

Laboratory Report No. 176
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RESEARCH & DEVELOPMENT DIVISION
MONTREAL

RESTRICTED

INVESTIGATIONS ON THE EFFECT OF HUMAN SMOKING CONDITIONS
ON THE BIOLOGICAL ACTIVITY OF TOBACCO SMOKE CONDENSATE

AUTHOR: C. McBride
M.H. Billimoria

Issued By: S.R. Massey

Date Issued: January 23, 1986

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Summary and Conclusions

The objective of this study was to investigate the effect of human smoking conditions on the specific biological activity of tobacco smoke condensate. From a preliminary study, two human smoking parameters, puff duration and puff interval, appeared to have some influence. It was felt important to investigate the apparent effects of these two parameters while controlling for puff number. Two commercial Canadian ventilated products, Brand A and Brand B (declared tar deliveries of 4 and 11 mg respectively) were selected for use in the studies. The results of the present investigation can be summarized as follows:

No statistically significant differences in the specific biological activities of either cigarette, as determined by the Ames Test, were found for the tobacco smoke condensate generated under different human smoking conditions relative to: 1) standard smoking conditions or
2) each other.

It appears, therefore, that the standard procedure for the determination of the specific biological activity of cigarette smoke is an accurate indicator of the specific biological activity to which a smoker may be exposed.

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Introduction

Most studies of the biological activity of tobacco smoke condensate have been concerned with the relative differences between tobacco and product types. The majority of these studies have used standard smoking conditions (35 ml puff, 2.0 s duration, 58.0 s interval) to generate the smoke condensate. It is known, however, that actual human smoking conditions can vary from standard conditions. In light of this, it was felt prudent to assess the effect, if any, of human smoking conditions relative to standard conditions, on the Ames biological activity of smoke condensate. The Ames Test was considered to be the most appropriate short term mutagenic testing procedure to follow.

A preliminary investigation, carried out in 1984, indicated that human smoking conditions, specifically puff duration and puff interval may affect the specific biological activity of smoke condensate. It was decided, at that point, to focus attention on the effect of varying these two smoking parameters.

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1. Method

1.1 Cigarette Selection

Two commercial Canadian brands, Brand A and Brand B (declared tar deliveries 4 and 11 mg. respectively), were selected for investigation. These two products are typical examples of Canadian cigarettes representing the extremes of the usual delivery range associated with ventilated products.

All cigarettes were selected for weight, pressure drop and ventilation level. Details of the cigarette specifications are given in Table 1.

1.2 Human Smoking Behaviour

Out of a pool of 95 previously recorded human profiles, 6 profiles were selected for further investigation using the following criteria.

- 1) Group A: all smoking parameters except puff duration to remain constant.
- 2) Group B: all smoking parameters except puff interval to remain constant.

where necessary, profiles were truncated to control for puff number and butt length; details are given in Table 2.

1.3 Duplication

The Human Smoking Behaviour Research group carried out the duplication of each profile using the ITL smoke duplicator¹. In order to satisfy the requirements of the Ames Test in terms of condensate concentration, each profile was duplicated 10 times. Concurrently, with duplication, each brand was smoked under standard machine conditions to

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obtain a control. The duplicated deliveries for each profile are given in Table 3.

1.4 Mutagenicity Testing

The smoke condensate for each profile and the companion control samples for the two groups of smoking conditions were passed to the Microbiological group for mutagenicity testing. The condensate on the pads was extracted with DMSO and tested for mutagenicity by the standard procedure followed in this laboratory^{2,3}. Salmonella typhimurium TA-98 was used to compare the mutagenicities of the condensates. The microbiological procedures and preparation of the Sprague-Dawley rat liver post-mitochondrial supernatant (S-9), were according to procedures described by Ames et al.⁴

1.5 Statistical Analysis

The mutagenicities of the condensates were calculated in two ways:

1. Regression analysis of log transformed data in which regression lines were checked for parallelism by an ANOVA test⁵. The intercepts, using a common slope, were employed to express mutagenicities.
2. Converting each dose point to a specific activity (number of revertants per µg condensate). Means and standard deviations were calculated for each condensate from a total of 20 points. An ANOVA was performed to determine whether the differences between means were significant or not.

