

TABLE 20.

DIURNAL VARIATIONS IN PARTICULATE PAH* CONCENTRATIONS (NG/M³)
IN CONTRA COSTA AIR SAMPLES COLLECTED JANUARY 13 - 15, 1982

<u>Sampling Location</u>	<u>CHR</u>	<u>BAA</u>	<u>BBF + BEP</u>	<u>BKF</u>	<u>BAP</u>	<u>BGP</u>	<u>COR</u>	<u>BO + ISO</u>
Richmond								
Night	1.5	1.8	3.6	0.7	2.0	3.1	1.3	6.7
Day	0.6	0.6	1.5	0.4	0.8	2.9	1.4	4.4
Night	2.6	2.7	5.2	1.1	2.6	4.6	2.1	7.7
Martinez								
Night	1.0	0.7	0.7	0.1	0.4	0.5	0.2	4.3
Day	0.4	0.2	0.6	0.1	0.2	0.7	0.5	1.4
Night	1.2	1.2	3.0	0.6	1.5	2.1	0.8	5.7
Concord								
Night	2.6	2.7	4.6	1.0	2.6	3.3	1.3	11.9
Day	0.7	0.5	1.3	0.4	0.6	1.9	1.0	3.6
Night	2.3	2.6	4.5	0.8	2.6	3.6	1.3	9.5
Pittsburg								
Night	1.2	1.4	2.5	0.5	1.3	1.7	0.7	6.3
Day	0.6	0.5	1.0	0.2	0.5	1.5	0.8	2.1
Night	1.3	1.4	2.5	0.5	1.1	1.9	0.7	7.1

*Abbreviations used are as follows: CHR = chrysene, BAA = benz(a)anthracene, BBF + BEP = benzo(b)fluoranthene + benzo(e)pyrene, BKF = benzo(k)fluoranthene, BAP = benzo(a)pyrene, BGP = benzo(ghi)perylene, COR = coronene, BO + ISO = benzanthrone + isomers. Values listed as 0.1 are \leq 0.1.

episode when transport was W to E. The diurnal patterns of pollutant levels reflected the fact that during the August episode, the inversion base was lower by day than by night (thereby reducing the mixing volume over the sampling area) and auto emissions were higher by day. At all four sampling sites, daytime levels were higher than nighttime.

Less uniform diurnal patterns were observed during the October and January episodes. In October, different patterns emerged at different sampling sites (Cf Tables 16, and 19). At Richmond, daytime pollutant levels were again generally higher than those found at night. By contrast, at Concord nighttime levels of several key pollutant (mutagenicity, PAH and Pb) were higher than the daytime levels. This may reflect transport of pollutants from other parts of the region into Concord at night. Less clear-cut diurnal differences were observed at Pittsburg and Martinez.

During the January episode (Cf Table 17), mutagenicity and most other particulate pollutant concentrations were fairly constant from day to night. However this constancy did not extend to a number of organic species, which showed clear diurnal variations (Cf Table 20). Concentrations of BAP and several lower molecular weight PAH (e.g. CHR, BAA) were 3 to 4 times higher at night than during the day. Coronene, an automotive tracer PAH, was an exception and showed little diurnal variation. The diurnal patterns for the PAH were especially clear at Concord and Martinez. We can interpret this behavior in terms of the winter episode meteorology and speculate on a possible novel emission source. During the January episode, a moderately strong NE gradient was associated with a nighttime lowering of the inversion base and consequential pollutant build-up, followed by a clean-out during the day. Emissions from residential wood combustion, especially over residential sections near the Concord and Martinez stations may have contributed to the dramatic build-up of PAH concentrations at night.

Behavior of Air Particulate Extracts in a Nitroreductase Mutant

Information about the chemical nature of mutagens in air extracts may be

obtained by comparing the mutagenic responses in strain TA98 with those in strain TA98NR, the nitroreductase deficient mutant. Lower activities in TA98NR relative to TA98 would suggest the presence of reducible NO₂-organic mutagens such as nitroarenes in the extracts. As shown in Tables 21-23, most air particulate extracts from the three episodes were indeed less active in TA98NR. Samples collected in August were about four times less active while those collected in October and January were about half as active in the nitroreductase deficient mutant. These episode differences suggest that atmospheric conditions during the summer favor nitroarene mutagenic NO₂-organic formation. Station differences in the TA98NR/TA98 response ratio were also noted. Ratios were generally lowest at Pittsburg or Martinez and highest at Richmond. On the basis of these observations, chemical searches for nitroarenes might best be focused on aerosols collected in summer, especially at Pittsburg. Finally, no day vs night differences in the TA98NR/TA98 ratio were observed. If present, nitroarenes do not appear to be formed and degraded rapidly on a diurnal cycle such as seen for ozone.

B. Correlations Between Mutagenicity and Other Air Pollutants.

Relationships between mutagenic variables and other pollutants were explored further by means of correlation analysis. Spearman rank order correlation coefficients between mutagenic variables and other pollutant variables were calculated. The most informative correlations for mutagen source identification employed three mutagenic density variables (TA98 ± S9 and TA98NR-S9). Results are summarized in Tables 24-26.

In both the August and October episodes, mutagenicity was strongly correlated with pollutants derived predominantly from vehicles. Significant ($p < 0.05$) correlations of mutagenicity vs Lead, COR, and NO₂ were found, with correlation coefficients ranging from 0.73 to 0.95. During August NO₃⁻ was also well correlated with mutagenicity.

During October, CO, NO, BAP and ZINC were all correlated with at least one mutagenic variable. In general, fine fraction ($d < 2.5 \mu\text{m}$) lead was better

TABLE 21

MUTAGENIC DENSITY OF EXTRACTS OF HI-VOL SAMPLES
 COLLECTED AUGUST 6 - 7, 1981;
 COMPARISON WITH NITROREDUCTASE MUTANT

	Revertants* per m ³		
	<u>TA98</u>	<u>TA98NR</u>	<u>TA98NR/TA98</u>
Richmond			
Day	6.2	1.7	0.27
Night	0.8	0.2	0.25
Day	5.0	1.2	0.24
Martinez			
Day	5.6	1.5	0.27
Night	3.6	1.2	0.33
Day	6.2	1.3	0.21
Concord			
Day	12.1	2.7	0.22
Night	7.3	2.0	0.27
Day	11.7	2.4	0.21
Pittsburg			
Day	9.2	1.7	0.18
Night	6.9	1.3	0.19
Day	12.4	1.9	0.15
Controls			
(Revertants per µg)			
2-Nitrofluorene	414	34	0.08
Quercetin	7.1	6.1	0.86

*Direct-Acting (-S9)

TABLE 22

MUTAGENIC DENSITY OF EXTRACTS OF HI-VOL SAMPLES
 COLLECTED OCTOBER 20 - 22, 1981;
 COMPARISON WITH NITROREDUCTASE MUTANT

Revertants* per m³

	<u>TA98</u>	<u>TA98NR</u>	<u>TA98NR/TA98</u>
Richmond			
Night	1.0	1.1	1.10
Day	6.2	3.5	0.57
Night	2.7	2.5	0.92
Martinez			
Night	7.3	2.5	0.21
Day	4.3	2.6	0.28
Night	6.2	3.9	0.21
Concord			
Night	14.0	6.9	0.49
Day	7.3	3.9	0.53
Night	10.8	5.4	0.50
Pittsburg			
Night	8.9	3.9	0.44
Day	9.6	3.8	0.40
Night	10.0	3.1	0.31
Controls (Revertants per µg)			
2-Nitrofluorene	567	102	0.18
4-Nitroquinoline oxide (4-NQO)	1274	847	0.66

* Direct-Acting (-S9)

TABLE 23

MUTAGENIC DENSITY OF EXTRACTS OF HI-VOL SAMPLES
COLLECTED JANUARY 13 - 15, 1982;
COMPARISON WITH NITROREDUCTASE MUTANT

	Revertants* per m ³		
	<u>TA98</u>	<u>TA98NR</u>	<u>TA98NR/TA98</u>
Richmond			
Night	7.5	5.4	0.72
Day	7.6	3.6	0.47
Night	11.8	5.6	0.47
Martinez			
Night	7.1	2.9	0.41
Day	3.5	1.9	0.54
Night	7.3	4.4	0.60
Concord			
Night	6.2	4.5	0.74
Day	5.4	3.1	0.57
Night	5.7	3.0	0.53
Pittsburg			
Night	6.5	3.1	0.48
Day	5.9	1.5	0.25
Night	10.9	6.2	0.57
Controls (Revertants per µg)			
2-Nitrofluorene	635	88	0.14
Quercetin	2.3	2.2	0.96
4-Nitroquinoline oxide	745	584	0.78

*Direct-Acting (- S9)

TABLE 24

SPEARMAN RANK CORRELATIONS^a BETWEEN MUTAGENIC DENSITY (REV/M³)
AND SELECTED AIR POLLUTANTS^b FOR THE AUGUST 6-7, 1981 SAMPLING EPISODE

	<u>TA98+S9</u>	<u>TA98-S9</u>	<u>TA98NR-S9</u>
SO ₄	- 0.05 (0.87)	- 0.11 (0.73)	- 0.18 (0.58)
NO ₃	<u>0.83</u> (0.001)	<u>0.77</u> (0.003)	<u>0.82</u> (0.001)
BSO	0.32 (0.31)	0.12 (0.72)	0.13 (0.68)
BAP ^c	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
COR	<u>0.70</u> (0.01)	<u>0.82</u> (0.001)	<u>0.85</u> (0.001)
IRON	0.62 (0.03)	0.46 (0.13)	0.27 (0.39)
IRONF	<u>0.80</u> (0.002)	0.56 (0.06)	<u>0.58</u> (0.05)
NICKEL	0.29 (0.36)	0.07 (0.82)	0.09 (0.79)
NICKELF	0.29 (0.36)	0.04 (0.91)	0.11 (0.74)
ZINCF	0.47 (0.12)	0.45 (0.14)	0.51 (0.09)
DC LEAD	<u>0.92</u> (<u>< 0.001</u>)	<u>0.86</u> (<u>< 0.001</u>)	<u>0.92</u> (<u>< 0.001</u>)
LEADF	<u>0.94</u> (<u>< 0.001</u>)	<u>0.87</u> (<u>< 0.001</u>)	<u>0.95</u> (<u>< 0.001</u>)
IP	<u>0.64</u> (0.02)	0.44 (0.16)	0.38 (0.23)
O ₃	0.58 (0.10)	0.58 (0.10)	0.24 (0.53)
CO	0.07 (0.85)	0.24 (0.54)	- 0.10 (0.80)
NO	- 0.09 (0.81)	- 0.08 (0.83)	0.02 (0.96)
NO ₂	<u>0.88</u> (0.002)	<u>0.73</u> (0.02)	<u>0.84</u> (0.005)
SO ₂	0.46 (0.21)	<u>0.71</u> (0.03)	0.42 (0.26)

^aSignificant ($p \leq 0.05$) correlations are underlined (the significant level for each correlation coefficient is given in parenthesis).

^bSee text for abbreviations.

^cAll values \leq limit of quantitation (0.1 ng/m³).

TABLE 25

SPEARMAN RANK CORRELATIONS BETWEEN MUTAGENIC DENSITY (REV/M³)
AND SELECTED AIR POLLUTANTS* FOR THE OCTOBER 20-22, 1981 SAMPLING EPISODE

	<u>TA98+S9</u>	<u>TA98-S9</u>	<u>TA98NR-S9</u>
SO ₄	0.10 (0.78)	0.30 (0.37)	0.13 (0.70)
NO ₃	0.38 (0.25)	0.31 (0.21)	0.40 (0.22)
BSO	<u>0.66</u> (0.03)	<u>0.61</u> (0.05)	0.52 (0.10)
BAP	<u>0.64</u> (0.03)	<u>0.66</u> (0.02)	0.48 (0.11)
COR	<u>0.87</u> (<u>< 0.001</u>)	<u>0.83</u> (<u>< 0.001</u>)	<u>0.78</u> (0.003)
IRON	0.47 (0.15)	0.37 (0.26)	0.45 (0.17)
IRONF	<u>0.60</u> (0.05)	0.38 (0.24)	0.53 (0.09)
NICKEL	- 0.12 (0.72)	0.29 (0.38)	0.20 (0.55)
NICKELF	0.16 (0.64)	0.31 (0.35)	0.23 (0.50)
ZINCF	<u>0.71</u> (0.01)	<u>0.75</u> (0.01)	<u>0.60</u> (0.05)
DC LEAD	<u>0.93</u> (<u>< 0.001</u>)	<u>0.86</u> (<u>< 0.001</u>)	<u>0.76</u> (0.01)
LEADF	<u>0.94</u> (<u>< 0.001</u>)	<u>0.89</u> (<u>< 0.001</u>)	<u>0.75</u> (0.01)
IP	0.22 (0.51)	0.18 (0.60)	0.36 (0.27)
O ₃	- 0.66 (0.08)	- 0.62 (0.10)	- 0.54 (0.17)
CO	<u>0.76</u> (0.02)	<u>0.81</u> (0.01)	<u>0.79</u> (0.01)
NO	<u>0.90</u> (0.001)	<u>0.87</u> (0.003)	<u>0.80</u> (0.009)
NO ₂	<u>0.92</u> (0.001)	<u>0.74</u> (0.02)	<u>0.79</u> (0.01)
SO ₂	0.34 (0.37)	0.37 (0.33)	0.43 (0.25)

*See text for abbreviations. Significant ($p < 0.05$) correlations are underlined (the significant level for each correlation coefficient is given in parenthesis).

TABLE 26

SPEARMAN RANK CORRELATIONS BETWEEN MUTAGENIC DENSITY (REV/M³)
AND SELECTED AIR POLLUTANTS* FOR THE JANUARY 13-15, 1982 SAMPLING EPISODE

	<u>TA98+S9</u>	<u>TA98-S9</u>	<u>TA98NR-S9</u>
SO ₄	- 0.15 (0.65)	- 0.24 (0.44)	- 0.18 (0.57)
NO ₃	- <u>0.84</u> (0.001)	- 0.47 (0.12)	- 0.44 (0.15)
BSO	0.11 (0.73)	0.21 (0.51)	0.35 (0.27)
BAP	0.42 (0.17)	0.36 (0.25)	<u>0.63</u> (0.03)
COR	<u>0.64</u> (0.02)	0.34 (0.28)	0.46 (0.13)
IRON	<u>0.68</u> (0.04)	0.38 (0.31)	0.43 (0.24)
IRONF	<u>0.67</u> (0.05)	0.30 (0.43)	0.48 (0.19)
NICKEL	0.50 (0.17)	- 0.03 (0.93)	0.50 (0.17)
NICKELF	0.47 (0.20)	- 0.07 (0.86)	0.41 (0.27)
ZINCF	<u>0.87</u> (0.003)	<u>0.68</u> (0.04)	<u>0.77</u> (0.02)
DC LEAD	0.69 (0.06)	0.67 (0.07)	0.57 (0.14)
LEADF	<u>0.80</u> (0.01)	0.53 (0.14)	<u>0.67</u> (0.05)
IP	- 0.13 (0.75)	- 0.03 (0.95)	0.31 (0.42)
O ₃	- 0.04 (0.92)	- 0.32 (0.41)	- 0.54 (0.13)
CO	0.29 (0.44)	0.21 (0.59)	0.34 (0.38)
NO	0.22 (0.58)	0.08 (0.83)	0.18 (0.65)
NO ₂	0.63 (0.07)	0.33 (0.38)	0.42 (0.26)
SO ₂	- 0.17 (0.65)	- 0.50 (0.17)	- 0.04 (0.92)

*See text for abbreviations. Significant ($p \leq 0.05$) correlations are underlined (the significant level for each correlation coefficient is given in parenthesis).

correlated with mutagenicity than total inhalable ($d \leq 15 \mu\text{m}$) lead. This is consistent with the fact that most of the mutagenic activity is found in fine particulates. However mutagenic correlations with both lead variables were high. The strongest correlation between mutagenicity and lead occurred during the fall episode. As shown in Figure 14, mutagenic density (revertants/ m^3) as determined in TA98+S9 was linearly related to fine fraction lead over a ten-fold range of lead concentrations ($\leq 100\text{ng}/\text{m}^3$ - $800\text{ng}/\text{m}^3$), with a correlation coefficient of 0.98. In contrast to Pb, fine fraction Ni bore no apparent relationship to mutagenicity during the October sampling (Fig. 15). This implies that most mutagens sampled in August and October were derived from automobiles. Nevertheless possible contributions to mutagenicity from industrial sources during the August episode were also suggested by the significant correlation ($r=0.71$) between direct-acting (-S9) mutagenic density and SO_2 (Cf Table 24).

Mutagenic correlations with zinc were especially interesting. Significant positive correlations between fine fraction zinc and all three mutagenic variables were observed in fall and winter, but not in summer. Zinc is a complex source tracer which has been attributed to both mobile sources (tire and road dust) and stationary sources (incineration, manufacturing) (37). As noted above, a significant stationary source of Zn in Contra Costa County is the Pittsburg Works of U.S. Steel (36). In Los Angeles, Zn is emitted from 40 separate source classes (37). Thus we have avoided using zinc as a source-related predictor variable in multivariate analysis.

In contrast to the high correlations between mutagenicity and automobile pollutants observed in August and October, correlations in January were uniformly much lower for most variables of interest. Only ZINCF, LEADF, IRON, COR and BAP variables were significantly and positively correlated with mutagenic density. These correlations were not strong and lower values of LEADF and COR imply relatively weaker contributions from vehicular emissions in January than in August and October.

