ames and his colleagues, a considerable amount of time was spent on examining the activation step in the test procedure. This was done on account of the fact that the target organ of tobacco smoke is primarily the lung, and this organ is known to contain the enzymes necessary for the metabolism of carcinogenic compounds. However, every rat and guinea pig lung preparation that we tried, failed to activate cigarette smoke condensate to intermediate(s) mutagenic towards Salmonella typhimurium TA-98. On account of the metabolic differences

between rat and guinea pig tissues, we also compared liver and kidney preparations for their metabolic capabilities, and having found that rat liver was the most suitable, have decided to use this tissue for our studies on the mutagenicity of tobacco smoke condensates.

The Ames test is most frequently used by others for a quick yes or no answer for chemicals of unknown activity, and it takes one operator about a week to test several chemicals. However, when the test is adapted for quantitative studies, as we have done for differentiation of cigarettes, several replications are called for, and the time period to complete the testing is extended considerably.

Cigarette samples:

Two series of cigarettes have been chozen, viz., three samples from the Janus B11 series, and fifteen samples from the cigarettes made for the Agriculture Canada Tobacco and Health Study (Experiment No. 40, 1973), and kindly supplied by Dr. B. Zilkey. The former was chosen because it has been examined by a variety of biological tests, while the latter was selected because it included a wide variety of tobacco materials produced under controlled conditions. Although each series has its own controls, a laboratory control cigarette (Players Check 20) was always included so as to permit inter-comparison of samples tested over different periods of time.

On account of the metabolic studies referred to earlier, the examination of the Janus samples was not begun until about the middle of May.

and was completed a month later. The summer holidays and a delay in statistical analysis, combined with a very busy schedule at McGill University, have delayed the presentation of results till now. Work on the Zilkey series is progressing well, and it is hoped that the laboratory work will be finished by the middle of November. A memorandum will be issued as soon as possible.

Results and Discussions:

In this memo are presented the results of the examination of the Janus samples in the Ames test. A brief description of the Janus samples is presented in Table 1, while the mutagenic activities of these samples are presented in Tables 2 and Figures 1 and 2. From these illustrations it will be clear that the dose responses were linear. However, from Table 2 it will be seen that in the case of all four cigarettes, the variances at highest and lowest dose levels differed considerably. Consequently, a logarithmic transformation was carried out to make the range of values as well as the variances more uniform. The regression lines were then checked for parallelism by a standard ANOVA test, following which the intercepts were employed to express the mutagenicities of the four condensates. These are also presented in Table 2. A Student-Newman-Keul test was performed to test the intercepts for significant differences and the results are shown in Table 3.

From Table 3, it will be seen that both B11-1 and Players Check 20 cigarettes are very similar in their mutagenic activity, but are significantly different from B11-2 and B11-3, the two types of cigarettes

made from reconstituted tobaccos. It will also be seen that cigarette B11-2 does not differ from cigarette B11-3, a result that conflicts with that obtained by the mouse carcinogenicity test, in which B11-3 is significantly lower than B11-2, and both of these significantly lower than B11-1 (please see Table 4). The results obtained by the Ames test also differ from those obtained by the Sebaceous gland test, in which B11-2 was not significantly different from B11-1, but B11-3 was significantly lower than both B11-1 and B11-2 (see Table 4). The promotion test told a similar story in that B11-3 was lower than both B11-1 and B11-2.

This lack of correlation between these testing procedures is not surprising when one considers the complexity of tobacco smoke and the procedures employed in the tests. In the skin painting test the condensates are stored for long periods of time, while in the bacterial test they are used within a day or two of their collection. One has also to contend with the promotion activity of tobacco smoke when performing the skin painting test. It must also be remembered that not all carcinogens are positive on mouse skin, and other routes and species have to be used to detect their carcinogenic potential. Since 90% of the known carcinogens (detected by different routes in different species of animals) respond positively in the Ames test, the chances of the latter test responding to more carcinogens in the smoke than the mouse skin test are greater. Further, because of the complexity, as well as expense of biological tests such as mouse skin painting, they are not often replicated, which leaves their interpretation open to question.

whereas the Ames test, because of its simplicity, lends itself to replication and thorough statistical analysis quite readily.