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2. Results and Discussion

It can be seen from Table 4 and Figures 1 and 2, that for varying puff duration, there were no significant differences in the specific biological activity of the human smoking profiles, nor any differences between the control and the human smoking profiles. There were also no significant differences in biological activity when puff interval was varied (Table 5, Figures 3 and 4).

The earlier study indicated that an increase in puff duration resulted in a decrease in biological activity, while an increase in puff interval (decrease in puff number) led to an increase in specific biological activity. However, these preliminary results were obtained from single experiments. In the study reported here, puff duration and interval were varied while puff number was controlled. Furthermore, sufficient replicates were conducted for statistical analyses. In light of these points, the preliminary findings appear questionable.

Other researchers have reported that an increase in flowrate during a puff, decreased the specific biological activity (GR & DC Status Review Notes, November 1984 - April 1985 and the Biological Conference, Montreal, Canada 1985). It was not the intent of the study described here, to investigate the effect of flowrate on biological activity. However, it can be seen from Table 2 that flowrates did vary within Group A smoking conditions (varying puff durations). However, the information in Table 4 indicates that there were no differences in specific biological activity which could be attributed to differences in either puff duration, as discussed previously, or flowrate. The reasons for these different results are not clear and should perhaps be further investigated.

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The results of this study appear to indicate that the Ames biological activity of smoke condensate is unaffected by changes in the puff duration or interval of a smoking regime and possibly unaffected by changes in flowrate. Therefore these findings support the use of standard smoking conditions to monitor the biological activity of tobacco products.

It should be noted, however, that the cigarettes used in this study were Canadian products which are manufactured with flue-cured tobacco. Previous work has shown that the most influential factor in relative Ames biological activity is tobacco type⁶. Flue-cured tobacco is less biologically active than other tobacco types. Furthermore the differences in biological activity between Canadian cigarettes (flue-cured tobacco) are small. Therefore these results may not be applicable to all tobacco types and a further study using other tobaccos may be warranted.

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References:

1. McBride, C., Design and Operation of the Imperial Tobacco Ltd. Smoke Duplicator. R & D Report No. 170.
2. Billimoria, M.H., Imperial Tobacco Ltd., Research Laboratory Report No. 162, 1981.
3. Idem, Imperial Tobacco Ltd., memo dated 18 October 1978.
4. Ames, B.N., McCann J. and Yamaski, E., *Mutat. Res.*, 31 (1975) 347.
5. Brownlee, K.A., *Statistical Theory and Methodology in Science and Engineering*, J. Wiley & Sons, N.Y., 1960.
6. Billimoria, M.H., A Review of BAT Studies of the Mutagenicity of Cigarette Smoke in the Ames Salmonella/Microsome Test (in preparation).

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TABLE 1

CIGARETTE SPECIFICATIONS

	<u>Brand A</u>	<u>Brand B</u>
DPM (mg/cigt.)	3.7	11.1
Nicotine (mg/cigt.)	0.42	0.93
Water (mg/cigt.)	0.37	1.11
CO (mg/cigt.)	3.8	11.4
P.D. (mm W.G.) open	75	94
Dilution (%)	63	37
Paper Porosity (C.U.)	36.8	35.2

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TABLE 2

DETAILS OF THE SMOKING CONDITIONS USED TO GENERATE SMOKE CONDENSATE

Smoking Condition	No. of Puffs (Butt Length) (mm)	Mean Puff Volume (ml)	Mean Maximum Flowrate (ml/s)	Mean Average Flowrate (ml/s)	Mean Puff Duration (s)	Mean Puff Interval (s)	Mean Total Puff Volume (ml)
Standard Conditions		35		17.5	2.0	58	
<u>Group A - Varying Puff Duration</u>							
S #9-328-13	9 (35)	50.8	21.0	16.2	3.0	48.7	457.4
S #7-328-11	8 (37)	53.6	35.0	25.0	2.1	49.0	419.10
S #6-919-12	9 (37)	46.1	51.7	40.2	1.1	48.8	461.1
<u>Group B - Varying Puff Interval</u>							
S #1-328-1	6 (38)	42.3	42.0	33.0	1.2	65.4	253.9
S #5-328-9	7 (37)	36.8	49.4	32.7	1.2	36.0	257.5
S #6-328-5	7 (37)	39.5	43.9	30.5	1.3	28.5	276.5