An interesting relationship between fine fraction lead and mutagenic density (+S9) for the three episodes was observed (Figure 16). Higher correlations

FIGURE 14

MUTA (TA98+S9) VS LEAD (F)

OCTOBER 1981 EPISODE

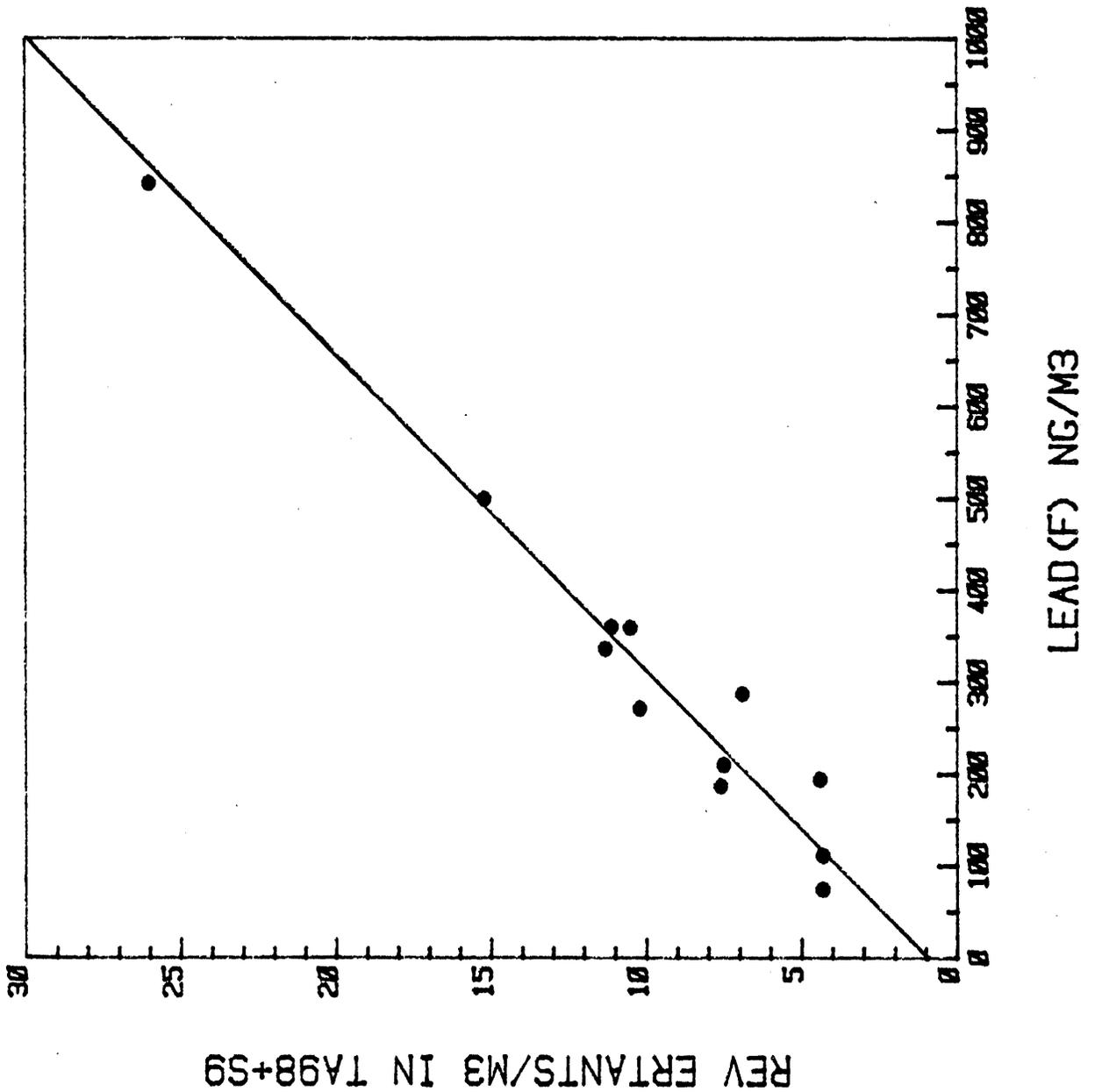


FIGURE 15
 MUTA (TA98 +S9) VS NICKEL (F)
 OCTOBER 1981 EPISODE

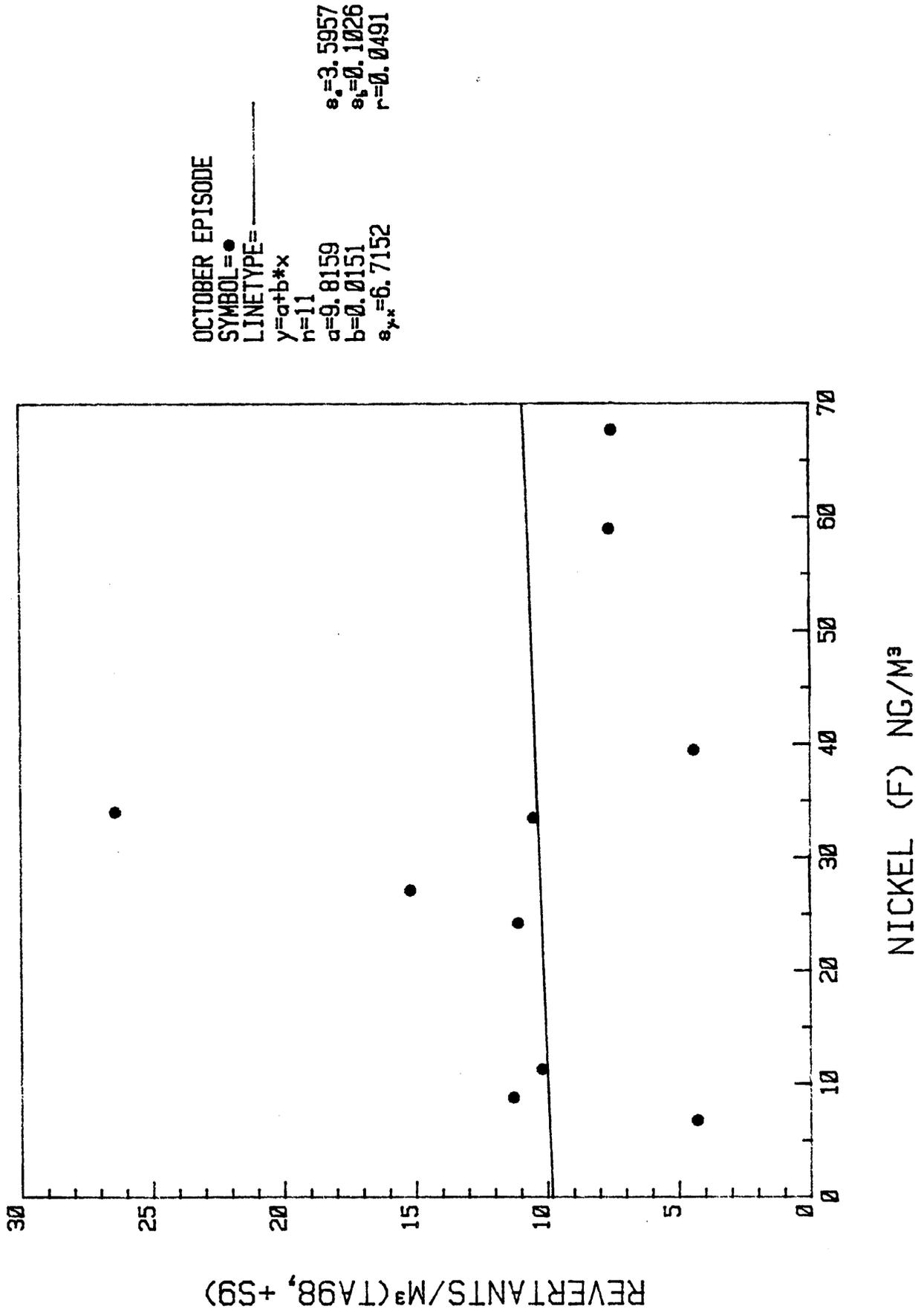
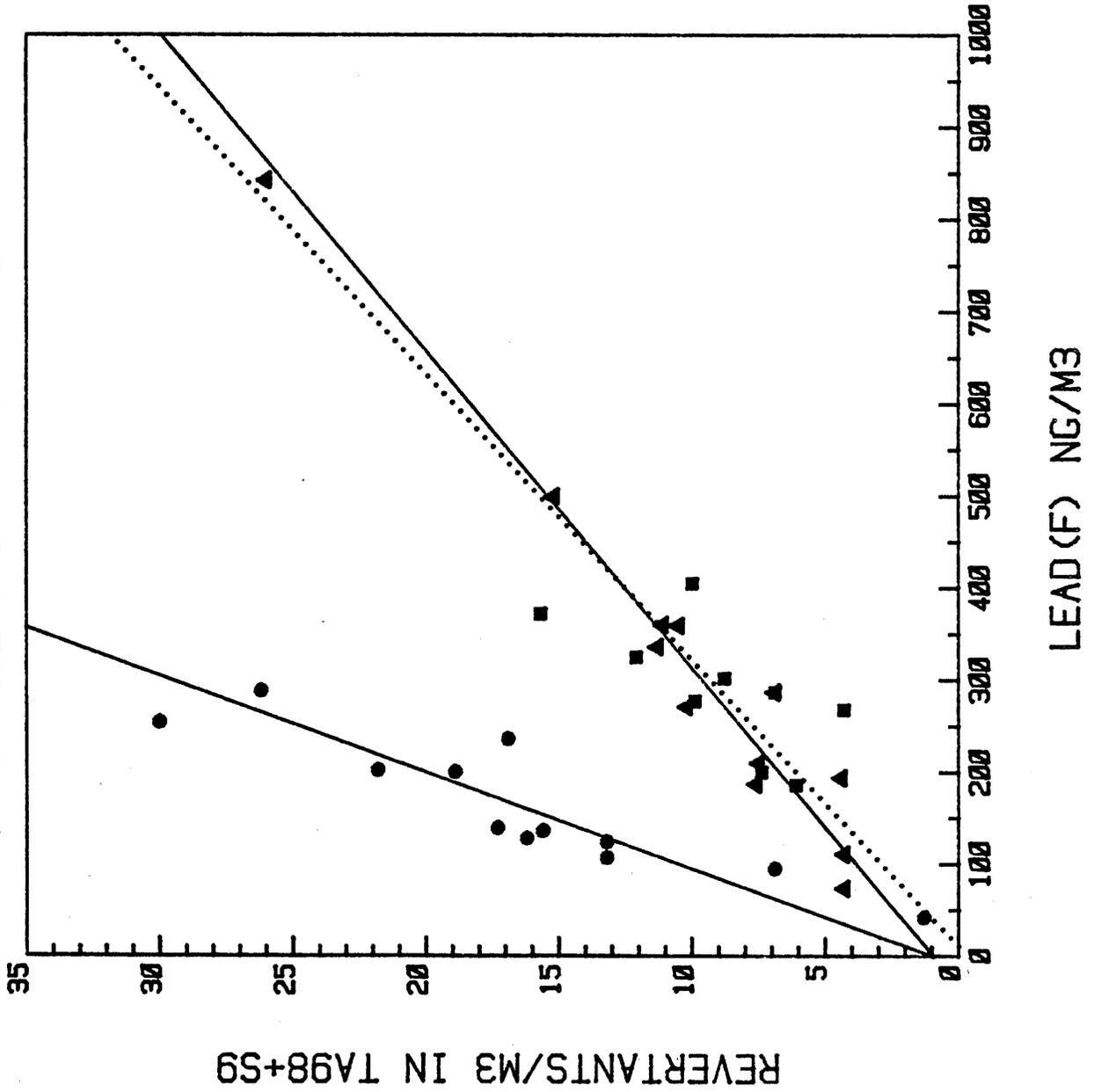


FIGURE 16

MUTAGENS (+S9) VS LEAD (F)
1981-1982 EPISODE COMPARISONS



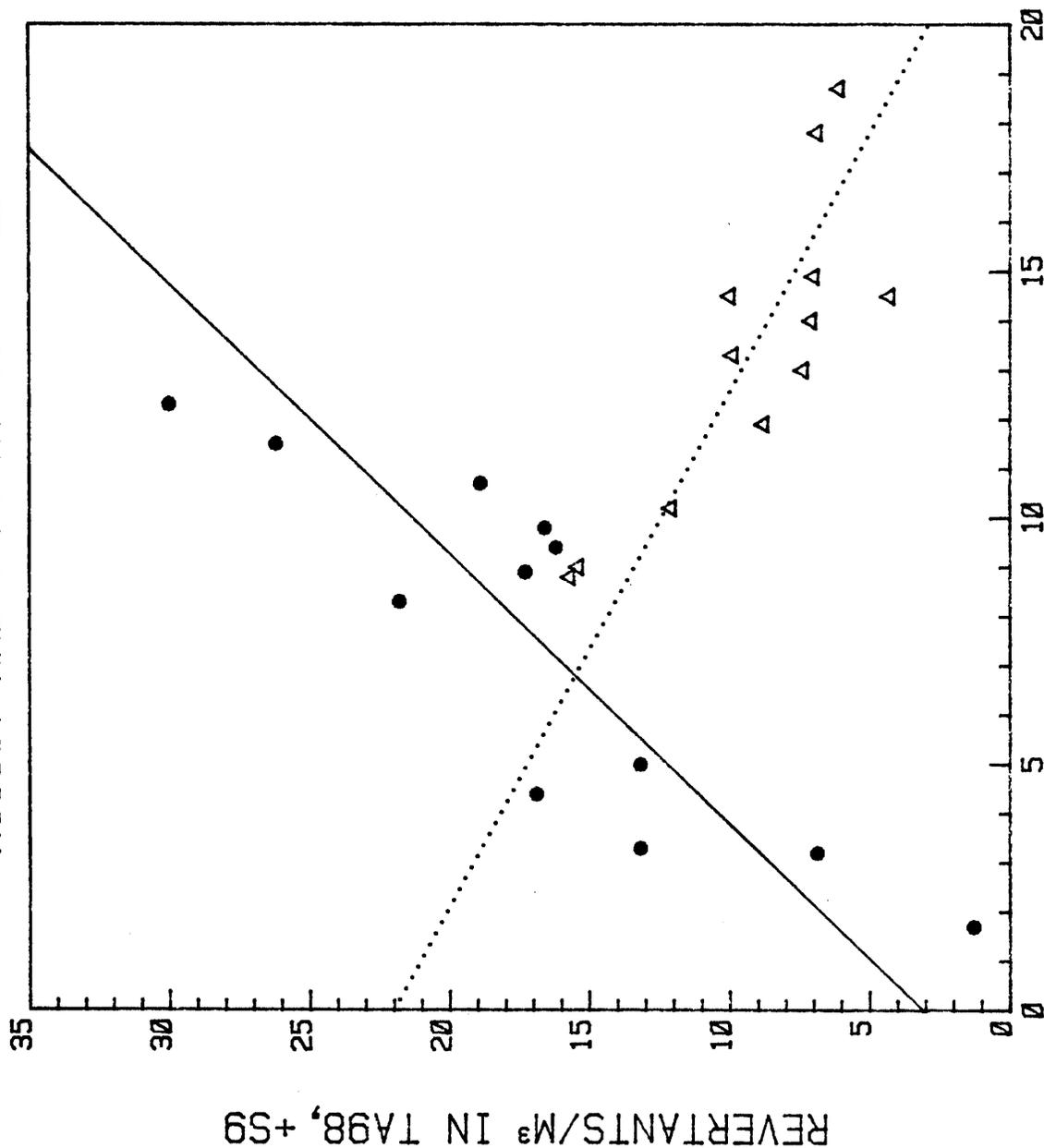
were found in August ($r=0.90$) and October ($r=0.98$) than in January ($r=0.67$). However, the slopes of the curves obtained in fall and winter (i.e., ca. 0.03 revertants/ng Pb) were virtually identical to each other while the slope in summer was three times higher (i.e. ca. 0.1 revertants/ng Pb). Thus automotive emissions unit weight of lead in August resulted in higher mutagenic densities than emissions in October or January.

The most dramatic change in the correlations between episodes involved NO_3^- (Figure 17). During August, mutagenic density (rev/m^3 in TA98 +S9) was positively and strongly correlated with NO_3^- ($r = 0.86$). During January, mutagenic density was again strongly correlated with NO_3^- ; however the correlation this time was negative with a correlation coefficient of -0.84 . Since particulate NO_3^- measurements were made on hi-vol filter samples and such measurements contain artifacts due to gas phase nitric acid (38), interpretation of these results is difficult.

The observation that the correlation between mutagenicity and nitrate is significantly positive in the summer and negative in the winter episode should be studied further. At present the following interpretation of these correlations can be provided (B. Appel, personal communication). Let us assume that the active mutagens are predominantly nitro-PAH, formed in the atmosphere or as artifacts on filters and that the rate of their formation is proportional to the HNO_3 concentration. If the concentration of HNO_3 is controlled by the equilibrium $\text{NH}_3 + \text{HNO}_3 \rightleftharpoons \text{NH}_4\text{NO}_3$, then conditions favoring high particle NO_3^- (i.e. low temperature, high relative humidity) will lead to low HNO_3 . Low HNO_3 in turn leads to low nitroPAH formation and low mutagenicity. Perhaps this is relevant to the episode data. In winter, the observed NO_3^- is approximately equal to the true particulate NO_3^- with little gas phase HNO_3 present. However during the summer, the observed NO_3^- equals the sum of the true particulate NO_3^- plus the gas phase HNO_3 . In summer, HNO_3 may account for half or more of the observed NO_3^- . Thus, if the observed NO_3^- is correlated with HNO_3 (as would probably be the case), then a positive correlation between observed NO_3^- and mutagenicity is expected.

FIGURE 17

MUTA (TA98 +S9) VS NO₃
AUGUST AND JANUARY COMPARED



AUGUST EPISODE DATA
 SYMBOL=●
 LINETYPE=——
 $y = a + b * x$
 $n = 12$
 $a = 3.0915$
 $b = 1.8237$
 $s_{y,x} = 4.1195$
 $s_e = 2.7797$
 $s_b = 0.3407$
 $r = 0.8610$

JANUARY EPISODE DATA
 SYMBOL=△
 LINETYPE=.....
 $y = a + b * x$
 $n = 12$
 $a = 21.9963$
 $b = -0.9543$
 $s_{y,x} = 2.1347$
 $s_e = 2.8469$
 $s_b = 0.2077$
 $r = -0.8238$

Finally, the relationship between mutagenic density and nitrates may depend to some extent on atmospheric conversion rates. The slower conversion of nitric oxide to nitrogen dioxide and aerosol nitrate during winter is revealed in the seasonal NO/NO_x and NO/NO_3 ratios and this may contribute to the seasonal variations.