When the results of the Ames test are compared with the NMFI data (Table 4), it will be found that both of these tests show that B11-2 and B11-3 are much lower than B11-1, but not different from each other. It will be interesting to see if this correlation between the Ames and NMFI tests is obtained again when we compare the results of the Zilkey cigarettes.

Conclusions:

This study with the Janus cigarettes suggests that the Ames test is suitable for detecting biological differences between smoking materials as widely different as flue-cured tobacco and reconstituted tobacco sheet made therefrom. While it is unlikely that the Ames test will pick up differences between different brands of cigarettes made from the same kind of tobacco, the results obtained are encouraging enough to warrant an examination of cigarettes made from tobaccos, such as air-cured, sun-cured and the fermented Turkish and Perique, as well as other types of smoking products such as cigarillos, cigars and pipe tobaccos. Since the mutagenic activity of chemical agents is of great concern to modern man, a biological assay based on this property should be considered a valuable addition to the arsenal of tests already available to the tobacco industry.

TABLE 1

DESCRIPTION OF JANUS B11 SAMPLES

Code	Description
PL CK 20	Laboratory control: lamina 73%, PCL 8% and CRS 19%.
B11-1	Control: lamina 60%, CRS 40% blend.
B11-2	Montreal PCL sheet from B11-1 blend.
B11-3	Schweitzer SRT sheet from B11-1 blend.

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TABLE 2

MUTAGENICITY OF SMOKE CONDENSATES FROM B11 CIGARETTES

Cigarette No.	Dose µg Condensate per Plate	No. of His+ Revertants - Mean ± S.D.	Regressions from Logarithmic Transformation of Data		
			Correlation Coefficient (Dose Response)	Slope	Intercep
PL C 20	50	53.0±15.8	0.9658	0.7357	3.928
	100	105.0±21.29			
	200	238.9±55.06			
	400	455.2±63.36			
B11-1 (Control)	50	47.2±14.76	0.9617	0.7219	3.826
	100	98.7±22.47			
	200	202.7±37.78			
	400	395.5±62.22			
B11-2 (PCL)	50	31.9±10.24	0.9661	0.7633	3.435
	100	70.2±12.45			
	200	143.8±25.95			
	400	310.4±58.92			
B11-3 (SRT[illegible] Schweitzer)	50	31.4±10.65	0.9675	0.7458	3.415
	100	67.2±11.87			
	200	137.3±22.60			
	400	283.8±35.44			

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TABLE 3

The Student-Newman-Keul test for significance of observed differences in mutagenicity.

No. of steps apart - P	Mutagenicity in terms of intercepts from transformed data				Least significant range (Wp) at	
	PL CK 20	B11-1	B11-2	B11-3	Levels of significance	
	3.93	3.83	3.44	3.42	5%	1%
	Differences in intercepts					
2	PL CK vs B11-1 0.10	B11-1 vs B11-2 0.39*	B11-2 vs B11-3 0.02		0.13	0.17
3	PL CK vs B11-2 0.49*	B11-1 vs B11-3 0.41*			0.15	0.19
4	PL CK vs B11-3 0.51*				0.17	0.20

* Significant differences at both 1 and 5% levels.

TABLE 4

A COMPARISON OF THE JANUS B11 SERIES CIGARETTES IN SEVERAL BIOLOGICAL AND BIOCHEMICAL TESTS

Cigarette	Mouse Skin Tumorigenicity - % animals Showing Tumours	Sebaceous gland - Dose to Produce 50% Suppression	Promotion - % animals bearing hyperplasias & neoplasias	NMFI	Ames Mutagenicity Test
B11-1 (Control)	46 T R. 100.0	22.2	24	6.0	3.83
B11-2 (PCL)	39* 85.2	24.9	23	4.5*	3.44*
B11-3 (SRT)	17* 40.9	38.9*	17*	4.3*	3.42*

*Significantly different from appropriate control.

