

EXAMINATION OF CIGARETTE SAMPLES

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Four cigarette samples were received for analysis of their genotoxic potential in 3 *in vitro* bioassays. The samples were as detailed below :

Standard reference cigarette (CODE CK28).

Untreated Test cigarette (CODE 837).

2 Variables of the Test Cigarette prepared by different treatments (CODES 645 and 772).

These samples were evaluated using the Chromosome damage assay (CHO cells) , the Micronucleus assay (V79 cells) and the Unscheduled DNA synthesis assay (hepatocytes).

Statistical analysis was performed using the students t test with a pooled standard deviation.

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RESULTS

1. Chromosome Aberration Assay.

This assay was performed on one occasion, using triplicate cultures, up to a maximum level of 75 ug / ml PMWNF (Particulate Matter Water and Nicotine Free). The degree of cytotoxicity of each sample is shown in Figure 1.

For chromosome aberrations the CK28 sample produced a clear positive dose response. The other 3 samples gave either a shallower response (837 and 772) or a flat negative response (645) (Table 1, Figures 2 - 5). Doses above 75 ug / ml were not scored due to the high cytotoxicity of the condensates.

Table 1.
Summary of Chromosomal aberration data from 4 cigarette samples.

Sample Code	Dose ug/ml (PMWNF)	Aberrations / 100 cells (mean \pm SD)
Control	1% DMSO	4.85 \pm 2.43
EMS	2 mg ml ⁻¹	71.25 \pm 13.50 +++
CK 28	25	10.33 \pm 2.88 +++
	50	22.33 \pm 6.81 +++
	75	37.00 \pm 9.54 +++
837	25	5.33 \pm 3.78 NSD
	50	10.0 \pm 1.73 +++
	75	17.63 \pm 5.03 +++
772	25	8.33 \pm 1.35 ++
	50	6.33 \pm 4.16 NSD
	75	12.00 \pm 4.00 +++
645	25	4.67 \pm 2.52 NSD
	50	6.00 \pm 2.64 NSD
	75	2.50 \pm 2.12 NSD

SD Standard deviation

+++ Significantly different from control at 99 % confidence limits (CL).

++ Significantly different from control at 95 % CL.

NSD No significant difference from control.

EMS Ethyl methane sulphonate.

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A statistical cross comparison of all samples at 75 ug / ml was performed and the following is a summary of the findings :

Differences between cigarette samples at 75 ug / ml PMWNF in the chromosome damage assay.

Code	CK28	837	772	645
CK28		++	++	+++
837			NSD	++
772				NSD
645				

+++ Significant difference at 99% CL.

++ Significant difference at 95% CL.

NSD No significant difference.