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TABLE 3

COMPARISON OF THE DELIVERIES OBTAINED FROM THE DIFFERENT SMOKING CONDITIONS

Smoking Condition	Brand A				Brand B			
	Tar	Nicotine	H ₂ O	CO	Tar	Nicotine	H ₂ O	CO
	(mg/cigt.)				(mg/cigt.)			
Group A -								
Puff Duration								
Std. Conditions	4.0	0.40	0.42	--	10.6	0.99	0.99	--
S #9-328-13	4.8	0.45	0.29	3.9	9.8	0.83	0.69	9.4
S #7-328-11	5.5	0.52	0.44	5.5	11.0	0.93	0.93	12.5
S #16-919-12	7.3	0.67	0.61	7.0	13.0	1.13	1.21	15.5
Group B -								
Puff Interval								
Std. Conditions	4.8	0.47	0.40	--	11.0	0.98	0.70	--
S #6-328-5	3.1	0.27	0.18	3.3	5.9	0.48	0.49	6.8
S #1-328-1	3.8	0.32	0.22	3.9	6.7	0.57	0.50	8.3
S #5-328-9	2.8	0.23	0.15	2.9	5.0	0.41	0.41	6.7

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TABLE 4

MUTAGENIC ACTIVITIES* BY THE AMES TEST OF SMOKE CONDENSATE GENERATED UNDER DIFFERENT PUFF DURATIONS

Cigarette and Profile Code	Puff Duration (s)	Regressions from Logarithmic Transformation of Data			Mutagenicities # of His ⁺ Revertants of TA98/ug of Condensate Mean ± S.D.	Significance of Differences Between Mutagenicities (Student-Newman-Keul-t-Test)			
		Correlation Coefficient	Slope	Intercept (Mutagenicity)		Std. Conditions	S#9-328-13	S#7-328-11	S#16-919-2
Brand A									
Std. Conditions	2.0	0.983	1.156	0.762	2.109 ± 0.276	--	N.S.	N.S.	N.S.
S #9-328-13	3.0	0.969	1.153	0.694	1.929 ± 0.329	--	--	N.S.	N.S.
S #7-328-11	2.1	0.980	1.202	0.686	1.913 ± 0.352	--	--	--	N.S.
S #16-919-2	1.1	0.983	1.166	0.692	1.915 ± 0.266	--	--	--	--
Brand B									
Std. Conditions	2.0	0.988	1.179	0.456	1.486 ± 0.198	--	N.S.	N.S.	N.S.
S #9-328-13	3.0	0.980	1.278	0.422	1.374 ± 0.258	--	--	N.S.	N.S.
S #7-328-11	2.1	0.991	1.211	0.432	1.395 ± 0.189	--	--	--	N.S.
S #16-919-12	1.1	0.990	1.206	0.459	1.494 ± 0.195	--	--	--	--

* Calculated from 4 replications at 5 concentrations of condensate, each in triplicate.

** Calculated separately at each concentration of smoke condensate.

N.S. = not significantly different.

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TABLE 5
MUTAGENIC ACTIVITIES* BY THE AMES TEST OF SMOKE CONDENSATE GENERATED UNDER DIFFERENT PUFF INTERVALS

Cigarette and Profile Code	Puff Interval (s)	Regressions from Logarithmic Transformation of Data			Mutagenicity # of His ⁺ Revertants of TA98/ug of Condensate Mean ± S.D.	Significance of Differences Between Mutagenicities (Student-Newman-Keul-t-Test)			
		Correlation Coefficient	Slope	Intercept (Mutagenicity)		Std. Conditions	S#1-320-1	S#5-320-9	S#6-320-5
Brand A									
Std. Conditions	50	0.994	1.260	0.516	1.814 ± 0.243	--	N.S.	N.S.	N.S.
S #1-320-1	65	0.992	1.157	0.484	1.703 ± 0.187	--	N.S.	N.S.	N.S.
S #5-320-9	36	0.976	1.284	0.515	1.806 ± 0.339		--		N.S.
S #6-320-5	28.5	0.986	1.241	0.514	1.821 ± 0.285				--
Brand B									
Std. Conditions	50	0.987	1.174	0.546	1.592 ± 0.205	--	N.S.	N.S.	N.S.
S #1-320-1	65	0.984	1.202	0.536	1.553 ± 0.207	--	N.S.	N.S.	N.S.
S #5-320-9	36	0.992	1.239	0.616	1.796 ± 0.255		--		N.S.
S #6-320-5	28.5	0.983	1.184	0.587	1.715 ± 0.232				--