FIGURE 1: THE MUTAGENICITY OF JANUS B11 CIGARETTES IN THE AMES TEST

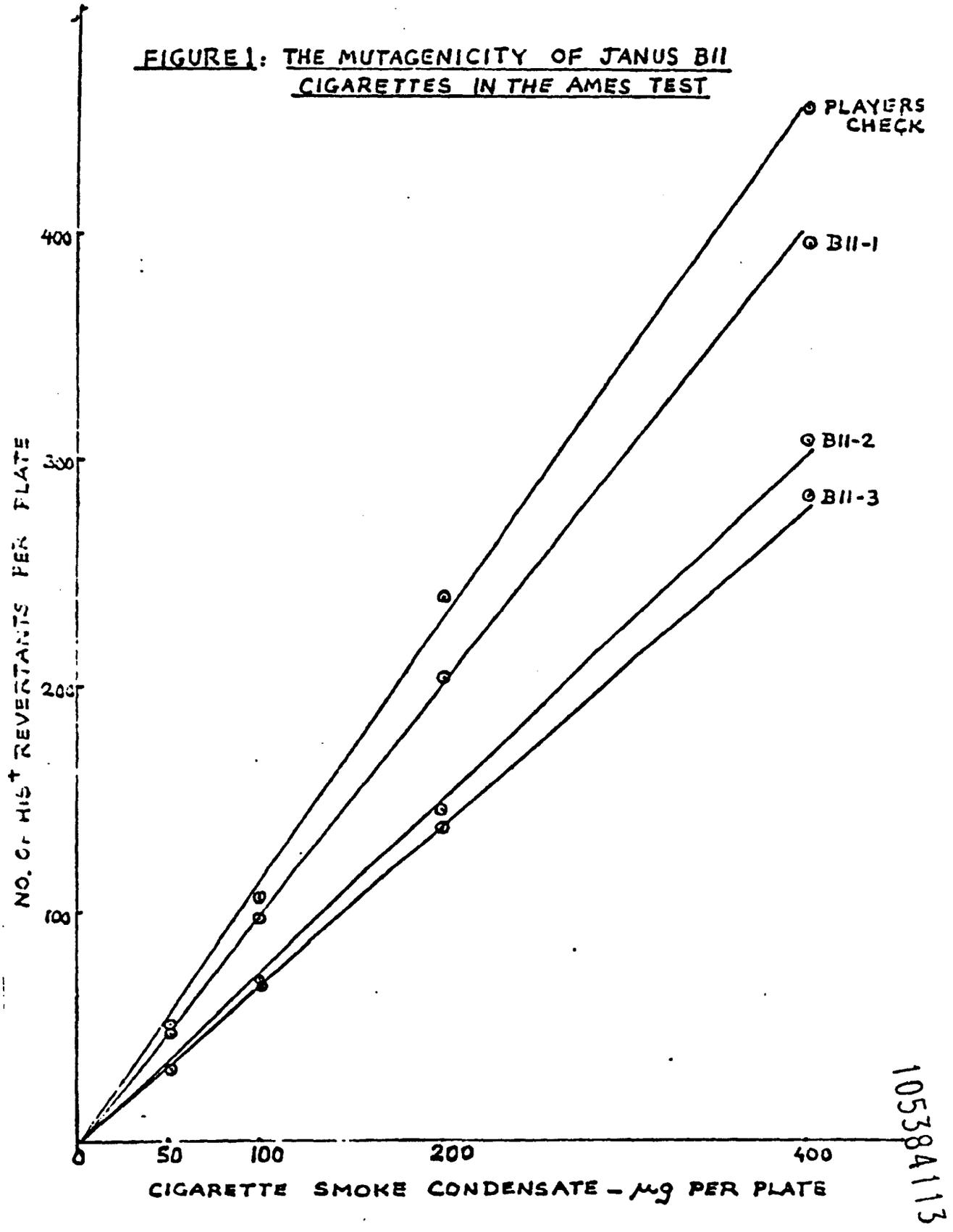

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