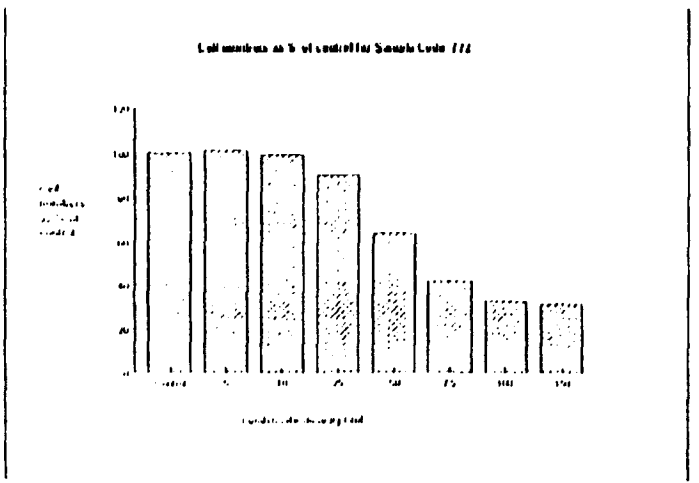
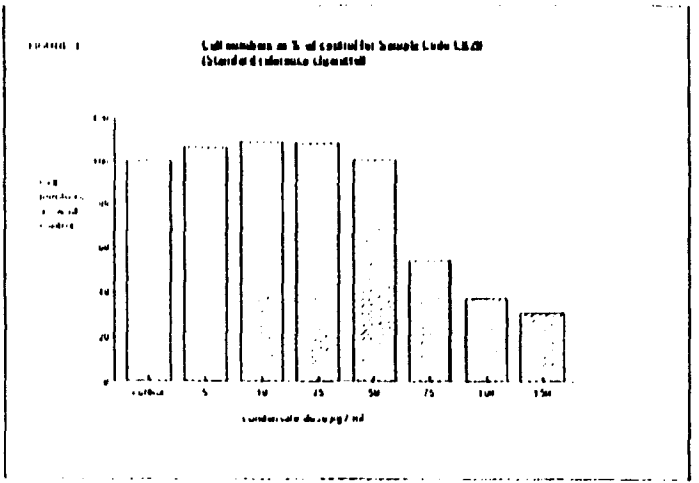
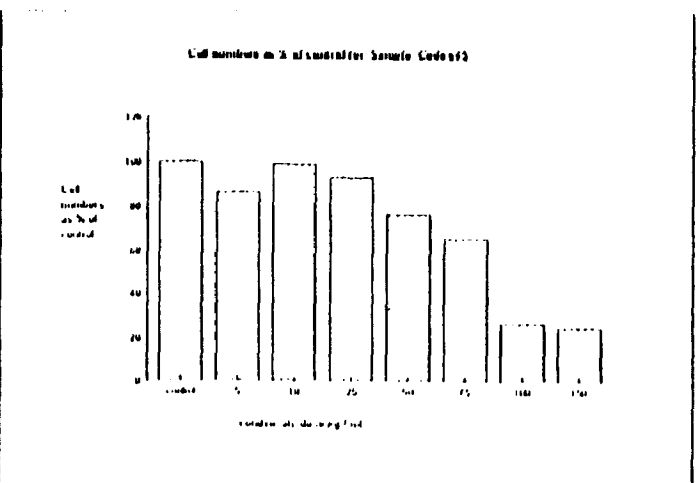
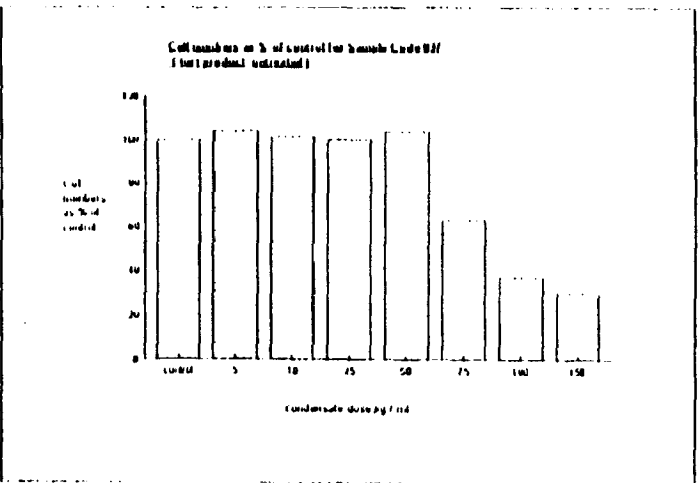
Summary - Chromosome Aberration Assay.

At the highest tested dose of 75 ug / ml the data indicate that all condensates, except that from treated sample 645, induced a statistically significant increase over controls in the levels of aberrations seen in CHO cultures (at 99% confidence limits).

Closer analysis of the results suggests that treatment method 772 produced no significant differences in the clastogenic activity of condensate when compared to the untreated sample 837.

Treatment method 645, however did produce a condensate with significantly reduced activity in CHO cultures when compared to its untreated version 837 (95 % CL).

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FIGURE 2

Induced Aberrations per 100 cells in CHO cultures exposed to Canadian reference cigarette (CX28)...