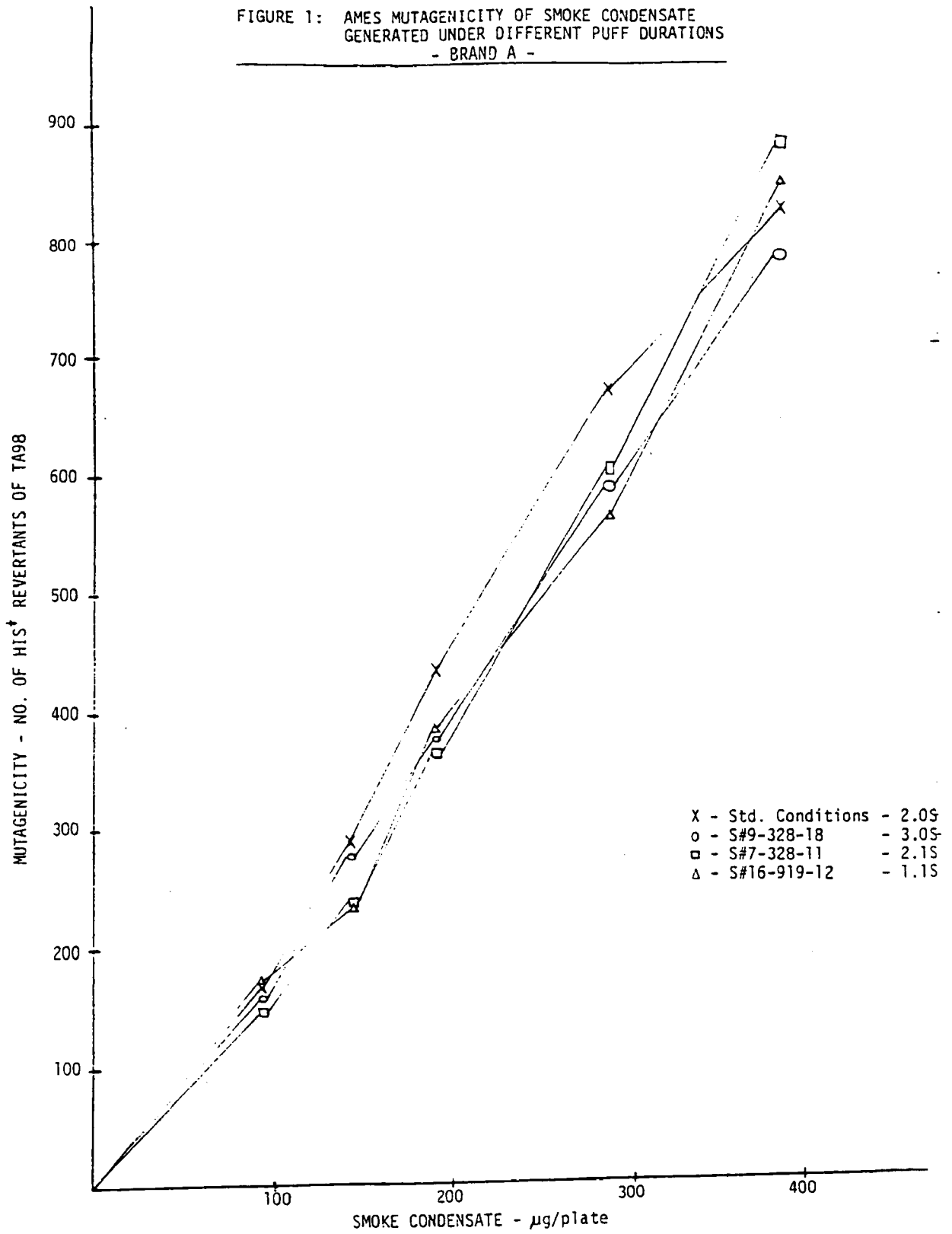
* Calculated from 4 replications at 5 concentrations of condensate, each in triplicate.