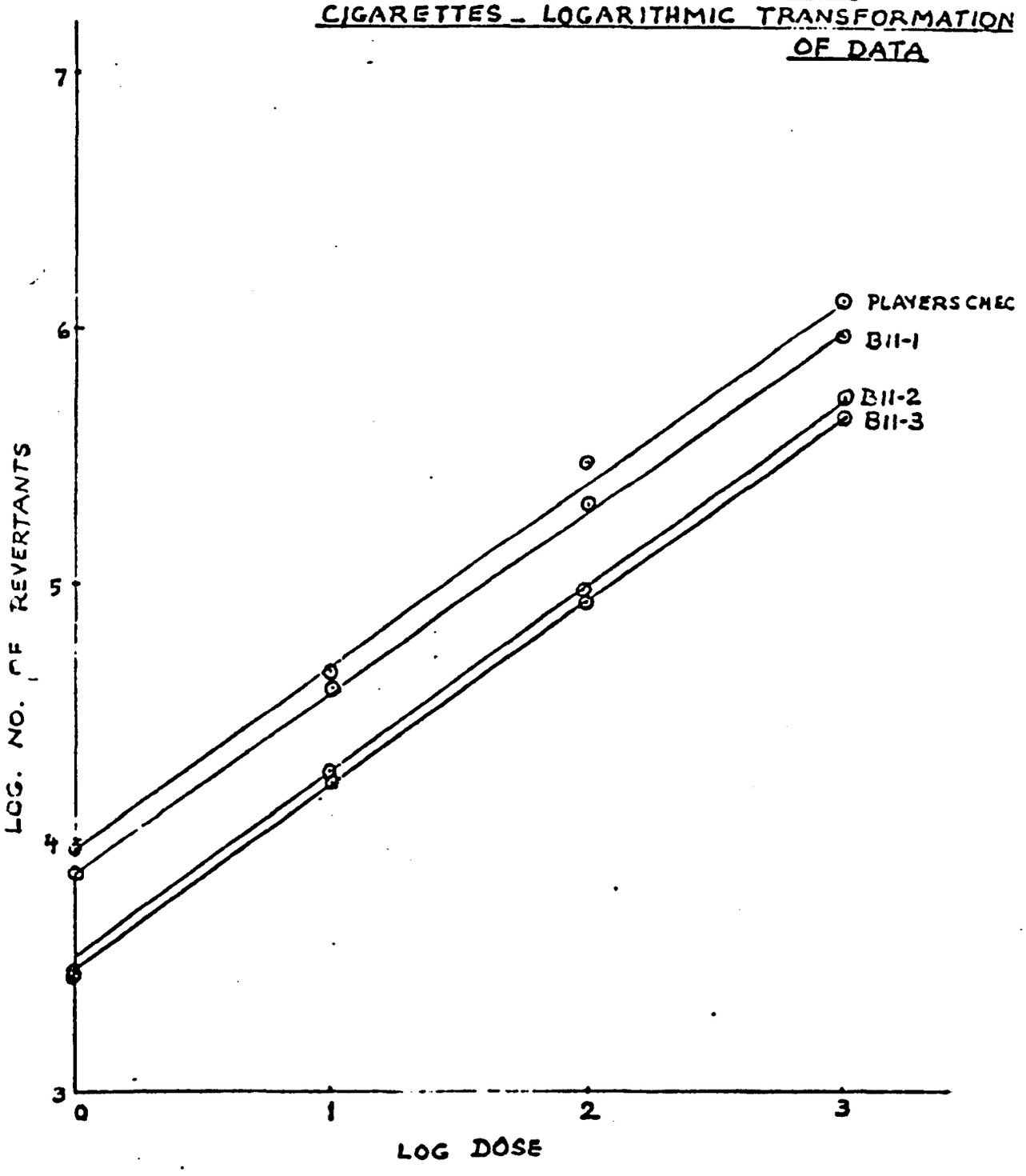
FIGURE 2 : THE MUTAGENICITY OF JANUS B11 CIGARETTES - LOGARITHMIC TRANSFORMATION OF DATA



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