Correlations (not shown) between mutagenic specific activity (revertants/mass) variables and other air pollutant variables were also calculated. In general, specific activity correlations were lower than but qualitatively similar to those found for the corresponding mutagenic density variables. Finally correlations between mutagenicity variables and other pollutants were made by station but these correlations were not readily interpretable. Apparently, the sources and meteorological conditions prevailing during each of the intensive sampling episodes were different enough from one another so as to obscure consistent patterns of correlation.

C. Source Identification During Three Pollution Episodes Using Factor Analysis - A First Approach.

Following Daisey and Kneip (35), factor analysis was used in the development of multiple regression models for mutagen source apportionment to help identify the principle types of emission sources and select the best set of sources tracers for entry into a multiple regression model. In this first approach, mutagenicity was considered to be the response variable (and therefore removed from the data sets used for factor analysis). Seven particulate pollutant variables were used for factor analysis: DCLEAD, NICKEL, IRON, $\text{SO}_4^{=}$, NO_3^- , BSO and PAH (cf. Table 2). Metal concentrations were obtained on dichotomous samples. The other pollutants were measured on hi-vol samples. In the present study, we defined PAH as the sum of the eight unsubstituted PAH and benzanthrone as measured by HPLC. This PAH variable represents no more than 0.1 percent of the total organic extractable mass of air particles.

Results of chemical analysis of these seven pollutants were sorted by episode, to create three separate data bases. In applying factor analysis, the Principle

Axis method was used initially. However factor patterns obtained by this method did not have key potential tracer variables (e.g. Pb and Ni) well resolved in separate factors. To improve resolution, the Principle Axis factor patterns were subjected to Varimax rotation. This produced factor patterns with better separation of tracer pollutants. A summary of the factor patterns for each of the episodes obtained by this method is shown in Tables 27-29. The most highly loaded variables in each factor are given for each factor, along with the loadings of each variable in parenthesis. In all three episodes, the seven variables were resolved into five factors which accounted for essentially all (>96%) of the variance in the data.

Episode I (August 6-7, 1981).

Table 27 shows the factor pattern obtained during the summer episode. The basic assumption is that each factor can be associated with some underlying structure in the data (i.e. source emissions and atmospheric processes in this instance). Factor No. 1 was strongly loaded with sulfate and also contained BSO. It explained 23 percent of the variance and can be related to sulfate-associated secondary particulates (35, 36). Primary vehicular particulate emissions were identified with Factor No. 2 because the latter was heavily loaded with PAH and DCLEAD (37). It accounted for 27 percent of the variance. A third factor contained NICKEL, associated with fuel-oil fly ash emissions, (37) and explained 17 percent of the total variance. Factor No. 4 contained IRON, explained another 17 percent of the variance and was associated with resuspended crustal material (soil) (37). The fifth factor contained NO_3^- associated secondary particulate (38), and it explained 13 percent of the variance. Thus in episode I, the factor associated with vehicular particulate (No. 2) accounted for 27 percent of the variance; The factor associated with industrial (refinery) particulates (No. 3) accounted for another 17 percent of the variance; the factor related to resuspended crustal matter (No. 4) accounted for 17 percent of the variance; and the two factors associated with secondary aerosols (No. 1 and No. 5) accounted for a total of 36 percent.

Table 27

POLLUTION FACTORS^a CONTRIBUTING TO THE VARIANCE IN THE CONCENTRATIONS OF SEVEN PARTICULATE POLLUTANTS: AUGUST 6 - 7, 1981 EPISODE

	FACTOR					
	1	2	3	4	5	
Most Significant Variables ^b (Factor Loadings)	SO ₄ (0.96) BSO (0.69)	PAH (0.91) DCLEAD (0.90)	NICKEL (0.91)	IRON (0.87)	NO ₃ (0.83)	
Percent of Total Variance Explained by Each Factor	23	27	17	17	13	Sum: 97
Source-Related Descriptors	Sulfate-associated secondary particulate	Vehicular primary particulate	Fuel-oil fly ash	Resuspended crustal material	Nitrate-associated secondary particulate	

^aInitial factor method: Principle Axis; Rotation method: Varimax

^bVariables with factor loadings > 0.5

Table 28

POLLUTION FACTORS^a CONTRIBUTING TO THE VARIANCE IN THE CONCENTRATIONS OF SEVEN PARTICULATE POLLUTANTS: OCTOBER 20 - 22, 1981 EPISODE

	FACTOR					Sum:
	1	2	3	4	5	
Most Significant Variables ^b (Factor Loadings)	PAH (0.98) DCLEAD (0.98) BSO (0.73)	NICKEL (0.91)	NO ₃ (0.89)	IRON (0.88)	SO ₄ (0.87)	
Percent of Total Variance Explained by Each Factor	36	16	15	15	14	96
Source - Related Descriptors	Vehicular primary particulate	Fuel-oil fly ash	Nitrate-associated secondary	Resuspended crustal material	Sulfate-associated secondary particulate	

^aInitial factor method: Principle Axis; Rotation method: Varimax

^bVariables with factor loadings ≥ 0.5

Table 29

POLLUTION FACTORS^a CONTRIBUTING TO THE VARIANCE IN THE CONCENTRATIONS OF SEVEN PARTICULATE POLLUTANTS: JANUARY 13 - 15, 1982 EPISODE

	FACTOR					Sum:
	1	2	3	4	5	
Most Significant Variables ^b (Factor Loadings)	IRON (0.95) DCLEAD (0.90)	BSO (0.88) PAH (0.87)	NO ₃ (0.99)	NICKEL (0.96)	SO ₄ (-0.83)	
Percent of Total Variance Explained by Each Factor	27	25	18	15	12	97
Source-Related Descriptors	Crustal material; vehicular primary particulate	Novel organic source	Nitrate-associated secondary	Fuel-oil fly ash	Not sulfate-associated secondary particulate	

^aInitial factor method: Principle Axis; Rotation method: Varimax

^bVariables with factor loadings ≥ 0.5

Episode II (October 21-22, 1981).

The factor pattern obtained for the fall episode (Table 28) was similar to that found in summer suggesting common source-types. Five factors were again resolved. Factor No. 1 contained DCLEAD and PAH as well as BSO and explained more than a third (36 percent of the variance). NICKEL was associated with Factor No. 2 which explained 16 percent of variance. A NO_3^- factor (No. 3) and an IRON factor (No. 4) each explained 15 percent and a SO_4^- factor (No. 5) explained 14 percent. Together these five factors explained 96 percent of the total variance. Thus in the autumn episode vehicle-associated pollution factor (No. 1) accounted for 36 percent while the industrial (refinery) pollution factor (No. 2) accounted for 16 percent of the total variance. The secondary aerosol factors (No. 3 and No. 5) accounted for 29 percent and the crustal factor for 15 percent of the variance. The factor patterns in summer and fall were therefore quite similar; the major difference involved BSO; during summer it was associated with the SO_4^- factor (No. 1) and during fall it was associated with the vehicular LEAD/PAH factor (No. 1).

Episode III (January 13-15, 1982).

The factor pattern obtained in the winter episode (Table 29) was qualitatively different from those seen in summer and fall, perhaps indicating seasonal changes in emission sources and atmospheric transformations. IRON (the crustal-tracer) and LEAD (the primary vehicular tracer) were unresolved and found together in factor No. 1 which accounted for 27 percent of the variance. Organics (both PAH and BSO) were associated with factor No. 2 which contained none of the three elemental tracers (LEAD, NICKEL, IRON) or two secondary pollutant tracers (SO_4^- , NO_3^-). The novel organic pollution factor explained one quarter of the variance. It may reflect contributions from residential wood combustion during the winter. A third factor contained NO_3^- and explained 18 percent of the variance and a fourth contained NICKEL and explained another 15 percent of the variance. The fifth factor, which explained 12 percent of the variance, was strongly but negatively associated with SO_4^- . It is difficult to provide a ready explanation

of this negative association in terms of sources. Negative factor loading by sulfate may result because of slow conversion of sulfur dioxide to sulfate during winter time conditions.

Factor Patterns by Station

Factor patterns were also obtained for each of the four stations. Results are summarized in Tables 30-33. Attempts to interpret these factor patterns were complicated by differences in source emission patterns and atmospheric conditions prevailing during the three sampling episodes. Automotive, industrial and crustal source tracers were not well resolved. However, the novel organic factor which appeared in the winter episode was also observed at Concord (Table 32, Factor No. 5) and Martinez (Table 33, Factor No. 4). These two sampling stations are the most likely to be subject to emissions from residential wood combustion. The novel organic factor was not present at Pittsburg or Richmond.

D. Multivariate Correlations Between Mutagenicity and Chemical Pollutants Using Factor Analysis - A Second Approach.

In the first approach described above, mutagenicity test results were used as response variables (and removed from the data sets used for factor analysis). In a second approach, mutagenicity results were included in the data base with the other particulate pollutants. If factor patterns show consistent correlations between mutagenicity and pollutant variables, some additional information about sources may be gained. The factor patterns for each episode using this second approach are summarized in Tables 34-39. In general, the patterns obtained including mutagenicity data were similar to those obtained when mutagenicity data were excluded. The differences are discussed below. Mutagenic density responses in TA98 with and without S9 were compared separately with the seven particulate pollutants (DCLEAD, NICKEL, IRON, SO_4^- , NO_3^- , BSO, PAH).

During the August episode, mutagenicity (both indirect and direct-acting) was associated with a single factor (No. 1) which also contained DCLEAD,

Table 30

POLLUTION FACTORS^a CONTRIBUTING TO THE VARIANCE IN THE CONCENTRATIONS OF SEVEN PARTICULATE POLLUTANTS: PITTSBURG STATION EPISODE DATA

	FACTOR				Sum:
	1	2	3	4	
Most Significant Variables ^b (Factor Loadings)	PAH (0.98) DCLEAD (0.93) NO ₃ (0.90) BSO (0.86) SO ₄ (0.53)	NICKEL (0.99)	IRON (-0.98)	SO ₄ (0.72)	99
Percent of Total Variance Explained by Each Factor	53	16	19	11	
Source-Related Descriptors	Vehicular, nitrate and sulfate-associated secondary particulate; organics	Fuel-oil fly ash	Not crustal material	Sulfate-associated secondary particulate	

^aInitial factor method: Principle Axis; Rotation method: Varimax

^bVariables with factor loadings ≥ 0.5

Table 31

POLLUTION FACTORS^a CONTRIBUTING TO THE VARIANCE IN THE CONCENTRATIONS OF SEVEN PARTICULATE POLLUTANTS: RICHMOND STATION EPISODE DATA

	FACTOR				Sum:
	1	2	3	4	
Most Significant Variables ^b (Factor Loadings)	PAH (0.93) NICKEL (0.88) BSO (0.66) DCLEAD (0.58)	SO ₄ (0.89)	NO ₃ (0.87)	IRON (0.86) DCLEAD (0.52)	
Percent of Total Variance Explained by Each Factor	37	18	21	19	95
Source - Related Descriptors	Fuel-oil fly ash; vehicular primary particulate	Sulfate-associate secondary particulate	Nitrate-associated secondary particulate	Crustal material; vehicular primary particulate	

^aInitial factor method: Principle Axis; Rotation method: Varimax

^bVariables with factor loadings > 0.5

Table 32

POLLUTION FACTORS^a CONTRIBUTING TO THE VARIANCE IN THE CONCENTRATIONS OF SEVEN PARTICULATE POLLUTANTS: CONCORD STATION EPISODE DATA

	FACTOR				
	1	2	3	4	5
Most Significant Variables ^b (Factor Loadings)	NICKEL (0.95) IRON (0.61)	DCLEAD (0.95)	NO ₃ (0.99)	SO ₄ (0.94)	PAH (0.91) BSO (0.65)
Percent of Total Variance Explained by Each Factor	22	18	17	15	24
Source - Related Descriptors	Fuel-oil fly ash; crustal material	Vehicular primary particulate	Nitrate-associated secondary particulate	Sulfate-associated secondary particulate	Novel organic source
					Sum: 96

^aInitial factor method: Principle Axis; Rotation method: Varimax

^bVariables with factor loadings ≥ 0.5

Table 33

POLLUTION FACTORS^a CONTRIBUTING TO THE VARIANCE IN THE CONCENTRATIONS OF SEVEN PARTICULATE POLLUTANTS: MARTINEZ STATION EPISODE DATA

	FACTOR				Sum:
	1	2	3	4	
Most Significant Variables ^b (Factor Loadings)	DCLEAD (0.95) NO ₃ (0.91) SO ₄ (0.65) BSO (0.61)	NICKEL (0.97) SULFATE (0.57)	IRON (0.96)	PAH (0.88) BSO (0.67)	96
Percent of Total Variance Explained by Each Factor	37	22	17	20	
Source - Related Descriptors	Vehicular primary, nitrate-and sulfate-associated secondary aerosol	Fuel-oil fly ash; sulfate associated secondary particulate	Crustal material	Novel organic source	

^aInitial factor method: Principle Axis; Rotation method: Varimax

^bVariables with factor loadings ≥ 0.05

TABLE 34

FACTOR ANALYSIS^a SHOWING ASSOCIATIONS BETWEEN TOTAL INDIRECT MUTAGENIC DENSITY (REV/M³ IN TA98+S9) AND SOURCE-RELATED AIR POLLUTANTS:

August 6-7, 1981 Episode

	FACTOR				
	1	2	3	4	5
Most Significant Pollutant Variable (Factor Loadings)	DC Lead (0.96) PAH (0.88) NO ₃ (0.63)	SO ₄ (0.96) BSO (0.71)	Nickel (0.90)	Iron (0.86)	NO ₃ (0.73)
Percent of Total Variance Explained by Each Factor	36	21	16	15	9
Source-Related Descriptors	Vehicular primary & nitrate-associated secondary particulate	Sulfate-associated secondary particulate	Fuel-oil fly ash	Resuspended crustal material	Nitrate-associated secondary particulate
Mutagenicity ^c Factor Loadings	0.88	- 0.10	0.20	0.16	0.35
					SUM:

a. Initial factor method: Principle axis; Rotation method: Varimax.
 b. Variables with factor loadings > 0.5.
 c. Mutagenic variable: (Total mutagenic density) Revertants/M³ in TA98+S9.

TABLE 35

FACTOR ANALYSIS^a SHOWING ASSOCIATIONS BETWEEN DIRECT-ACTING
MUTAGENS AND SOURCE-RELATED AIR POLLUTANTS:

August 6-7, 1981 Episode

	FACTOR				
	1	2	3	4	5
Most Significant Pollutant Variable ^b (Factor Loadings)	DC Lead (0.97) PAH (0.83) NO ₃ (0.65)	SO ₄ (0.96) BSO (0.70)	Nickel (0.91)	Iron (0.87) BSO (0.50)	NO ₃ (0.70)
Percent of Total Variance Explained by Each Factor	37	21	16	15	8
Source-Related Descriptors	Vehicular primary & nitrate associated secondary particulate	Sulfate- associated secondary particulate	Fuel-oil fly ash	Crustal material	Nitrate-associated secondary particulate
Mutagenicity ^c Factor Loadings	<u>0.93</u>	- 0.07	0.07	0.10	0.26
					SUM:
					97

a. Initial factor method: Principle axis; Rotation method: Varimax.
b. Variables with factor loadings > 0.5. Factor loadings are in parenthesis.
c. Mutagenic variable: (Direct-acting mutagenic density) Revertants/M³ in TA98-S9.

TABLE 36

FACTOR ANALYSIS^a SHOWING ASSOCIATIONS BETWEEN TOTAL INDIRECT MUTAGENIC DENSITY (REV/M³ IN TA98+S9) AND SOURCE-RELATED AIR POLLUTANTS:

October 20-22, 1981 Episode

FACTOR

	1	2	3	4	5	
Most Significant Pollutant Variable ^b (Factor Loadings)	PAH (0.97) DC Lead (0.97) BSO (0.70)	Nickel (0.91)	Iron (0.88)	NO ₃ (0.89)	SO ₄ (0.86)	
Percent of Total Variance Explained by Each Factor	43	15	14	13	12	SUM: 97
Source-Related Descriptors	Vehicular primary particulate	Fuel-oil fly ash	Resuspended crustal material	Nitrate-associated secondary particulate	Sulfate-associated secondary	
Mutagenicity ^c Factor Loadings	<u>0.99</u>	- 0.03	0.08	0.03	0.05	

a. Initial factor method: Principle axis; Rotation method: Varimax.

b. Variables with factor loadings > 0.5.

c. Mutagenic variable: (Total mutagenic density) Revertants/M³ in TA98+S9.

TABLE 37

FACTOR ANALYSIS^a SHOWING ASSOCIATIONS BETWEEN DIRECT-ACTING
MUTAGENS AND SOURCE-RELATED AIR POLLUTANTS:

October 20-22, 1981 Episode

	FACTOR				
	1	2	3	4	5
Most Significant Pollutant Variable ^b (Factor Loadings)	PAH (0.98) DC Lead (0.97) BSO (0.73)	Nickel (0.93)	Iron (0.89)	NO ₃ (0.87)	SO ₄ (0.84)
Percent of Total Variance Explained by Each Factor	37	16	14	15	13
Source-Related Descriptors	Vehicular primary particulate	Fuel-oil fly ash	Crustal material	Nitrate- associated secondary particulate	Sulfate- associated secondary particulate
Mutagenicity ^c Factor Loadings	<u>0.69</u>	0.03	0.10	0.44	0.42
					SUM:

a. Initial factor method: Principle axis; Rotation method: Varimax.

b. Variables with factor loadings ≥ 0.5 .

c. Mutagenic variable: (Direct-acting mutagenic density) Revertants/M³ in TA98-S9.