** Calculated separately at each concentration of smoke condensate.

N.S. = not significantly different.

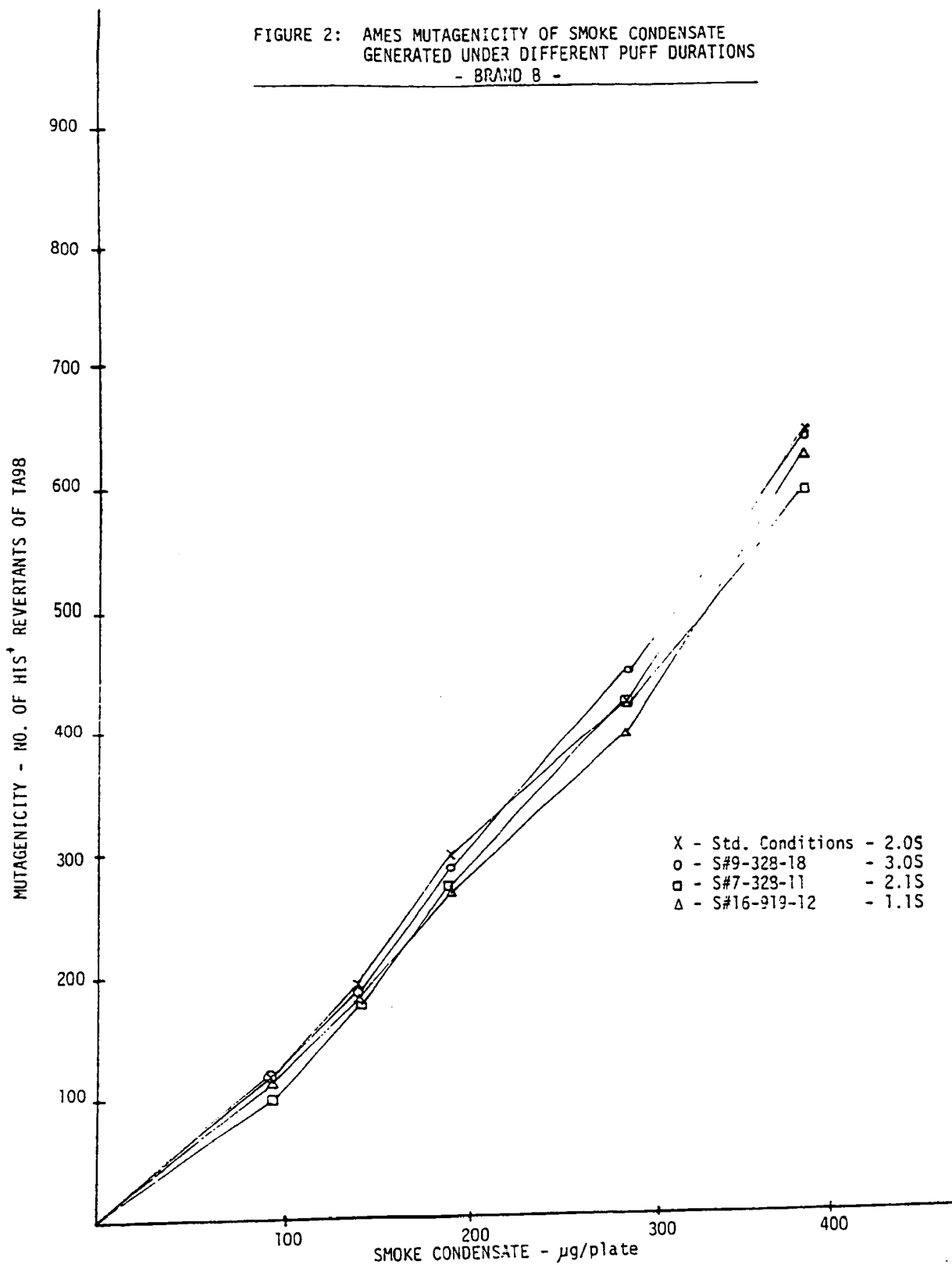
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FIGURE 1: AMES MUTAGENICITY OF SMOKE CONDENSATE
 GENERATED UNDER DIFFERENT PUFF DURATIONS
 - BRAND A -