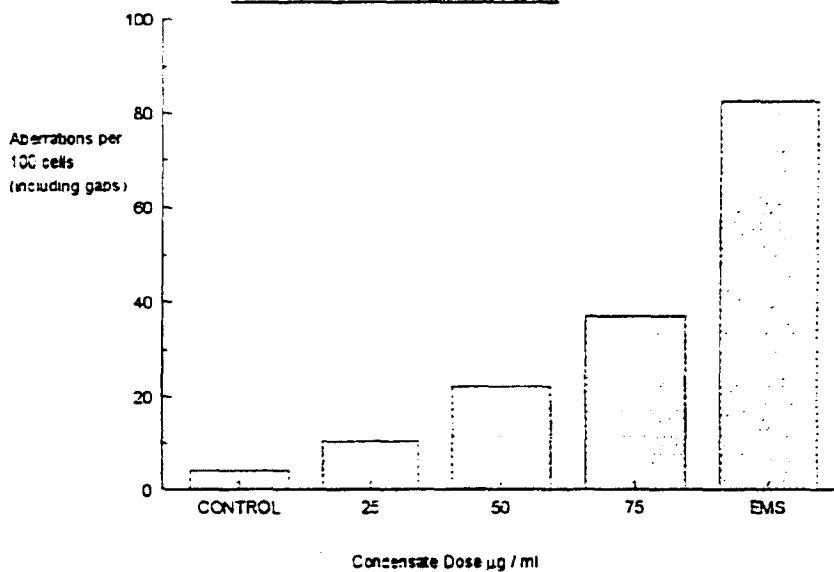
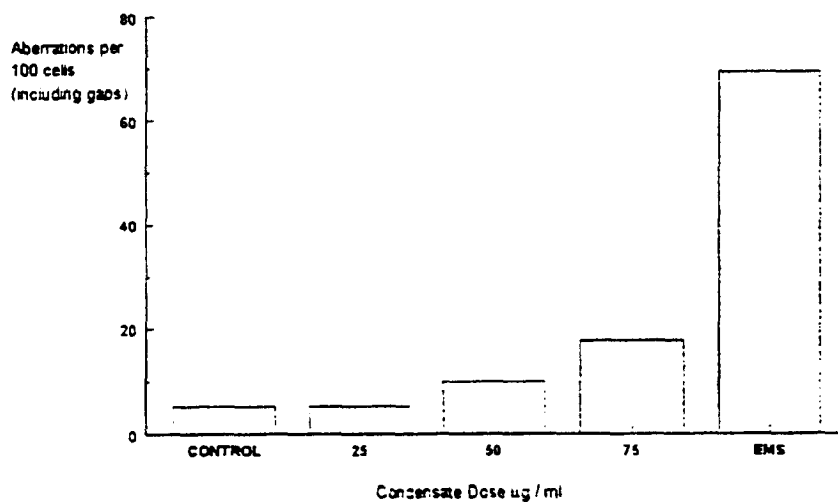


FIGURE 3

Induced Aberrations per 100 cells in CHO cultures exposed to cigarette smoke condensate Code 837...



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FIGURE 4

Induced Aberrations per 100 cells in CHO cultures exposed to cigarette smoke condensate Code 772.