TABLE 38

FACTOR ANALYSIS^a SHOWING ASSOCIATIONS BETWEEN TOTAL INDIRECT MUTAGENIC DENSITY (REV/M³ IN TA98+S9) AND SOURCE-RELATED AIR POLLUTANTS:

January 13-15, 1982 Episode

	FACTOR					
	1	2	3	4	5	SUM:
Most Significant Pollutant Variable ^b (Factor Loadings)	Iron (0.94) DC Lead (0.89)	BSO (0.91) PAH (0.84)	NO ₃ (- 0.98)	Nickel (0.96)	SO ₄ (- 0.82)	
Percent of Total Variance Explained by Each Factor	25	23	24	15	10	97
Source-Related Descriptors	Crustal material; vehicular primary particulate	Novel organic source	Not nitrate-associated secondary particulate	Fuel-oil fly ash	Not sulfate-associated secondary particulate	
Mutagenicity ^c Factor Loadings	0.38	0.11	<u>0.82</u>	0.29	0.05	

a. Initial factor method: Principle axis; Rotation method: Varimax.

b. Variables with factor loadings ≥ 0.5 .

c. Mutagenic variable: (Total mutagenic density) Revertants/M³ in TA98+S9.

TABLE 39

FACTOR ANALYSIS^a SHOWING ASSOCIATIONS BETWEEN DIRECT-ACTING
MUTAGENS AND SOURCE-RELATED AIR POLLUTANTS:

January 13-15, 1982 Episode

	FACTOR						
	1	2	3	4	5	6	
Most Significant Pollutant Variable ^b (Factor Loadings)	Iron (0.92) DC Lead (0.92)	BSO (0.89) PAH (0.82)	NO ₃ (0.96)	Nickel (0.96)	None	SO ₄ (-0.82)	
Percent of Total Variance Explained by Each Factor	25	23	17	14	10	10	99
Source-Related Descriptors	Crustal material; vehicular primary particulate	Novel organic source	Nitrate- associated secondary particulate	Fuel-oil fly ash	Unknown	Not sulfate- associated secondary particulate	
Mutagenicity ^c Factor Loadings	0.26	0.28	- 0.45	0.15	<u>0.79</u>	- 0.00	

a. Initial factor method: Principle axis; Rotation method: Varimax.

b. Variables with factor loadings > 0.5.

c. Mutagenic variable: (Direct-acting mutagenic density) Revertants/M³ in TA98-S9.

NO_3^- , and PAH (Tables 34 and 35). In October, both mutagenicity variables were associated primarily with a factor containing DCLEAD, PAH AND BSO (Factor No. 1, Tables 36 and 37). The association was especially strong for total indirect mutagenicity. This implies that particulate mutagens and PAH were derived primarily from vehicles.

In contrast to the August and October patterns, the January episode pattern was more difficult to interpret (Tables 38 and 39). Total indirect mutagenic density (revertants/ m^3 in TA98+S9) was associated primarily with a factor (Table 38, No. 3) containing a strong negative NO_3^- factor loading. This result is consistent with the negative Spearman rank correlation between this mutagenic variable and NO_3^- ($r=-0.84$) calculated above (Cf Table 26, Figure 17). Direct-acting mutagenic density (revertants/ m^3 in TA98-S9) was not associated with any of the seven chemical pollutant variables used for factor analysis (Table 39, No.5). This suggests that direct-acting mutagens were derived from unrecognized emissions and/or atmospheric transformations in January.

A number of general comments on the results of factor analysis which apply to the analysis both without and with the inclusion of the mutagenicity test results can be made.

- (a) Nickel is always isolated in one factor.
- (b) Lead (dichot fine plus coarse fraction) is associated with PAH and BSO during summer and fall. During winter, lead is associated with iron. The moderately strong northeasterly gradient which characterized meteorological transport conditions in the winter episode may have contributed to the association of lead and iron. This is because the northeasterly gradient carries resuspended surface material (as well as other pollutants) from the Sacramento Valley delta into the Contra Costa region.
- (c) Sulfate is associated with BSO during the summer and is isolated in fall and winter, with a positive loading in fall and a negative loading

in winter. The rate of secondary sulfate formation is strongly dependent on humidity and temperature. The northeasterly gradient during the winter and the different rates of aerosol formation may contribute to the seasonal variability in the factor analysis.

- (d) The factor analysis indicates the mutagenic density (plus S9) is positively associated with nitrate during summer, weakly associated with nitrate during fall, and negatively associated with nitrate during the winter. The rate of secondary nitrate formation in the atmosphere is dependant on temperature and ozone concentrations, which are high in summer and low in winter. This may contribute to the seasonal variability of the nitrate factor.

During the summer and fall episodes, the factor patterns for the seven chemical pollutants were very similar whether or not the mutagenicity test results were present in the data base used for statistical analysis. However in the winter episode, the factor patterns with and without mutagenicity data were different. With respect to Total Indirect mutagenicity (+S9), the patterns with and without mutagenic data (Tables 29 and 38) are qualitatively the same except that the nitrate factor loading is positive without the mutagenicity and negative with the mutagenicity results present. Furthermore, with mutagenicity results present, mutagenic density is strongly loaded in the same factor which contains the negatively loaded nitrate (No. 3, Table 4). Some differences also exist between the factor patterns obtained with and without direct-acting mutagenicity (-S9) present in the data base. The salient difference between the two patterns (Tables 29 and 39) is that the mutagenicity variable is loaded most heavily into a new factor (No. 5, Table 39) which is not heavily loaded with any of the seven chemical pollutants. This (partial) isolation of mutagenicity in a separate factor suggests the existence of hither-to unknown sources of particulate mutagens in Contra Costa community air during winter inversions when transport is from the northeast.

E. Mutagen and PAH Source Apportionment by Multivariate Linear Regression.

The results of factor analysis suggested that the three metals (Pb, Ni and Fe) and two secondary pollutants ($\text{SO}_4^{=}$ and NO_3^-) were likely variables to use as source tracers for entry into multiple regression models. Since mutagens and carcinogen-containing PAH are both found predominantly on particles collected in the fine fraction ($d \leq 2.5 \text{ } \mu\text{m}$), fine-fraction lead (LEADF) and nickel (NICKELF) concentrations were used. (Note that fine fraction lead and nickel were used in the regression analysis and total fine plus coarse lead and nickel concentrations used in the factor analysis.) The fine fraction was used in the regression analysis because mutagens are known to be on small particles. The fine-fraction contains most of the lead and nickel. In contrast, most of the iron is on particles collected in the coarse fraction (2.5 - 15 μm). Thus for iron, the total fine plus coarse concentrations were employed in the multiple regression model.

Two mutagenic density variables (revertants/ m^3 in TA98 \pm S9) and PAH were used as response variables and an attempt was made to apportion mutagens and PAH using data from the three pollution episodes in a step-wise multiple regression model. This modeling yielded statistical significant and physically meaningful conclusions for both the summer and fall episodes but not for the winter episode. In winter, the residual terms in the regression model were as large or larger than the responsible variables themselves and thus no useful information about sources could be gained.

The results of multiple regression modeling of August and October are presented in the Tables 40 and 41. The linear equations obtained for both mutagenicity variables and PAH were highly significant ($p \leq 0.003$) with r^2 values ranging from 0.76 to 0.98. The step-wise technique of multiple regression used entered the most significant variable first, the next most significant variable second, etc. and only entered variables which met the 0.15 significance level into the model. For comparison with the step-wise technique, multiple regression was also carried out using as non-stepwise general linear model (GLM) which has no significance criterion for entry of variables. Results (not shown) obtained with the GLM were in good agreement

TABLE 40

MODELING OF PARTICULATE MUTAGENICITY AND PAH USING
STEPWISE MULTIPLE REGRESSION:

August 6-7, 1981 Episode

RESPONSE VARIABLES ^a	PREDICTOR VARIABLES ^b	
Total Indirect Mutagenicity (TA98 + S9)	$= (0.063 \pm 0.014)[LEADF] + (0.93 \pm 0.29)[NO_3] - 0.68$	(1)
	$n = 12, (p < 0.0001)$	
	$r^2 = 0.91$	
Direct-Acting Mutagenicity (TA98-S9)	$= (0.031 \pm 0.008)[LEADF] + (0.36 \pm 0.16)^c[NO_3] - 0.44$	(2)
	$n = 12, (p < 0.0001)$	
	$r^2 = 0.87$	
PAH	$= (0.0087 \pm 0.0019)[LEADF] - (0.032 \pm 0.010)[NICKELF]$ $+ (0.052 \pm 0.030)^c[SO_4] + 1.1$	(3)
	$n = 12, (p < 0.008)$	
	$r^2 = 0.76$	

^aConcentrations of mutagenicity are in revertants/m³, PAH in ng/m³.

^bThese variables met the 0.15 significance level for entry into the stepwise regression model; concentrations of LEADF and NICKELF are in ng/m³, of NO₃ and SO₄ in µg/m³.

^cRegression coefficient not statistically significant at the $p < 0.05$ level.

TABLE 41

MODELING OF PARTICULATE MUTAGENICITY AND PAH USING
STEPWISE MULTIPLE REGRESSION:

October 20-22 1981 Episode

RESPONSE VARIABLES ^a	PREDICTOR _b VARIABLES	
Total Indirect Mutagenicity (TA98 + S9)	$= (0.031 \pm 0.002)[LEADF] - (0.0021 \pm 0.0008)[IRON] + 2.2$	(4)
	$n = 11, (p < 0.0001)$	
	$r^2 = 0.98$	
Direct-Acting Mutagenicity (TA98-S9)	$= (0.010 \pm 0.002)[LEADF] + (0.19 \pm 0.07)[SO_4] - 0.1$	(5)
	$n = 11, (p < 0.0003)$	
	$r^2 = 0.77$	
PAH	$= (0.020 \pm 0.001)[LEADF] + (0.035 \pm 0.014)[NICKEL] - (0.24 \pm 0.06)[NO_3] + 1.0$	(6)
	$n = 11, (p < 0.0001)$	
	$r^2 = 0.98$	

^aConcentrations of mutagenicity are in revertants/m³, PAH in ng/m³.

^bThese variables met the 0.15 significance level for entry into the stepwise regression model; concentrations of LEADF and NICKEL are in ng/m³, of NO₃ and SO₄ in µg/m³.

with those obtained by the step-wise method.

During the August episode, particles associated with fine fraction lead and NO_3^- made positive contributions to both mutagenicity variables (Table 40, equations 1 + 2). The significant predictor variables for PAH were LEADF, NICKELF and $\text{SO}_4^{=}$ (Table 40, equation 3). However, the latter two variables, made approximately equal but opposite contributions. Nickel is a primary pollutant and maximum concentrations occur near the source, while aerosol sulfate is a secondary pollutant and maximum concentrations occur downwind. The association of aerosol sulfate with PAH at downwind receptor sites (i.e. the sampling sites) may reflect contributions from upwind sources of PAH.

During the October episode, fine particle lead was again a good predictor of both mutagenicity and PAH (Table 41, equations 4-6). IRON made an apparently negative contribution to Total Indirect Mutagenicity (equation 4). $\text{SO}_4^{=}$ made a positive contribution to Direct-Acting Mutagenicity (equation 5). In addition to LEADF, NICKELF made a positive contributions and NO_3^- a negative contribution to PAH (equation 6).

The regression equations were evaluated using the average concentrations of the predictor variables. Tracers assumed to be derived from various source-types were used. LEADF and NICKELF were used as primary vehicular and industrial tracers respectively and IRON as a tracer of resuspended crustal material. NO_3^- and $\text{SO}_4^{=}$ were used as secondary aerosol tracers. Contributions of these source-types to mutagenicity and PAH during the August and October episodes are presented in Table 42. The results were as follows: In summer, primary vehicular emissions (LEADF) contributed about two thirds of the mutagenicity while secondary NO_3^- associated emissions contributed the remaining third (equations 1 and 2). More than half (61%) of PAH was also derived from primary vehicular emissions (LEADF). Primary industrial emissions (NICKELF) had an apparent negative effect on PAH; $\text{SO}_4^{=}$ associated aerosols made a positive contribution to PAH; and half (48%) of the PAH was associated with residual (unexplained) sources (equation 3). In the fall episode, nearly all (95%) of the Total Indirect Mutagenicity was attributable to primary vehicular emissions (LEADF)

TABLE 42

CALCULATED SOURCE CONTRIBUTIONS TO PARTICULATE MUTAGENICITY AND
POM: COMPARISONS OF AUGUST AND OCTOBER 1981 EPISODESSOURCE CONTRIBUTIONS^a

Equation No.	Episode	Response Variable	SOURCE CONTRIBUTIONS ^a					
			Vehicular Primary (LEADF)	Industrial Primary (NICKELF)	Nitrate Associated Secondary (NO ₃)	Sulfate Associated Secondary (SO ₄)	Soil-Like (IRON)	Residual
(1)	August	Total Indirect Mutagenicity (+S9)	10.2 (62)	--	6.9 (42)	--	--	-0.7 (-4)
(2)		Direct-Acting Mutagenicity (S-9)	5.0 (69)	--	2.7 (37)	--	--	-0.4 (-6)
(3)		PAH	1.4 (58)	-0.8 (-33)	--	0.6 (25)	--	1.1 (48)
(4)	October	Total Indirect Mutagenicity (+S9)	9.7 (95)	--	--	--	-1.7 (-16)	2.2 (22)
(5)		Direct Acting Mutagenicity (-S9)	3.1 (48)	--	--	3.5 (54)	--	-0.1 (-2)
(6)		PAH	6.3 (129)	1.0 (20)	-3.4 (-69)	--	--	1.0 (20)

^a Concentrations are in revertants/m³ for mutagenicity and ng/m³ for PAH; the contribution of each source was calculated using the regression coefficients in equations 1-6 and average concentrations of the predictor variable; percentage contributions are given in parenthesis; dashes indicate no contribution using the stepwise regression technique.

(equation 4). Direct-acting mutagenicity was derived approximately equally from primary vehicular emissions (LEADF = 48%) and secondary sulfate-associated sources (SO_4^- = 54%) (equation 5). Finally, PAH was derived from vehicular emissions (LEADF = 129%), primary industrial emissions (NICKELF = 20%) and other residual sources (unknown = 20%). Nitrate-associated secondary aerosols made a negative contribution (-69%) to PAH. Little significance should be attached to sources which contribute $\leq 20\%$, because of the small size of the data base.

An overall summary of estimated source contributions to ambient particulate mutagenicity and PAH during the August and October episode is presented in Table 43. The results suggest the following interpretation: During the August episode, transportation-related pollutants (LEADF) accounted for sixty to seventy percent of the mutagenicity (both indirect and direct) and sixty percent of the PAH. Unknown sources accounted for the remaining PAH. During the October episode, transportation sources accounted for essentially all of the indirect and half of the direct-acting mutagenicity, as well as more than half of the PAH. Secondary SO_4^- associated sources (SO_4^-) accounted for about half of the direct-acting mutagenicity and primary industrial emissions (NICKELF) gave one-fifth of the PAH. The sources of mutagens during the August and October episodes are summarized graphically in Figure 18.

Finally, the possible role of secondary atmospheric transformations in the formation of mutagenic aerosols is suggested by the contributions to mutagenicity of nitrate-associated particles in summer and sulfate-associated particles in fall.

TABLE 43

SUMMARY ESTIMATES OF SOURCE CONTRIBUTIONS
TO AMBIENT PARTICULATE MUTAGENICITY AND PAH^a

SOURCE	TRACERS	CONTRIBUTIONS BY EPISODE					
		AUGUST 1981			OCTOBER 1981		
		MUTAGENICITY ^b		PAH ^b	MUTAGENICITY		PAH
		+ S9	- S9		+ S9	- S9	
Transportation	LEADF	10.2 (62)	5.0 (69)	1.4 (58)	9.7 (95)	3.1 (48)	6.3 (129)
Industry	NICKELF	—	—	-0.8 (-33)	—	—	1.0 (20)
Secondary Aerosols	NO ₃ ⁻	6.9 (42)	2.7 (37)	—	—	—	-3.4 (-69)
	SO ₄ ⁼	—	—	0.6 (25)	—	3.5 (54)	—
Crustal Resuspension	IRON	—	—	—	-1.6 (-16)	—	—
Residual	Unknown	- 0.7 (-4)	- 0.4 (-6)	1.1 (48)	2.2 (22)	-0.1 (-2)	1.0 (20)
Total		16.4	7.2	2.4	10.2	6.5	4.9

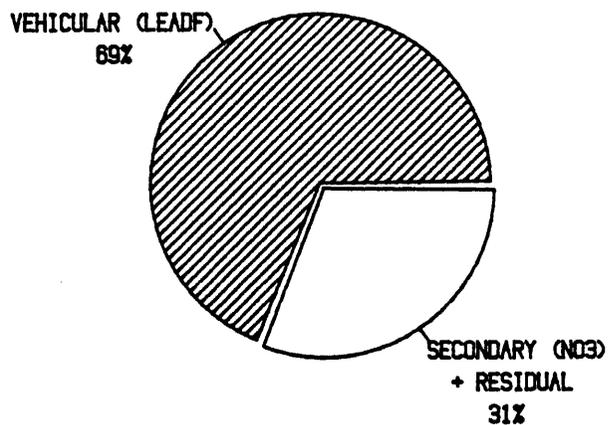
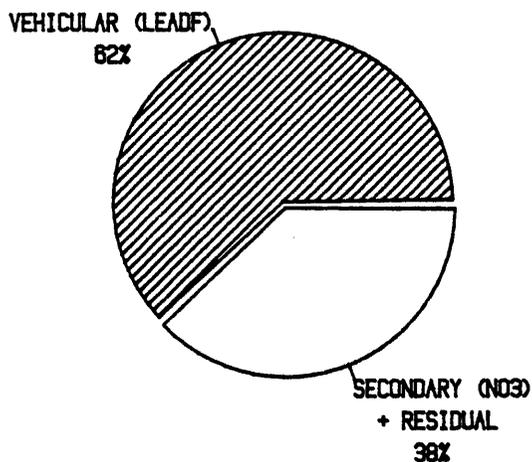
^aEstimates based on equations 1-6 above; percentage contributions are given in parenthesis.

^bConcentrations of mutagenicity are in revertants/m³; PAH in ng/m³.