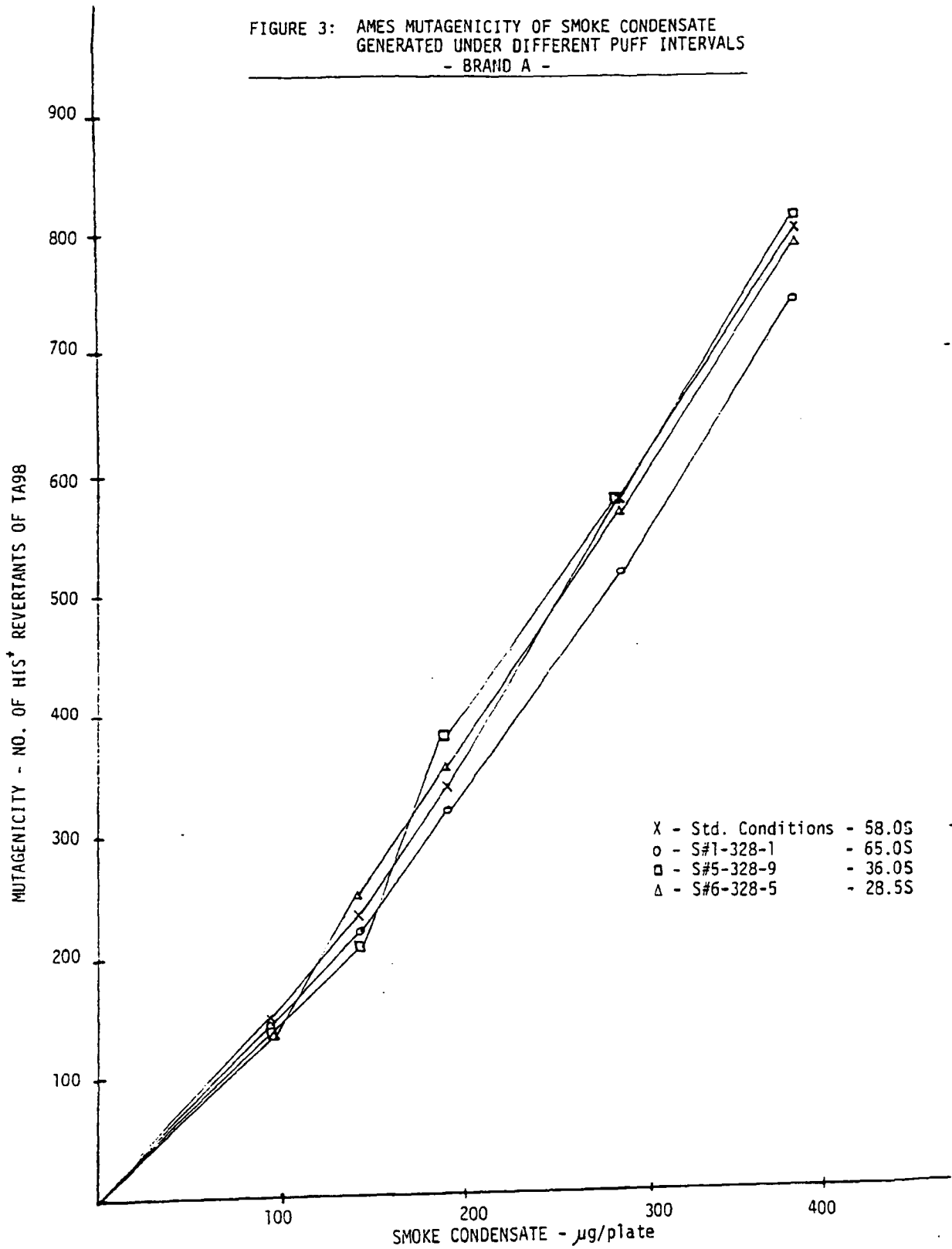
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FIGURE 2: AMES MUTAGENICITY OF SMOKE CONDENSATE
 GENERATED UNDER DIFFERENT PUFF DURATIONS
 - BRAND 8 -