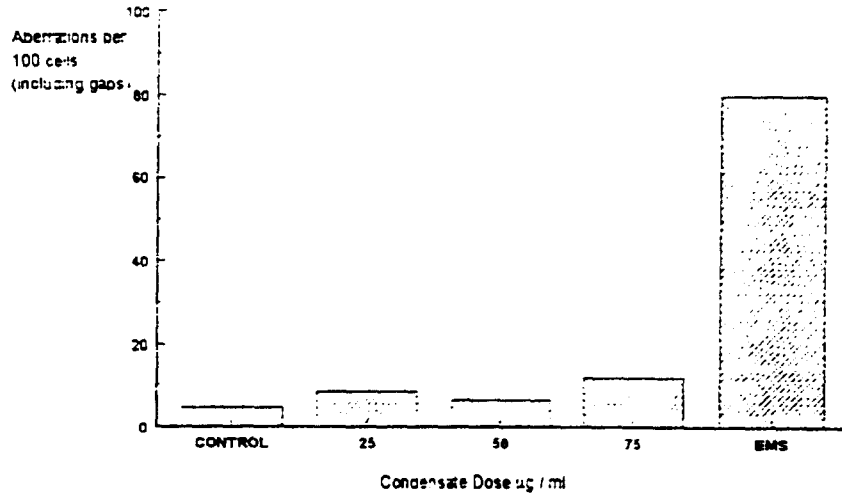
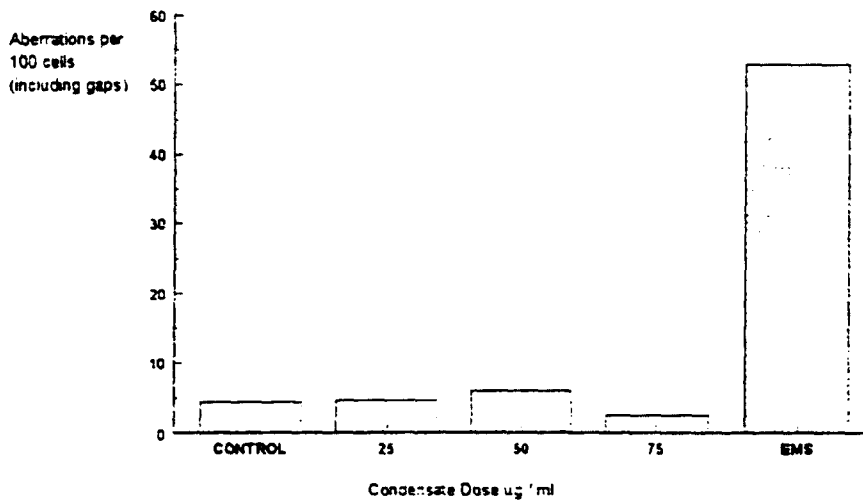


FIGURE 5

Induced Aberrations per 100 cells in CHO cultures exposed to cigarette smoke condensate Code 645.



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2. Micronucleus Assay :

Based on the results from previous studies, a dose of 30 ug / ml PMWNF, in triplicate cultures, was chosen for all cigarette samples. The standard micronucleus assay was performed using the V79 Chinese hamster lung cell line. Table 2 gives details of the cytotoxicity of the samples (as measured by the percentage of binucleate cells) together with the corresponding levels of induced micronuclei. These results are shown graphically in Figures 6 and 7.

Table 2 : Summary of induced micronuclei in V79 cells with 4 sample cigarettes.

Sample	% Binucleate cell population \pm SD	Micronuclei/1000 Binucleate Cells (mean \pm SD)
Control	71.25 \pm 6.46	8.35 \pm 4.78
Mitomycin C (0.1 ug ml ⁻¹)	39.03 \pm 3.31	26.66 \pm 3.50 +++
CK 28	27.68 \pm 5.16	31.60 \pm 1.96 +++
837	31.83 \pm 4.93	23.24 \pm 5.09 ++
645	42.97 \pm 3.03	22.18 \pm 1.46 +++
772	44.62 \pm 2.60	18.90 \pm 3.44 ++

+++ Significantly different from control at 99 % CL.

++ Significantly different from control at 95 % CL.

NSD No significant difference from control.

A statistical cross comparison of all samples was performed and the following table is a summary of the findings :

Differences between cigarette samples at 30 ug / ml PMWNF in the micronucleus assay.

Code	CK28	837	772	645
CK28		NSD	+++	+++
837			NSD	NSD
772				NSD
645				

+++ Significant difference at 99% CL.

NSD No significant difference.

Summary - Micronucleus Assay.

All four condensates induced levels of micronuclei which were significantly higher than those of the controls i.e. a positive response.

Closer statistical analysis indicated that neither treatment (samples 772 and 645) had produced any significant changes in the genotoxic potential of the condensate in comparison to that from the untreated ' parent ' cigarette (837).