FIGURE 18
 SOURCES OF AIR PARTICULATE MUTAGENS
 AUGUST 1981 EPISODE

TOTAL INDIRECT ACTING [+S9]

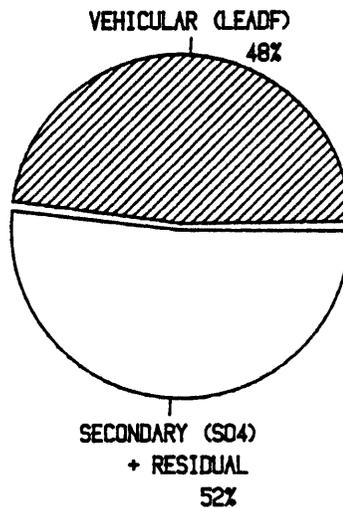
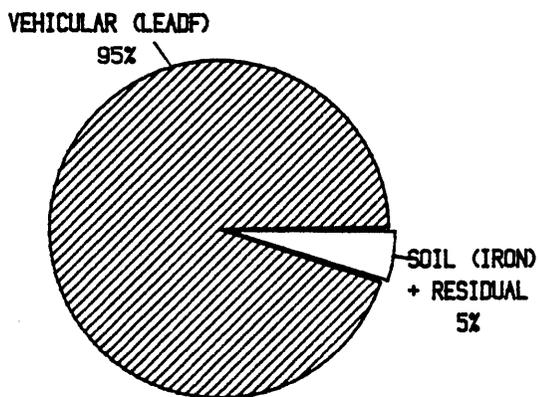
DIRECT ACTING [-S9]



OCTOBER 1981 EPISODE

TOTAL INDIRECT ACTING [+S9]

DIRECT ACTING [-S9]



V. CHRONIC SAMPLING: SEASONAL VARIATIONS AND TRENDS IN AMBIENT MUTAGENICITY AND PAH CONCENTRATIONS BETWEEN NOVEMBER 1979 AND JUNE 1982

A. Results and Comparisons by Station

Hi-vol samples were collected every sixth day between November 1979 and June 1982 at Concord, Richmond and Pittsburg and used to prepare composite samples for Ames and POM testing. As with episode sampling, the POM variable was again defined as the sum of the eight unsubstituted PAH and benzanthrone. Other particulate pollutants analyzed in the composites were MASS = TSP, LEAD, SO_4^- , NO_3^- and ORG = BSO. The logistical plan for sampling and analysis was as above (Figure 2).

The results of analyzing Contra Costa seasonal composites are presented in Table 44. Mutagenicity measurements were made in strains TA98 and TA98NR, both with and without S9 and results reported as revertants/ m^3 (mutagenic density). Results of PAH analysis are in units of ng/m^3 and the other pollutants are in ug/m^3 . No major differences in pollution levels between the three stations were observed. The 32-month average concentrations of each variable varied by less than a factor of two from station to station.

B. Seasonal Variations

In contrast to the small geographic fluctuations, dramatic seasonal variations in mutagenicity and PAH concentrations were observed. These are illustrated in Figures 19 and 20. The most extreme variations were in total indirect mutagenicity (+S9) and PAH. Concentrations of both variables were more than five times higher in the winter (November-February) than in the spring (March-June) season. The other particulate pollutants exhibited qualitatively similar but smaller seasonal variations. Concentrations of LEAD, NO_3^- and BSO were about three times higher in winter while concentrations of TSP and SO_4^- were higher by a factor of two (or less) in winter than in spring. (Figures 21-25). The good correlation among pollutants with respect to

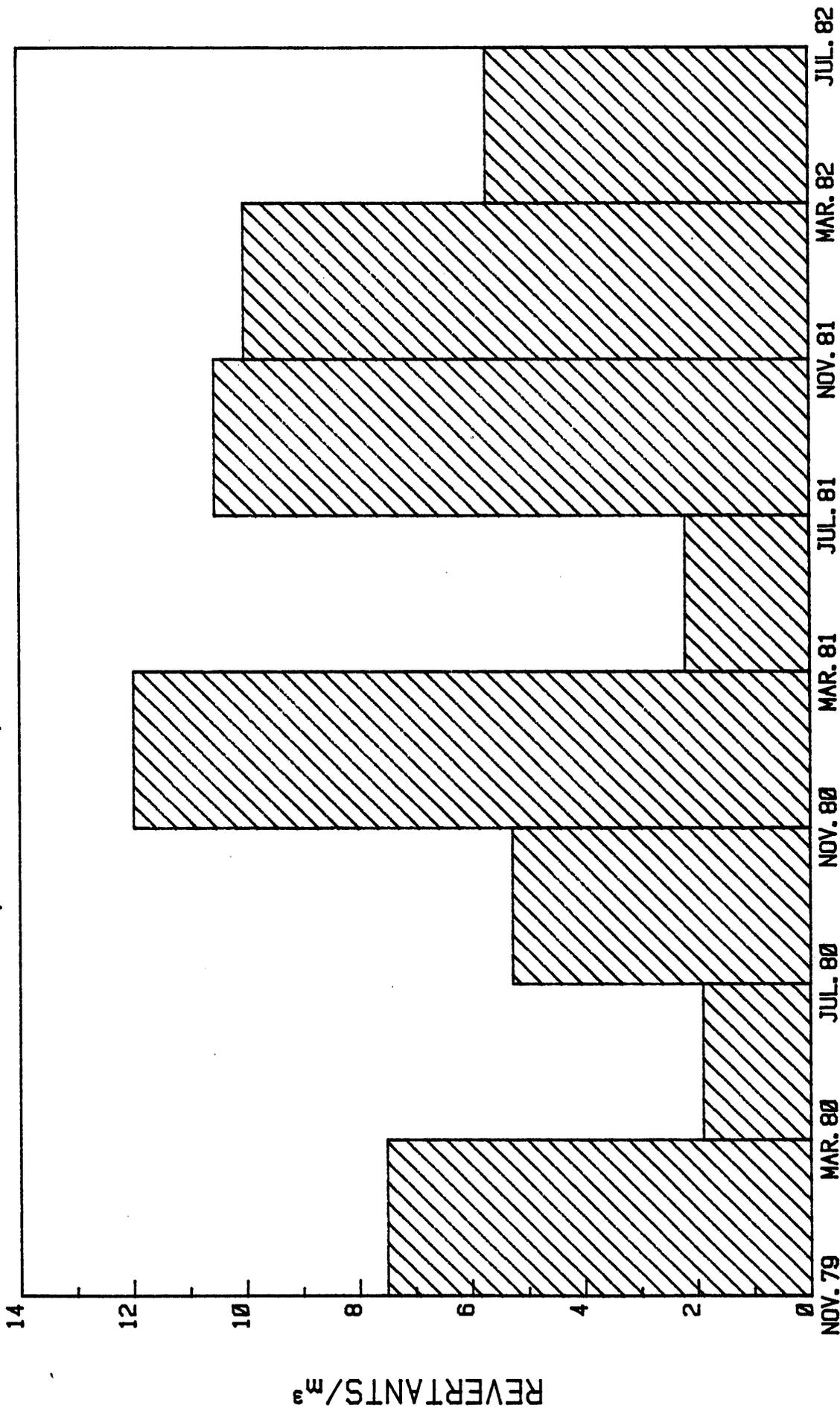
TABLE 44 CONTRA COSTA SEASONAL COMPOSITES

STATION	PERIOD	MASS	LEAD	804	NO3	DRQ	CHR	BAA	BBF+BEP	BKF	BAP	BGP	COR	BO+ISO	98+89	98-89	98NR+	78NR-
CONCORD	NOV-FEB 80	73	0.69	7.7	11.2	10.7	0.8	0.7	2.9	0.6	1.3	4.1	1.9	3.3	4.8	2.7	7.7	2.4
CONCORD	MAR-JUN 80	38	0.23	3.7	2.7	1.5	0.1	0.1	0.2	0.1	0.1	0.5	0.5	0.1	1.2	1.5	3.0	1.4
CONCORD	JUL-OCT 80	60	0.44	6.9	7.0	3.5	0.1	0.1	0.3	0.1	0.1	0.7	0.4	0.2	7.4	5.0	3.4	1.7
CONCORD	NOV-FEB 81	90	0.73	7.4	10.1	10.5	0.7	0.6	2.4	0.6	1.2	3.9	1.8	1.9	14.4	7.5	9.0	1.6
CONCORD	MAR-JUN 81	45	0.24	4.4	4.5	3.4	0.1	0.1	0.3	0.1	0.1	0.9	0.5	0.1	2.4	2.5	1.6	0.9
CONCORD	JUL-OCT 81	52	0.27	6.1	6.1	4.0	0.1	0.1	0.2	0.1	0.1	0.6	0.3	0.2	7.4	4.2	3.4	1.2
CONCORD	NOV-FEB 82	61	0.50	4.4	8.3	10.7	0.8	0.8	2.9	0.6	1.5	4.0	1.8	2.9	5.5	3.5	3.0	1.7
CONCORD	MAR-JUN 82	43	0.24	4.6	4.7	2.6	0.1	< 0.1	0.3	0.1	0.1	0.7	0.4	0.2	6.6	4.7	3.0	3.2
RICHMOND	NOV-FEB 80	64	0.57	9.1	9.2	5.6	0.2	0.2	1.2	0.3	0.3	2.7	1.5	0.9	12.3	6.8	6.9	3.3
RICHMOND	MAR-JUN 80	52	0.23	6.9	2.3	2.3	0.1	0.1	0.3	0.1	< 0.1	0.5	0.4	0.1	3.4	3.0	1.6	0.9
RICHMOND	JUL-OCT 80	60	0.30	8.5	4.3	3.1	0.1	< 0.1	0.2	< 0.1	< 0.1	0.4	0.3	0.1	4.0	2.3	2.2	1.0
RICHMOND	NOV-FEB 81	76	0.43	12.2	10.1	5.4	0.3	0.2	1.1	0.3	0.3	2.5	1.4	0.7	12.7	6.8	3.6	7.0
RICHMOND	MAR-JUN 81	65	0.21	6.9	3.5	3.6	0.1	0.1	0.3	0.1	0.1	0.6	0.4	0.1	2.4	2.8	1.8	0.6
RICHMOND	JUL-OCT 81	55	0.20	6.1	3.6	4.0	0.1	< 0.1	0.2	0.1	0.1	0.5	0.3	0.1	19.1	4.7	7.5	3.6
RICHMOND	NOV-FEB 82	63	0.43	3.2	6.9	5.1	0.5	0.4	1.4	0.4	0.5	2.6	1.3	1.3	11.3	5.7	6.8	3.2
RICHMOND	MAR-JUN 82	53	0.22	6.5	3.8	3.4	0.1	< 0.1	0.2	0.1	< 0.1	0.4	0.3	0.1	6.0	4.9	3.3	2.6
PITTSBURG	NOV-FEB 80	75	0.43	8.0	11.4	4.4	0.3	0.2	1.1	0.3	0.4	2.1	1.2	0.6	5.4	3.4	8.1	4.8
PITTSBURG	MAR-JUN 80	62	0.17	5.9	3.9	1.5	0.1	0.1	0.2	0.1	0.1	0.4	0.2	0.1	1.1	1.0	2.1	1.0
PITTSBURG	JUL-OCT 80	91	0.29	9.5	6.6	2.6	0.1	0.1	0.2	0.1	0.1	0.4	0.2	0.1	4.4	3.7	1.8	1.3
PITTSBURG	NOV-FEB 81	85	0.39	9.4	12.2	5.5	0.3	0.3	1.3	0.4	0.5	2.4	1.2	1.0	8.8	4.1	4.5	1.7
PITTSBURG	MAR-JUN 81	59	0.16	6.2	4.0	3.1	0.1	0.1	0.2	0.1	0.1	0.4	0.3	0.1	1.8	1.8	1.0	0.5
PITTSBURG	JUL-OCT 81	75	0.19	8.0	6.3	4.4	0.1	< 0.1	0.2	< 0.1	0.1	0.3	0.2	0.1	5.1	3.2	2.2	0.9
PITTSBURG	NOV-FEB 82	58	0.35	5.7	9.4	5.9	0.3	0.2	1.0	0.3	0.5	1.8	0.9	1.3	13.2	9.7	8.0	3.2
PITTSBURG	MAR-JUN 82	62	0.18	5.7	4.6	2.6	0.1	< 0.1	0.2	0.1	< 0.1	0.3	0.2	0.1	4.5	4.1	3.6	1.6

FIGURE 19

SEASONAL COMPOSITES

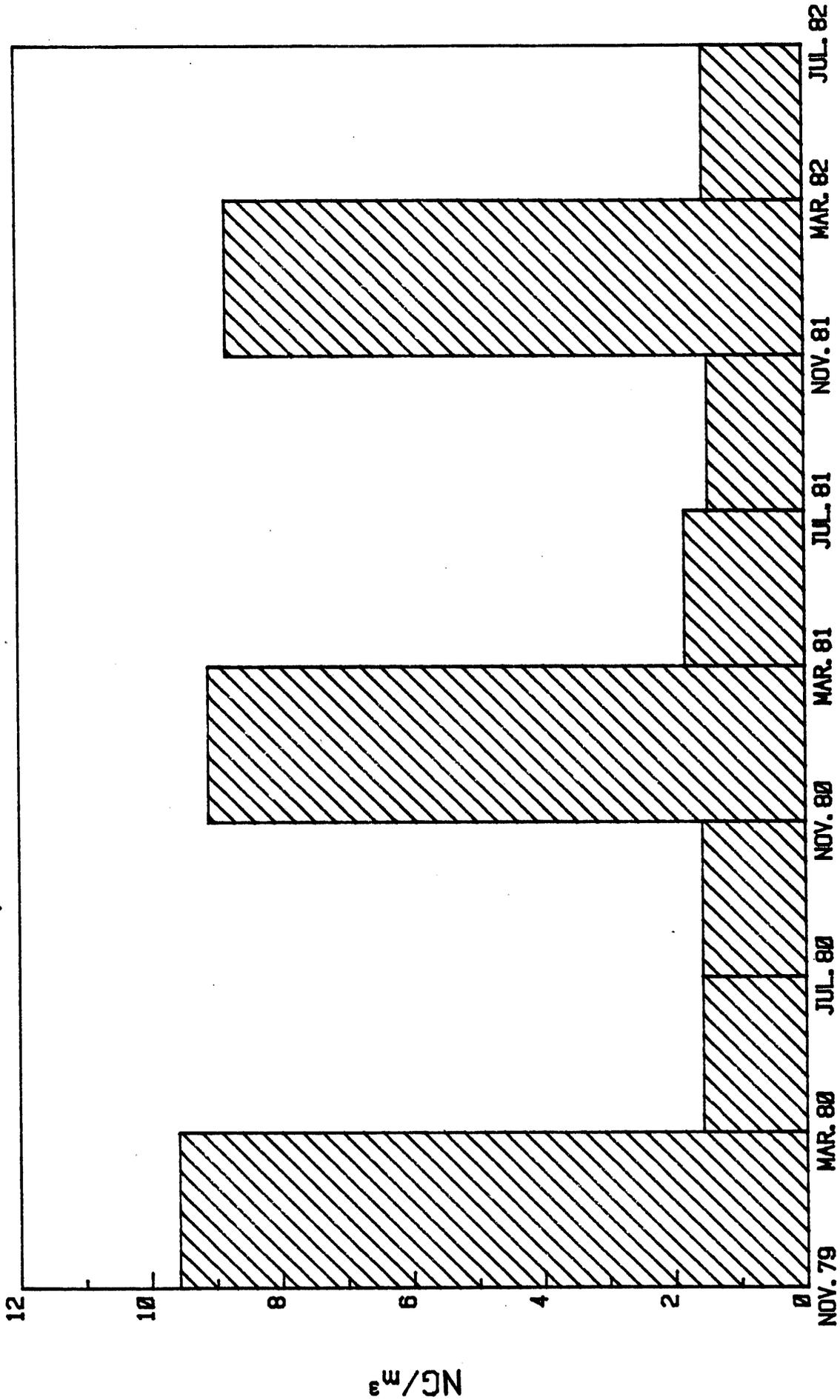
MUTAGENICITY, TA98 +S9, AVERAGE OF THREE STATIONS