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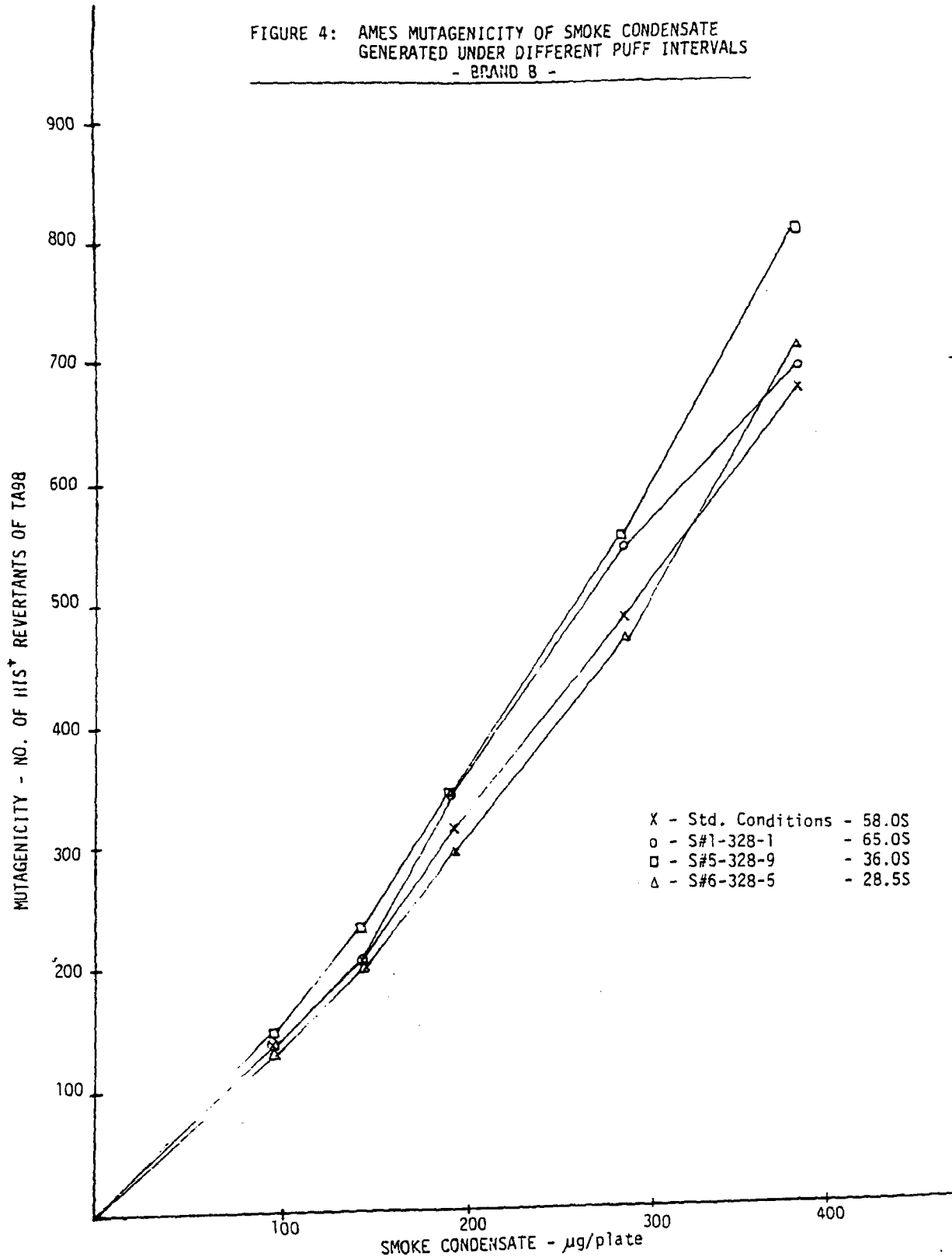
FIGURE 3: AMES MUTAGENICITY OF SMOKE CONDENSATE
 GENERATED UNDER DIFFERENT PUFF INTERVALS
 - BRAND A -



X - Std. Conditions - 58.05
 o - S#1-328-1 - 65.05
 □ - S#5-328-9 - 36.05
 Δ - S#6-328-5 - 28.55

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FIGURE 4: AMES MUTAGENICITY OF SMOKE CONDENSATE
 GENERATED UNDER DIFFERENT PUFF INTERVALS
 - BRAND B -



X - Std. Conditions - 58.05
 o - S#1-328-1 - 65.05
 □ - S#5-328-9 - 36.05
 Δ - S#6-328-5 - 28.55

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