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Fig 6

Binucleate Population of V79 Cells Exposed to Cigarette Smoke Condensate from 4 Cigarettes (30 µg / ml PMWNF)

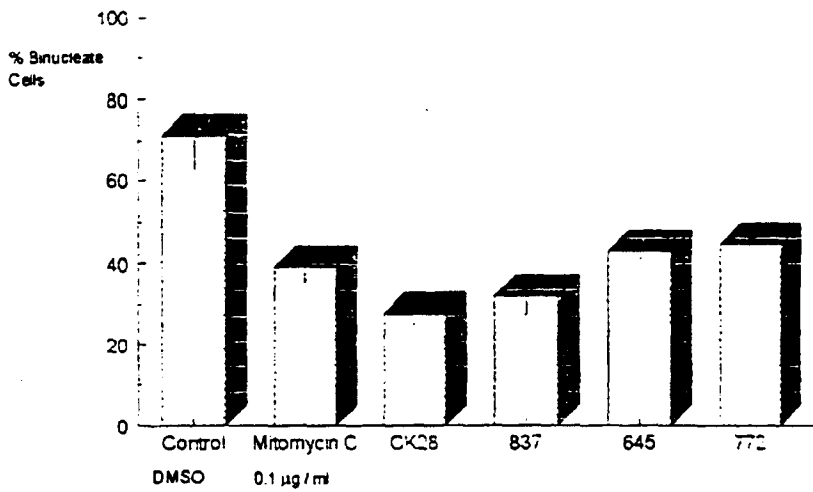
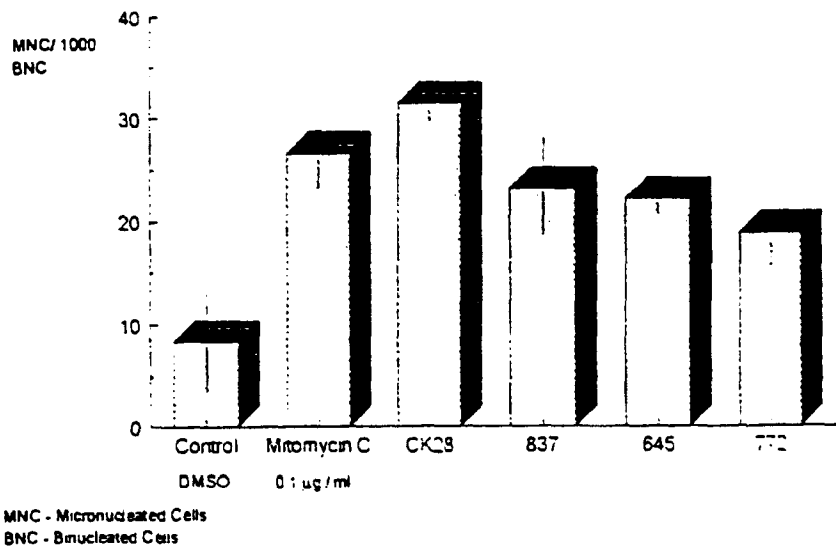


Fig 7

Induced Micronuclei of V79 Cells Exposed to Cigarette Smoke Condensate from 4 cigarettes (30 µg / ml PMWNF)



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3. Unscheduled DNA Synthesis assay

All 4 cigarette samples were tested at doses of 10, 25 and 50 ug / ml in the standard *in vitro* UDS assay using triplicate cultures of rat hepatocytes. Table 3 shows the mean net nuclear grains (NNG) induced per cell from each dose. (See also Figures 8-11).

Table 3 : Mean NNG per cell induced in hepatocytes by 4 sample cigarettes.

Sample Code	Mean NNG per cell with dose ug / ml		
	10	25	50
CONTROL	0.78 ± 0.94		
AAF	95.6 ± 54.0		
CK28	1.60 ± 0.30	8.00 ± 0.75 +++	TOXIC
837	-0.66 ± 2.11	2.20 ± 1.44 NSD	7.20 ± 4.55 ++
772	1.03 ± 0.70	2.36 ± 0.57 NSD	3.03 ± 0.42 ++
645	1.00 ± 0.68	0.97 ± 0.83 NSD	1.80 ± 1.15 NSD

+++ Significantly different from control at 99 % CL.