COMPOSITE PERIOD

FIGURE 20

SEASONAL COMPOSITES
PAH, AVERAGE OF THREE STATIONS

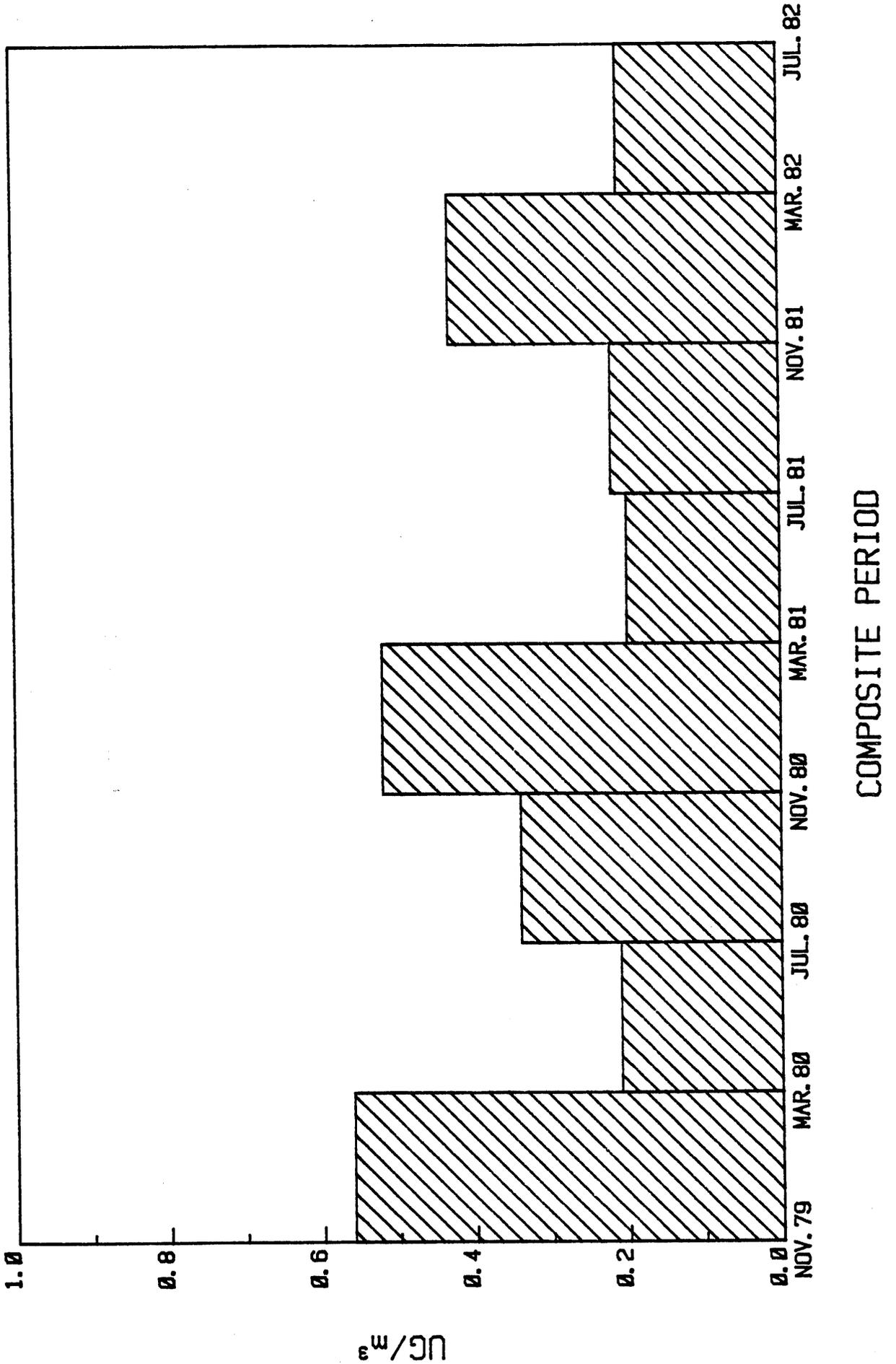


COMPOSITE PERIOD

NC/m³

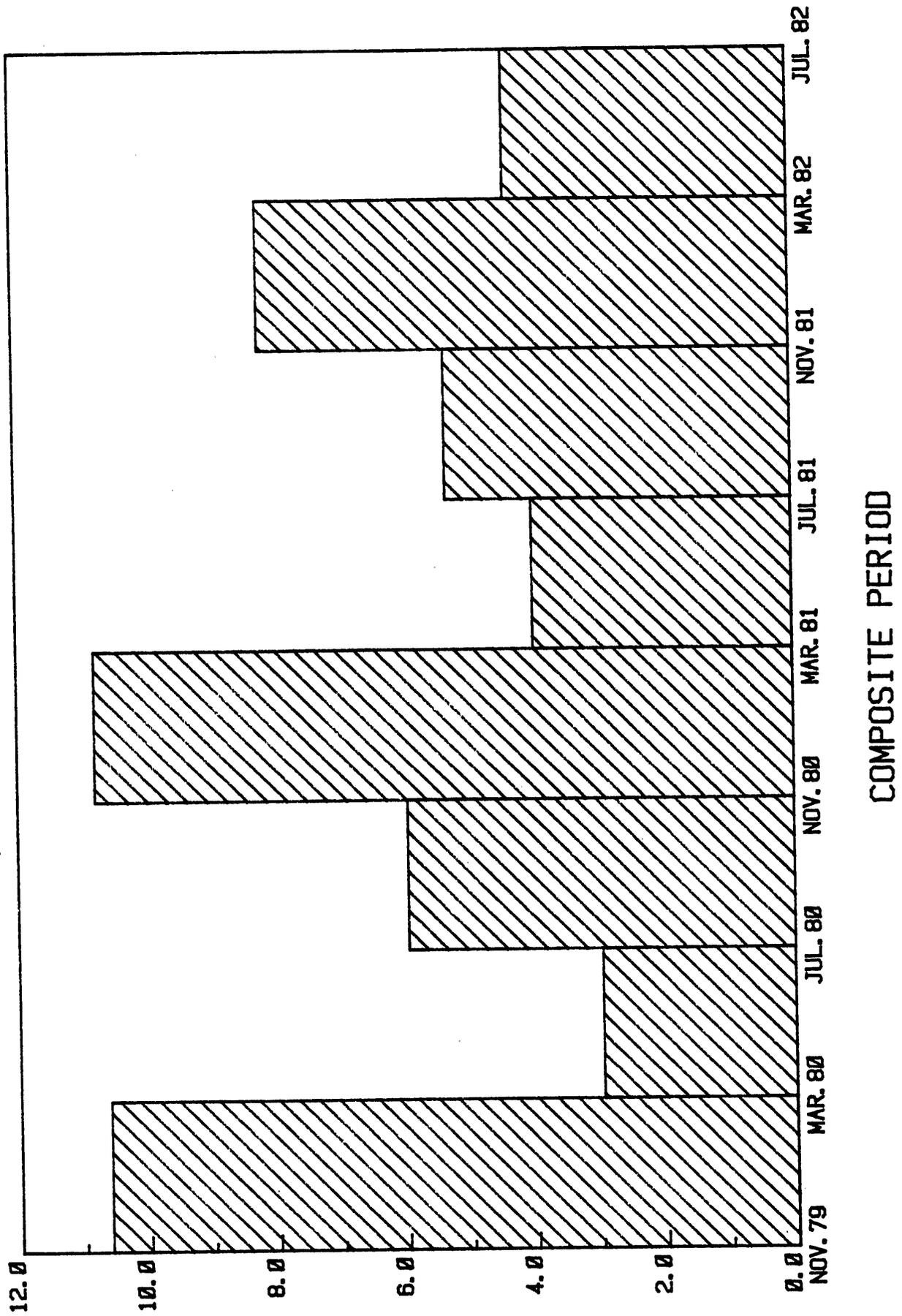
FIGURE 21

SEASONAL COMPOSITES
HI-VOL LEAD, AVERAGE OF THREE STATIONS



UC/m³

FIGURE 22
 SEASONAL COMPOSITES
 NITRATE, AVERAGE OF THREE STATIONS

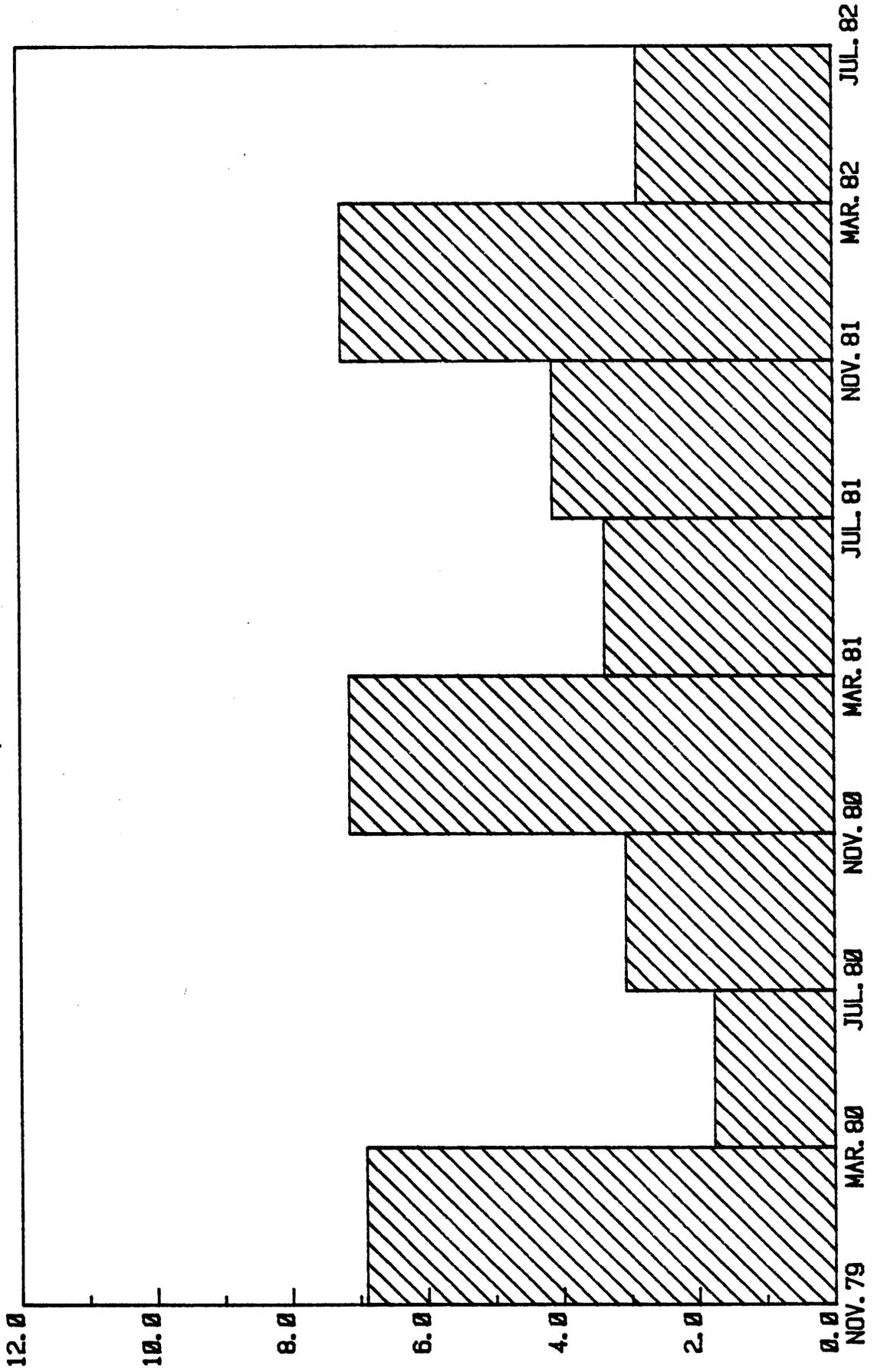


µM/gm

FIGURE 23

SEASONAL COMPOSITES

BENZENE SOL. ORGANICS, AVERAGE OF THREE STATIONS



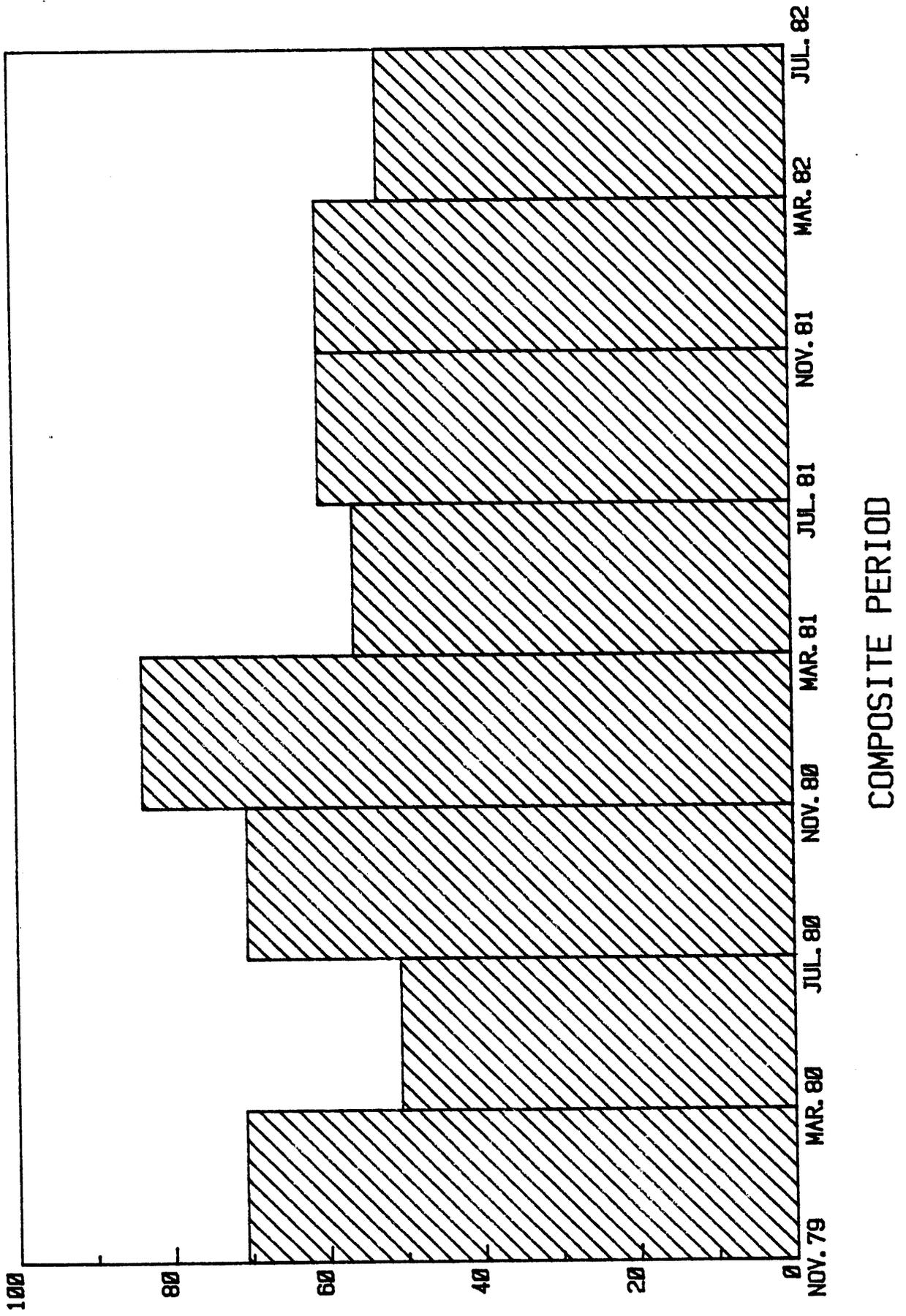
COMPOSITE PERIOD

$\mu\text{g}/\text{m}^3$

FIGURE 24

SEASONAL COMPOSITES

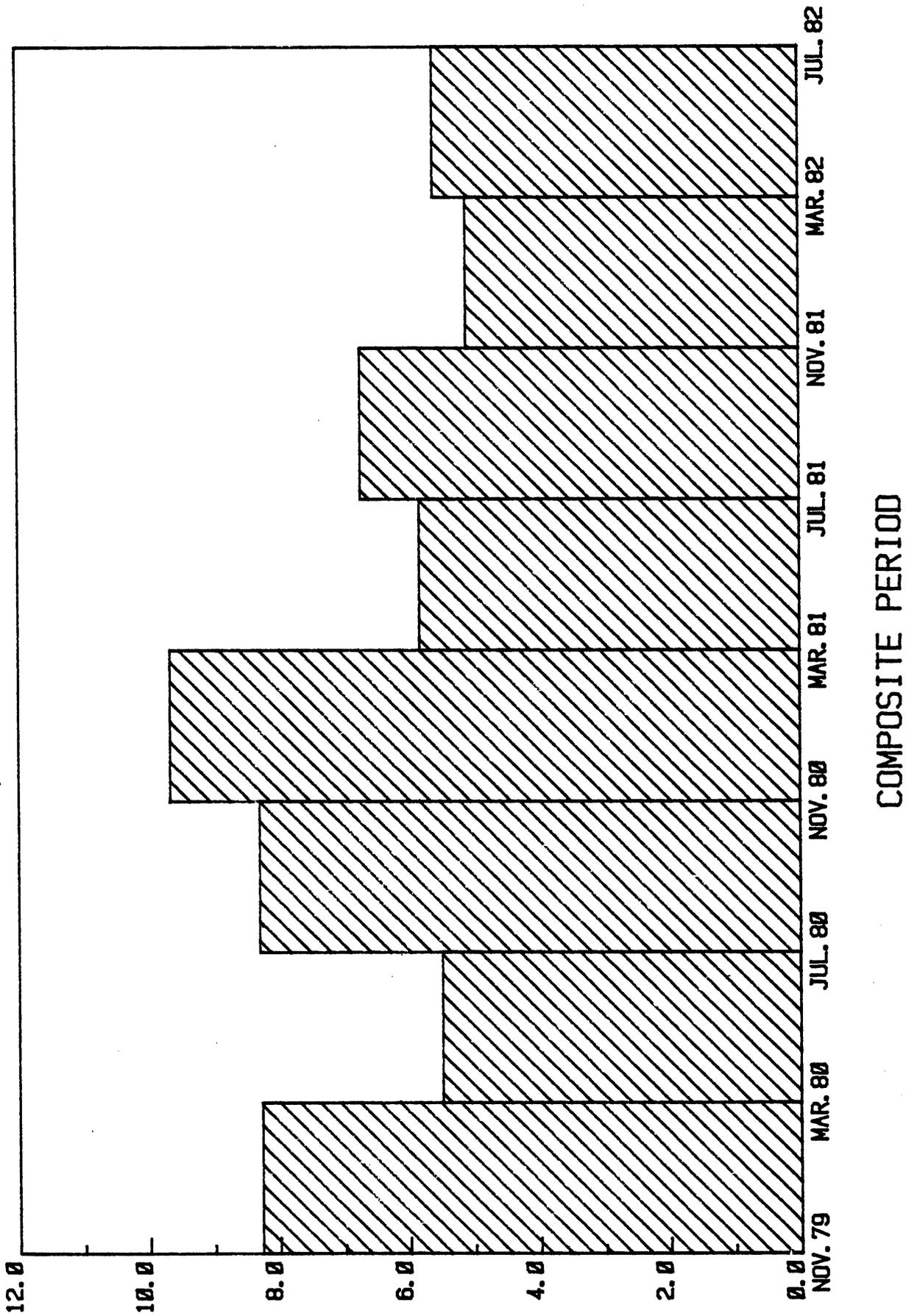
HI-VOL TSP MASS, AVERAGE OF THREE STATIONS



$\mu\text{g}/\text{m}^3$

FIGURE 25

SEASONAL COMPOSITES
HI-VOL SULFATE, AVERAGE OF THREE STATIONS



µg/m³

their seasonal patterns does not necessarily indicate a common source, but is more likely the result of meteorological variations. In the Bay Area, "ventilation", the volume of air available for the dispersal of pollutants, is generally less in winter months due to lower wind speeds and inversion heights (36). Thus higher concentrations of particulate pollutants during winter are to be expected.