++ Significantly different from control at 95 % CL.

NSD No Significant difference from control.

AAF 0.1mM 2-Acetyl aminofluorene

A further statistical cross comparison of the data at 25 ug / ml and 50 ug / ml was performed, the following tables summarise the findings ;

Differences between cigarette samples at 25 ug / ml PMWNF in the UDS assay.

Code	CK28	837	772	645
CK28		+++	+++	+++
837			NSD	NSD
772				NSD
645				

+++ Significantly different at 99% CL.

NSD No significant difference

Differences between cigarette samples at 50 ug / ml PMWNF in the UDS assay

Code	837	772	645
837		NSD	NSD
772			NSD
645			

NSD No significant difference.

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Summary - Unscheduled DNA Synthesis Assay.

Condensates CK28, 837 and 772 produced weakly positive dose responses in induced levels of unscheduled DNA synthesis. Condensate 645 produced no significant induction in UDS and can be considered to be negative in this assay. Further statistical analysis indicates that treatments 772 and 645 at both 25 and 50 mg/ml produced no significant changes in the genotoxic potential of the condensate of the untreated 'parent' cigarette 837.

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FIGURE 8 Mean NNG induced by exposure to cigarette smoke condensate Code CK28

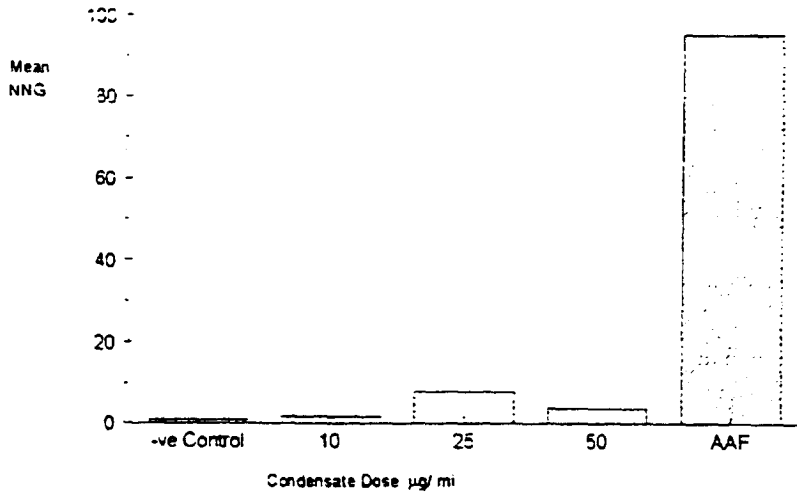
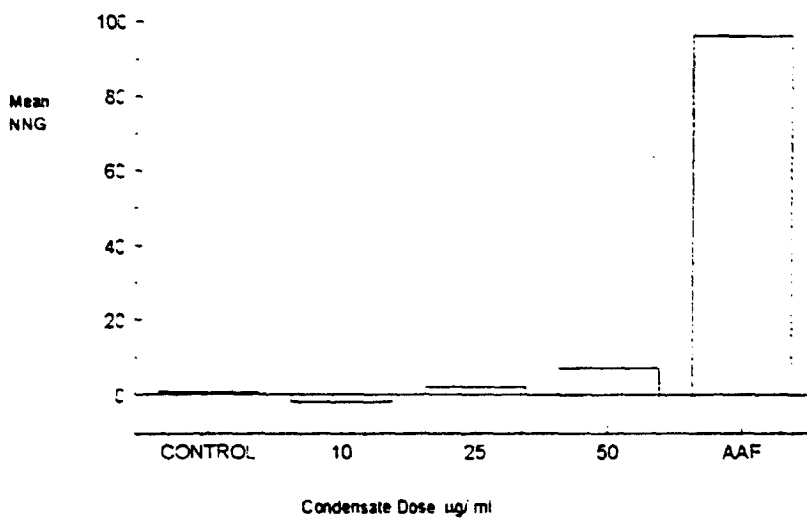


FIGURE 9 Mean NNG induced by exposure to cigarette smoke condensate Code 837



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<u>PMWNF Values</u>		<u>12-7-93</u>
[AMES RUN 4]		
<u>Cig. Code</u>	<u>PMWNF</u>	<u>mg/L</u>
93/0471	7.88	
93/0474	8.05	
93/0438	8.49	
93/0631	7.99	
93/0657	8.25	
93/0659	8.21	
93/0767	8.33	
93/0768	8.20	
E428	8.02	
90/1730	7.85	

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FIGURE 10 Mean NNG induced by exposure to cigarette smoke condensate Code 772.

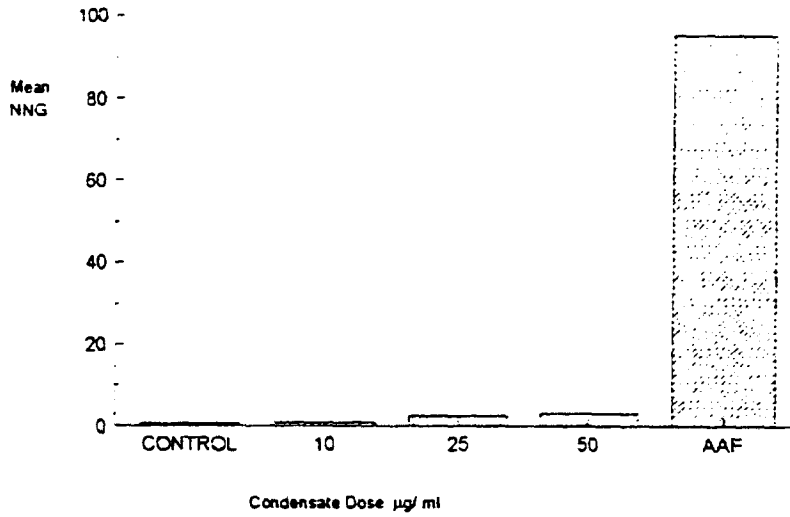
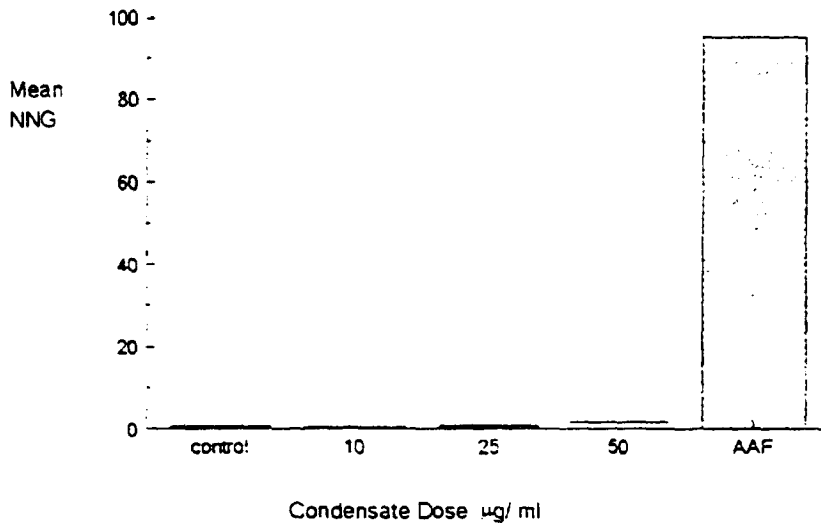


FIGURE 11 Mean NNG induced by exposure to cigarette smoke condensate Code 645.



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Card No.

1	7 88	1.97						
2	8 05	2.025						
3	8 49	2.123						
4	7 99	1.997						
5	8 25	2.063						
6	8 21	2.053						
7	8 33	2.083						
8	8 20	2.050						
9	8 02	2.005						
0	7 85	1.963						

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