C. Trends

One question addressed by this study is whether or not concentrations of ambient particulate mutagens and PAH have changed over recent times in Contra Costa County. No annual trends in mutagenicity or PAH were apparent over the 32-month period studied (cf Figures 19 and 20). The corresponding seasonal values were similar from one year to the next year. No major trends in other pollutants were observed either, although downward trends in LEAD, NO_3^- , TSP and $\text{SO}_4^{=}$ were suggested.

Thus, we conclude that annual average mutagen and PAH aerosol concentrations in Contra Costa County did not change significantly between 1979 and 1982. In previous air pollution studies in Contra Costa County (16), the mutagenic concentrations measured in composite samples collected between November 1978 and February 1979 averaged 7 revertants/ m^3 (-S9) and 11 revertants/ m^3 (+S9). In the present study, the averages for the November through February winter composites collected between 1979-1982 were 6 and 10 revertants/ m^3 without and with S9, respectively.

Finally, the results of these studies provide critical baseline information against which the impact of new or expanding technologies (e.g., increasing dieselization in California (27)) can be measured. Continued chronic routine monitoring at strategic sites in the state would seem prudent. This strategy would not only help define the nature of toxic air contaminants in California but also help to identify trends in their levels.

VI. GENERAL CONCLUSIONS AND IMPLICATIONS

A. Sources of Mutagens and Carcinogens During Pollution Episodes

The study has demonstrated the feasibility of integrating mutagenic, chemical and multivariate statistical methods for mutagen and carcinogen source identification.

We have shown that the source patterns during the three episodes were different and sources could be at least partially apportioned. Vehicular transportation sources were the predominate mutagenic contributors during the August and October 1981 episodes. In addition at least half of the PAH was also derived from automotive sources during summer and fall episodes. Industrial emissions may have contributed to direct acting mutagens in October, but this conclusion is not very firm. The source pattern during the January 1982 episode was the most complex and quantitative source apportionment yielded no useful information, as noted above. However qualitative results of factor analysis suggested possible contributions of residential wood combustion to PAH during the winter episode. Furthermore, simple two variable correlation analysis (Table 26) shows positive correlation between mutagenicity (TA98 \pm S9) and coronene, iron, zinc and lead and a significant negative correlation with nitrate.

Improvements could be made in the quantitative source apportionment method by introducing more complete and quantitative meteorological data than were available in this experiments into the multivariate statistical techniques. For example, Daisey and Kneip (35) used dispersion normalized concentrations with success in multiple regression modeling. It would also improve the technique if sampling were done at more stations.

B. Seasonal and Chronic Human Exposures

It is significant that concentrations of both carcinogenic and mutagenic pollutants vary widely as a function of season. Mutagenicity and PAH concentrations were measured to be at least five times higher in winter

than in spring due mostly to reduced ventilation in the Bay Area in winter. Thus in terms of human exposure, the winter is clearly the major seasonal contributor to the mutagenic and carcinogenic burdens of ambient air particles. In a typical recent year, Contra Costa residents inhaled more mutagens and PAH during the four-month winter season (November-February) than during the other two seasons combined because concentrations are so much higher in winter.

For purposes of discussion, it is useful to provide some estimate of the possible human risks associated with exposure to airborne mutagens and carcinogens at these current levels. In this study, composite air samples had an annual average mutagenic density (+S9) of ca. 7 rev/m^3 . This level may be compared with the mutagenicity in cigarette smoke condensates. The smoke condensate from one commercial cigarette gives approximately 17,500 revertants in the Ames test (39). Assuming that the average person breathes 20 m^3 per day, the number of "cigarette equivalents" per day is therefore ca. 0.01 or 3-4 cigarettes per year. A second type of risk estimate was made by Pike and Henderson (2) who used BAP as a surrogate for cancer risk and compared amounts of BAP in cigarettes with excess lung cancer in smokers. These authors calculated that daily breathing of community air containing 15 ng/m^3 BAP poses the same life-time lung cancer risk as smoking 1 cigarette per day. In the present study, annual levels of BAP averaged 0.3 ng/m^3 . Thus in terms of cancer risk, daily breathing of Contra Costa winter air may be considered equivalent to smoking about $0.3/15 = 0.02$ cigarettes per day or about 7 cigarettes per year. Considering the uncertainties in the in vitro bioassay and epidemiological data, and the assumptions and simplifications implicit in the calculations, the two-fold difference in the estimates derived from mutagenicity and BAP measurements is surprising small. Pike and Henderson conclude from their analysis that even at a BAP level as low as 1 ng/m^3 , the life-time lung cancer risk is "slightly greater than $1/1500$. Environmental regulations are usually made to keep such a risk to $1/10^5$ or even $1/10^6$ " (2). These risk-estimates neglect contributions from indoor air pollution. Also, the excess risk attributable to Contra Costa community air pollution (ca. $1/5000$) is less than one percent of the observed incidence of lung cancer from all causes

in Contra Costa County (between 1/20 and 1/10) (6). This is a number much too small to be identified by epidemiological tools, principally because smokers keep the background so high.

Presumably these possible excess risks will be less in the future if the recent downward trends in Bay Area air pollution levels continue. Air quality in the Bay Area has improved significantly over the past decade (36) as controls on stationary sources and vehicles have steadily reduced emissions. This has resulted in major reductions in concentrations of gaseous pollutants (notably ozone), total particulates and lead. Similar downward trends in polycyclic hydrocarbon concentrations are suggested by results of the present study. In San Francisco during the winter months of 1958-59, BAP concentrations ranged from 2.3 to 7.5 ng/m³ (5) while in the winters of 1979-82, the average BAP concentration in Contra Costa County was significantly lower (0.7 ng/m³). However, no downward trends in BAP, PAH or mutagenicity levels were observed within the brief 32-month period of this study, although the duration of our analysis was too short to have detected anything but major changes.

C. Chemical Nature of Particulate Mutagens

Aerosol extracts are extremely complex mixtures and much research on their chemical contents remains to be done. At present we know that Contra Costa aerosols contain predominantly direct-acting mutagens during warm-weather months and both direct- and indirect-acting mutagens during cold weather months. This conclusion is based on mutagenic testing of seasonal composites. However, both direct- and indirect-mutagens are clearly present during the hot August episode as well as during the cool October and cold January episodes. Thus sources and/or atmospheric conditions for production of both direct- and indirect-mutagens are present all year around.

As expected, PAH are among the indirect-acting mutagens found in Contra Costa aerosols. However the PAH species measured in this study made a very small contribution to the observed mutagenicity of air particle extracts. This was the case even during sampling periods when polycyclic hydrocarbon

concentrations reached their highest levels (i.e., during the January 1982 pollution episode, when the concentrations of BAP and BO averaged approximately 1 ng/m^3 and 4.5 ng/m^3 respectively). A mixture containing the PAH at their concentrations measured during the January episode was prepared and subjected to mutagenic testing. The simple mixture of pure chemicals showed activity in TA98+S9 but the amount was only about 1% of the indirect mutagenic activity observed in the complex mixtures extracted from the January episode air samples.

The question of NO_2 PAH in Contra Costa aerosols remains open. It seems likely that direct-acting nitroarenes are present in some urban aerosol extracts (40). However, the evidence in Contra Costa County is indirect and based on the behavior of extracts in the nitroreductase-deficient mutant, TA98NR, which lacks the ability to activate many nitro-compounds. Direct-mutagenicities of most Contra Costa samples were indeed much lower in TA98NR than in TA98. Decreases of about a factor of two or more were observed in at least half of the composite samples and more than three-quarters of the episode samples. Activities in TA98NR relative to TA98 were especially low during the summer intensive episode, when the most reactive atmospheric conditions prevailed. This makes it probable that direct-acting nitroarenes are present in the atmosphere (or formed on filters after collection via mechanisms such as proposed by Pitts and co-workers (25)). Further research is required to chemically identify the postulated nitroarene species in air extracts. Based on the indirect evidence provided by testing in TA98NR, we conclude that most of the Contra Costa samples analyzed contain compounds with a reducible NO_2 -group, like 1- NO_2 pyrene, which are directly active in the Ames test. Such compounds may account for half or more of the direct mutagenicity in air particulate extracts, especially in warm weather months.

D. Implication for ARB Regulatory Programs

Results of this study may be applied to ARB regulatory functions related to control of toxic air contaminants. Hopefully identification of sources can assist in the development of control strategies for mutagens and

carcinogens in community air. This is an area of significant long range public health concern.

In the present study, multivariate statistical methods were used to identify sources of mutagens and polycyclic aromatic carcinogens and to estimate their contributions to the ambient aerosol. It is important to recognize the limitations of these source apportionment efforts. As with any application of statistics, there is no assurance that the observations and conclusions represent cause and effect. In addition, the number of observations is small. Therefore, all conclusions are subject to revision as additional data become available. However, one salient conclusion does seem apparent. A major proportion of the mutagenicity of Contra Costa County aerosols collected during the August and October 1981 episodes can be accounted for by the variability in the fine-fraction lead concentration in these aerosols. This observation suggests that during the summer and fall pollution episodes, the majority of the mutagenicity in Contra Costa aerosols was due to vehicular emissions. The contribution of diesel exhaust emissions to mutagenic aerosols should be considered in future research. Furthermore, mutagens associated with NO_3^- , a secondary transformation product, contributed one third of the mutagenicity of aerosols collected during the summer episode.

The first implication of these conclusions for ARB regulatory programs is that emission standards and controls on vehicles are probably the most efficacious means of controlling ambient levels of particulate mutagens. The contribution of nitrate-associated aerosols to mutagenicity in summer suggests that regulation of secondary pollutant formation may have some impact on atmospheric levels of mutagenic compounds, but this is speculation.

Consideration should also be given to the following: Nickel is a tracer for fuel oil combustion and refinery operation. No significant statistical relationship was found between nickel and aerosol mutagenicity. The stepwise multiple regression equations indicate a negative relationship between fine nickel and PAH in the summer episode and a positive relationship in the fall episode. The monitoring site at Martinez is in close proximity to several refineries. Martinez experienced the highest average concentrations

of nickel and the lowest average aerosol mutagenic densities during the three episodes. Thus refinery emissions do not appear to contribute significantly to the mutagenicity of Contra Costa community air. This conclusion requires two caveats; it is based on only 108 hours of air sampling and it applies only to aerosols since vapor phase mutagens were not increased.

Another topic of possible interest for ARB regulatory programs concerns evidence that wood burning is a source of carcinogenic polycyclic hydrocarbons in Contra Costa air during winter. Several lines of evidence are presented in this report. First, diurnal patterns of selected PAH measured in the winter episode are consistent with night emissions from fireplaces. Because of meteorological factors, nighttime levels of most particulate pollutants measured in January were higher than daytime levels, but diurnal variations in certain PAH were the most dramatic. Specifically, concentrations of certain carcinogens (BAP, CHR, BAA) were three to five times higher by night than by day, especially in Concord and Martinez, the sampling stations located in the most residential environments. In a recent study of wood-burning in Waterbury, Vermont, Sexton *et al* (41) observed dramatic diurnal variations in concentrations of respirable particulates, with peak values at night exceeding afternoon levels by 5- to 10-fold. They concluded that wood burning was the major source of airborne particles in residential sections of the town. A second line of evidence in the present study employed a simple ratio technique to obtain information about PAH sources. As shown in Table 45, various investigators have measured the ratio of BAP to BGP for a number of combustion sources (1,42,43). Automobiles tend to have the lowest ratios, 0.2 to 0.5 while industrial sources tend to be ≥ 1 . The BAP/BGP ratios reported for wood combustion were 0.4 to 0.5. In this study, the average BAP/BGP ratios in the summer, fall and winter episodes were 0.17, 0.28 and 0.52 respectively. Clearly the ratios found in the summer and fall were characteristic of auto emissions whereas these in winter were more similar to the values reported for wood combustion. This is consistent with residential wood combustion being a major contributor of these PAH in winter. (The conclusions drawn on the basis of BAP/BGP ratios must be viewed as speculation for the following reasons:

TABLE 45

RATIO OF BENZ(A)PYRENE TO BENZ(GHI)PERYLENE FOR
SELECTED AIR EMISSION SOURCES

<u>Source-Type</u>	<u>BAP/BGP</u>	<u>Reference</u>
Vehicular	0.2 - 0.5	1
Industrial		
Petroleum refineries	0.65 - 1.7	"
Oil-burning powerplants	2 - 3	"
Coal-burning powerplants	0.9 - 6.6	
Wood Combustion		
Stoves	0.42	42
Fireplaces	0.52	42
Forest-fire	0.47	43
Contra Costa Community Air Pollution episodes:		
-Summer	0.17	This study
-Fall	0.28	" "
-Winter	0.52	" "

- (a) the data used for comparison are from different references, dating back to 1972.
- (b) Temperature differences probably influence, to an unknown extent, the observed ratios of BAP/BGP.
- (c) Even on the basis of the ratios used (Table 45), no clear cut distinction is possible between vehicular and wood burning emissions.)

A third type of evidence implicating wood combustion was obtained by factor analysis. During the winter episode, the factor analysis technique revealed a novel pollution factor containing both organic variables, PAH and BSO, and which explained 25 percent of the variance in the levels of particulate pollutants. However, this novel organic pollution factor did not contain any of the source-related tracers (LEAD, NO_3^- , NICKEL, $\text{SO}_4^{=}$, IRON). Furthermore, the factor was not present in the summer or fall episodes. Finally, the organic factor was only recognized in the pollution patterns at Concord and Martinez, the locations most subject to residential emissions. From these results, we conclude that residential wood combustion contributes seasonally to ambient PAH levels in Contra Costa County. If correct, this conclusion implies that a new control strategy may be needed.

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Dinitro and Mononitrobenzo(ghi)Perylenes and Mononitrocoronene Are Highly Mutagenic in the Ames Salmonella Assay

William A. Vance and Raymond Chan

Air and Industrial Hygiene Laboratory, California State Department of Health Services, Berkeley

Benzo(ghi)perylene (B(ghi)Per, (191-24-2)) and coronene (Cor, (191-07-1)) are major constituents of the polycyclic aromatic hydrocarbons (PAH) found in automobile exhaust and polluted air [eg, Grimmer et al, 1981]. Nitration of these PAH by NO₂ and traces of HNO₃, which are also formed in automobile exhaust, seems highly probable. To identify the presence of these nitroarenes in environmental samples and to examine their mutagenic potencies we synthesized and characterized nitro derivatives of both PAH. 5-NO₂B(ghi)Per (81316-87-2) and 1-NO₂Cor (81316-84-9) produced 405 and 340 revertants/nmole respectively in TA98 in the presence of 0.6 mg of microsomal enzymes (S-9) per plate in the Ames test. 5,8-diNO₂B(ghi)Per (83292-25-5) and 5,10-diNO₂B(ghi)Per (83292-26-6) produced 21,500 and 4,000 revertants/nmole in TA98 without microsomal activation. Mutagenicity for the dinitrobenzo(ghi)perylene was also high in TA98NR and TA97 but was reduced by 97% in TA98-1,8DNP. There is close similarity in the orientation and distances between reactive sites (nitrenium ion and carbocation) on the dinitrobenzo(ghi)perylene and 1,6-dinitropyrene (42397-64-8) and 1,8-dinitropyrene (42397-65-9).

Key words: dinitrobenzo(ghi)perylene, nitrobenzo(ghi)perylene, nitrocoronene, microsomal activation, TA98-1,8DNP, TA97

INTRODUCTION

Several laboratories [Pitts et al, 1978; Jager and Hanus, 1980; Tokiwa et al, 1981] have demonstrated that nitration of certain PAH occurs readily in an atmosphere containing as little as 1-1.22 ppm NO₂ and traces of HNO₃ (20 ppb) or light. This "facile" formation of nitroarenes would indicate that conditions in automobile exhaust would be conducive to formation of a broad spectrum of nitrated polycyclic aromatic hydrocarbons (NO₂ PAH). Many laboratories [eg, Schuetzle et al, 1981; Xu et al,

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Address reprint requests to Dr. W.A. Vance, AIHL Rm 334, CSDHS, 2151 Berkeley Way, Berkeley, CA 94704.

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1981; Yu and Hites, 1981; Gibson, 1982] are actively seeking to identify specific NO₂ PAH in automobile and diesel exhaust. Identification is often made by mass spectrometry but this can be limited in that unrelated NO₂ PAH such as 1-nitropyrene and 3-nitrofluoranthene produce similar electron impact mass spectra. With the availability of authentic standards one could better identify a NO₂ PAH in GC-MS by measuring retention time as well as fragmentation pattern. Since only a relative few of the NO₂ PAH are available as authentic standards we set out to synthesize the nitro derivatives of the PAH appearing on the EPA priority pollutant list. We have previously reported on nitrobenzo(e)pyrene [Vance et al, 1983a], nitrofluoranthenes, and nitrobenzo(k)fluoranthenes [Vance et al, 1983b]. We report here the mutagenic properties of nitrated derivatives of two major PAH found in automobile exhaust—benzo(ghi)perylene and coronene [Grimmer et al, 1981]. The recent demonstration by Ohgaki et al [1982] of the carcinogenicity in rats of two highly mutagenic NO₂ PAH is indicative of potential for carcinogenicity of other NO₂ PAH that are also highly mutagenic in the Ames Salmonella assay. We hope that by identifying the more toxic and mutagenic components of automobile exhaust, preventive measures can be taken to reduce or eliminate their formation or emission into the ambient air.

MATERIALS AND METHODS

Synthesis of NO₂ PAH was performed by slowly adding an equimolar amount of nitronium tetrafluoroborate (Aldrich) dissolved in anhydrous acetonitrile [Kuhn and Olah, 1961] to solutions of B(ghi)Per and Cor (Aldrich). Purification was achieved by adsorption of the nitro derivatives to silica gel, washing extensively with *n*-hexane, desorption with dichloromethane, and fractionation by preparative high-performance liquid chromatography. Identification of collected peaks was accomplished using electron impact mass spectrometry and high-resolution proton magnetic resonance spectroscopy (data available on request). Quantitation was done spectrophotometrically in acetonitrile using the absorption values from the major Soret bands (concentrations initially being determined gravimetrically). Mutagenic testing was performed according to Ames et al [1975] using Salmonella strains TA98, TA98NR [Rosenkranz and Speck, 1975], TA98-1,8DNP [Rosenkranz et al, 1981; McCoy et al, 1981c], and TA97 [Levin et al, 1982]. Microsomal enzymes were obtained commercially (Litton Bionetics, Kensington, Maryland) and were prepared from Aroclor 1254-induced rats. Revertant colonies were counted with a Biotran III Colony Counter (New Brunswick Scientific) after a 70-hr incubation.

RESULTS

The results of mutagenic testing of 5-NO₂B(ghi)Per and 1-NO₂Cor are enumerated in Table I and graphically represented in Figure 1. Mutagenic response with S-9 was determined using 0.6 mg S-9 protein per plate. It is interesting that mutagenicity for 1-NO₂Cor could not be effectively demonstrated in TA98NR with S-9, whereas this was possible for 5-NO₂B(ghi)Per.

The chemical synthesis of nitrobenzo(ghi)perylene produced more dinitro derivatives than mononitro derivatives. This seemed unusual since equimolar amounts of initial reactants were used. Also, nitration occurred very rapidly, suggesting that B(ghi)Per is a good nucleophile and that addition of the first nitro group may promote addition of the second nitro group. This observation may be important if gaseous

phase nitration occurs by mechanisms similar to those occurring with nitronium ions in solution.

Mutagenicity of diNO₂B(ghi)Per was dramatically increased over that of the mononitro derivative as shown in Figure 1C and 1D and Table I. The potent mutagenicity of these compounds was observed in both a -1 frameshift mutant (TA98) and a +1 frameshift mutant (TA97). The mutagenicity was markedly reduced

TABLE I. Mutagenicity of Nitrocoronene and Nitrobenzo(ghi)Perylenes in Salmonella

Compound	Concentration (nmol/plate)	TA98	TA98NR	TA98-1,8DNP (revertants/plate)	TA97	
1-NO ₂ coronene ^a	0	39	39			
	0.5	242	54			
	1	422	66			
	2	706	112			
	4	1,058	134			
	6	1,273	190			
	rev/nmol ^b		330	24		
5-NO ₂ benzo(ghi)perylene ^a	0	39	39			
	.5	86	82			
	1	180	122			
	2	528	324			
	4	1,652	1,076			
	6	1,784	1,123			
	rev/nmol		420	270		
5,8-diNO ₂ benzo(ghi)- perylene	0	30	20	25	104	
	0.01	314	42	nd	139	
	0.025	758	97	nd	427	
	0.05	1,416	168	nd	777	
	0.075	1,762	216	nd	nd	
	0.1	2,172	258	62	1,321	
	0.25	2,885	876	153	1,114	
	0.5	2,724	1,471	288	nd	
	1	2,835	1,554	480	nd	
	2	2,919	1,580	580	nd	
	rev/nmol		21,500	3,000	540	12,600
5,10-diNO ₂ benzo(ghi)- perylene	0	30	16	25	104	
	0.1	766	614	64	148	
	0.25	1,080	810	84	570	
	0.5	1,273	1,038	96	707	
	1	1,558	1,134	147	504	
	2	1,628	1,259	143	nd	
	rev/nmol		4,000	3,000	130	1,200
	1,8-diNO ₂ pyrene	0.005	1,054	1,116	56	nd
2-NH ₂ fluorene ^a	1 μg	843	848	113	244	
2-NO ₂ fluorene	4 μg	594	110	116	48	
4-NO ₂ quinoline-1-oxide	0.25 μg	401	316	275	460	

^aCompounds requiring S-9 protein (0.6 mg/plate) for optimal activity.

^bSpecific mutagenicities reported to two significant figures unless data allow more. Values are determined by linear regression analysis of linear region.

nd = Not determined.

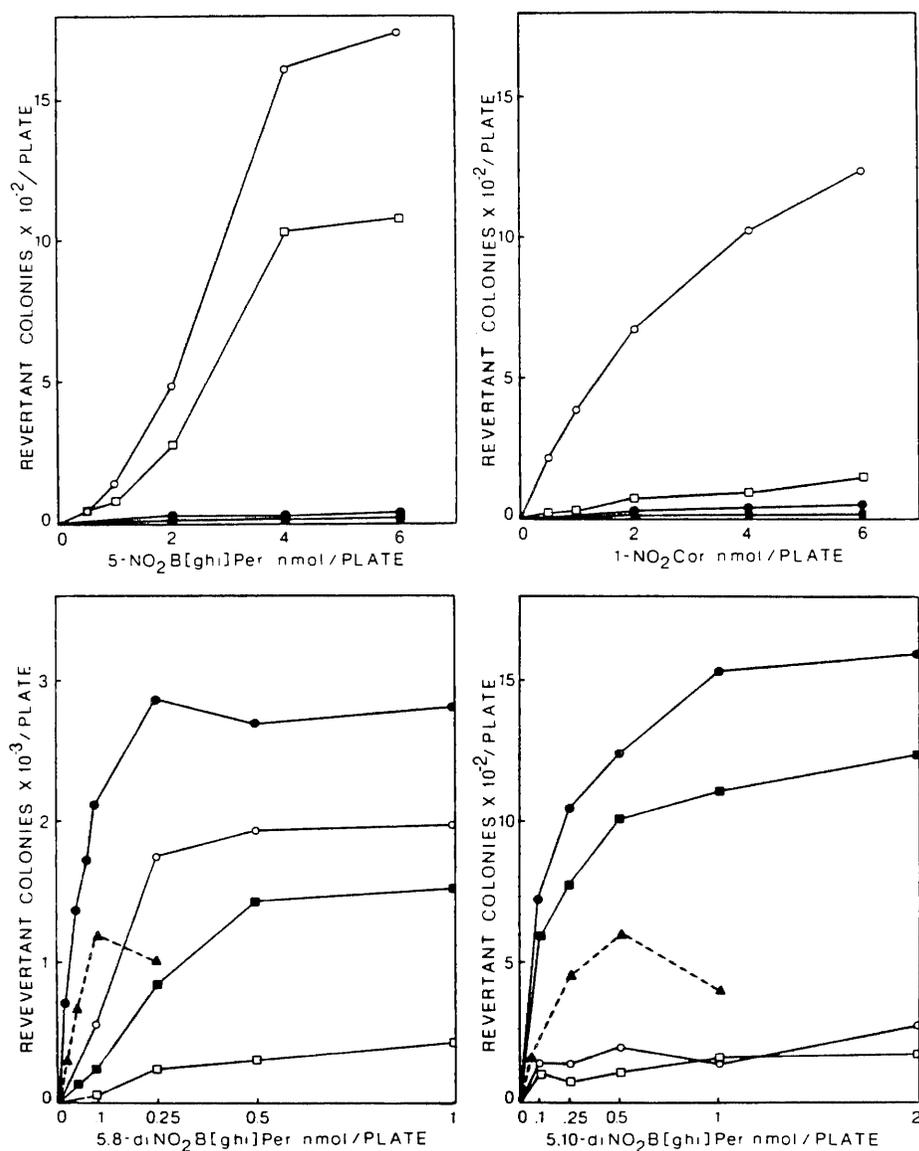


Fig. 1. Dose-response curves of nitroarenes in the Ames plate incorporation assay. Salmonella strains TA98 (●), TA98 + S-9 (○), TA98NR (■), TA98NR + S-9 (□), and TA97 (▲) were tested for mutagenic response to the amounts of nitroarenes indicated on the abscissa. Each point is the average of duplicate plates from replicate assays performed on different days. The cell titer for TA97, as determined by OD₆₅₀, was approximately half that of TA98 and TA98NR. S-9 protein was used at 0.6 mg/plate.

in TA98-1,8DNP, a mutant selected for its resistance to 1,8-dinitropyrene [McCoy et al, 1981c], and was not increased by addition of S-9 protein to the plate assay.

DISCUSSION

The role of microsomal enzymes (S-9) in activation or detoxification of mononitroaromatics is a subject of intense study. Metabolism may involve reduction to an aromatic amine [Poirier and Weisburger, 1974; Ball et al, 1983] where the reaction appears to be favored by low oxygen tension [El-Bayoumy et al, 1982]. The aromatic amine would presumably be converted to a mutagenic hydroxylamine by a mixed-function amine oxidase (EC 1.14.13.8) [Pelroy and Gandolfi, 1980] or by cytochrome P-450-dependent enzymes [Lotlikar and Hong, 1981; also reviewed by Hlavica, 1982]. It is also possible to generate hydroxylamines directly from nitroaromatics by "incomplete" enzymatic reduction [Howard and Beland, 1982; Howard et al, 1983], but isolation of intermediates and products is hampered by their oxygen sensitivity [Peterson et al, 1979]. The proximity of hydroxylamines to the ultimate mutagenic form of nitroarenes was previously indicated by the experiments of Wang et al [1980] and more recently by McCoy et al [1982] by generating N-hydroxy-2-aminofluorene *in situ* from 2-nitrosofluorene. It is also possible that nitroarenes may be made more mutagenic by hydroxylation of ring carbons similar to that observed by Fu et al [1982a,b] and Tong and Selkirk [1982] for 6-nitrobenzo(a)pyrene or by El-Bayoumy et al [1982] for 6-nitrochrysene and 1-nitropyrene. The issue of microsomal activation becomes more complex when, for example, induced versus noninduced liver preparations are used. Thus Greibrokk et al [1983] showed that 4-nitrobenzo(ghi)perylene had higher mutagenic activity with induced S-9 than noninduced S-9 whereas the opposite effect was shown for 3- and 8-nitrofluoranthenes. Pederson and Siak [1981], using fractionated S-9 preparations, found that membrane associated enzymes activated 1-nitropyrene and the cytosolic (soluble) enzymes had little effect. The results presented here for 5-nitrobenzo(ghi)perylene and 1-nitrocoronene indicate that Aroclor-induced microsomal enzymes increase the mutagenic response of these compounds in TA98 and TA98NR. It is interesting to note that other nitroarenes of five or more fused rings such as 6-nitrobenzo(a)pyrene [Nilsson et al, 1981; Pitts et al, 1982], 3-nitroperylene, 6-nitrochrysene [Nilsson et al, 1981; Greibrokk et al, 1983], 3-nitrobenzo(e)pyrene, and 3-nitrobenzo(k)fluoranthene [Vance et al, 1983a,b] show increased mutagenicity in the presence of liver microsomes. The biochemical mechanisms for activation of these compounds and their ultimate mutagenic form remain to be elucidated.

The role of microsomal enzymes in activation or detoxification of dinitroarenes may also prove to be one of the more exciting areas of investigation in the study of these highly mutagenic compounds. Pederson and Siak [1982] showed dramatic increases in mutagenicity for the dinitropyrene series (1,3-, 1,6-, and 1,8-) using the 110,000g supernatant plus NADPH whereas the resuspended pellet plus NADPH almost completely inhibited mutagenic activity in TA98NR. Löfroth [1983] observed marked reduction of mutagenic activity for dinitroperylenes in the presence of induced S-9 microsomes with the exception of a tentatively identified isomer, 3,6-dinitroperylene. This latter compound when tested in TA98 with S-9 enzymes and cofactors showed only a 20-30% reduction in mutagenic activity. Thus, if "detoxification" is

the result of nonspecific adsorption of the submicrogram quantities of dinitroarenes tested, then the effect is nonuniform as evidenced by the difference between the dinitrobenzo(ghi)perylene tested here and by Löfroth with the dinitroperylene [1983]. Further studies into the nature of the enzymes present in various S-9 preparations (cytosolic vs microsomal and induced vs noninduced) will be most helpful in determining which biochemical reactions activate and which detoxify nitroarenes.

The unusually high mutagenicity of diNO₂B(ghi)Per was a bit surprising. The size of these molecules would seem to preclude their effective interaction with DNA when compared to the dinitropyrenes. However, examination of the structures of these molecules, illustrated in Figure 2, shows the similarities in the relative positions and distances between the postulated nitrenium ion and carbocation [after Scribner and Naimy, 1975, and Scribner et al, 1979]. Two biological observations should be pointed out with respect to these compounds. First, even though the distance between the postulated reactive centers in 1,6-DNP and 1,8-DNP are identical, the latter is twice as mutagenic as the former [Rosenkranz et al, 1980]. Second, even though the distances between reactive centers in 5,10-diNO₂B(ghi)Per and 5,8-diNO₂B(ghi)Per

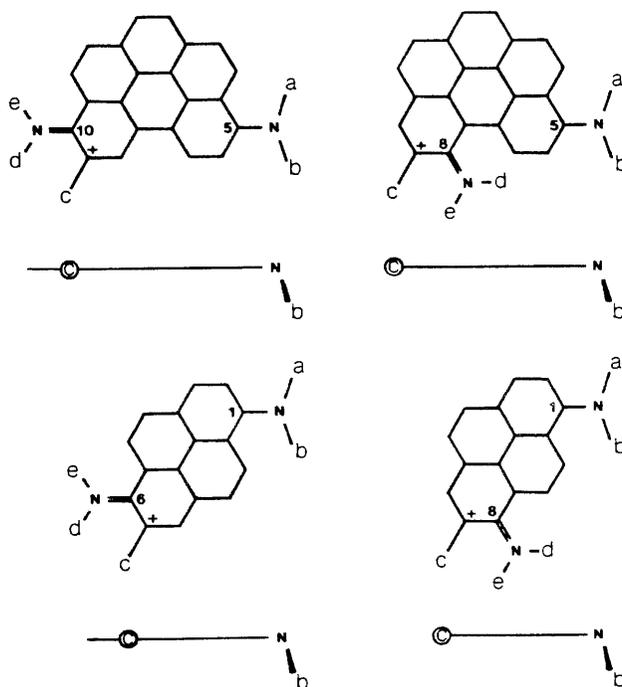


Fig. 2. Geometry of the reactive centers on activated dinitrobenzo(ghi)perylene and dinitropyrenes. Bond lengths and angles are drawn to scale where 1.4 Å was used as the average length for an aromatic C-C bond. Possible substituents on nitrogen are: a = H/CH₃COO or C-8 of guanine; b = same possibilities as a but not the same as a; d = H/CH₃COO or an electron pair; e = same possibilities as d but not the same as d. The point of attachment at c is presumed to be a primary amine. The approximate distances between points of attachment on B(ghi)Per are: c to b, 8.9 Å; c to a, 9.6 Å; and for pyrene: c to b, 8.2 Å; and c to a, 9.9 Å. Since amine bonds are free to rotate, these distances may be extremes of a range of values. Side profiles show that amines are sp³ and imines are sp² configuration.

are identical—the latter is four times more mutagenic than the former (Fig. 3; Table I). Acting as monofunctional compounds, it is possible that the relative position of the second nitrogen atom (as hydroxyacetamide, hydroxylamine, or amine) to the nitrenium ion formed by the first nitrogen atom strongly influences the ability of the molecule to react with DNA. Alternatively, these potentially bifunctional molecules could act as cross-linking agents as proposed in Figure 4. One site of addition to DNA could be via a nitrenium ion to the C-8 of guanine and the other via a carbocation to an exocyclic amine. Differences in mutagenicities between positional isomers then might be explained by stereochemical considerations—ie, whether or not the imido group is in front of or behind the carbocation as it approaches a reactive nucleophile. This explanation assumes that the formation of the C-9 carbocation by resonance stabilization of nitrenium ions at N^8 or N^{10} on B(ghi)Per occurs at comparable frequencies. A similar assumption is made for the C-7 carbocation formed from either N^6 or N^8 nitrenium ions on pyrene. We suggest the possibility of intrastrand cross-linking [eg, Levin et al, 1979] as an explanation only for the unusually high mutagenicities observed with certain dinitroarenes. For example, 1,3- and 1,8-dinitronaphthalene are very weak mutagens [McCoy et al, 1981a; Karpinsky et al, 1982] whereas 2,7-dinitrofluorene is moderately mutagenic in TA98 [McCoy et al, 1981b]. Löfroth [1983] has observed similar differences in the mutagenicities of dinitroperylene where a mixture of 3,9- and 3,10-dinitroperylene (1:2) showed a tenfold

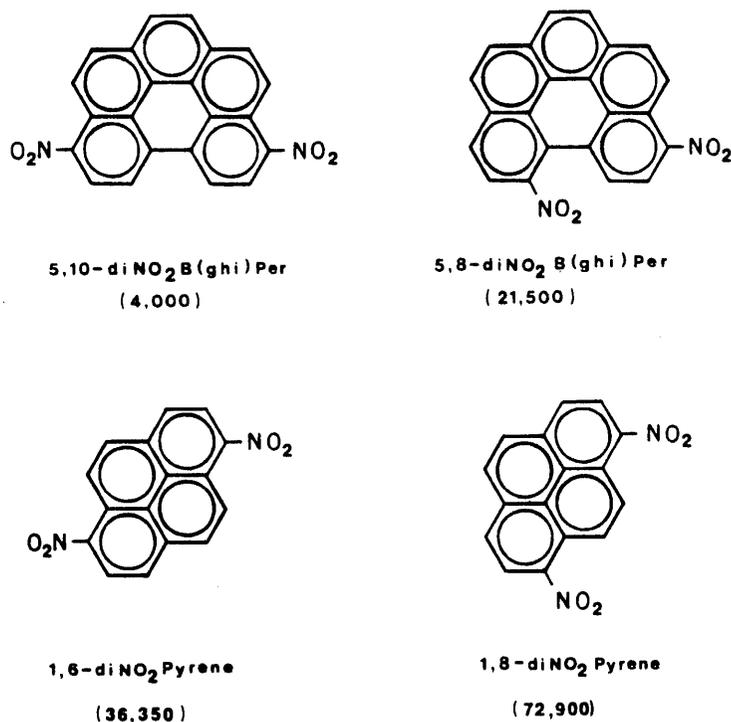


Fig. 3. Comparison of the structures and specific mutagenicities of four dinitroarenes. Values were determined in TA98 and are expressed in terms of revertants/nmole. Values for the dinitroperylenes are those of Rosenkranz et al [1980].

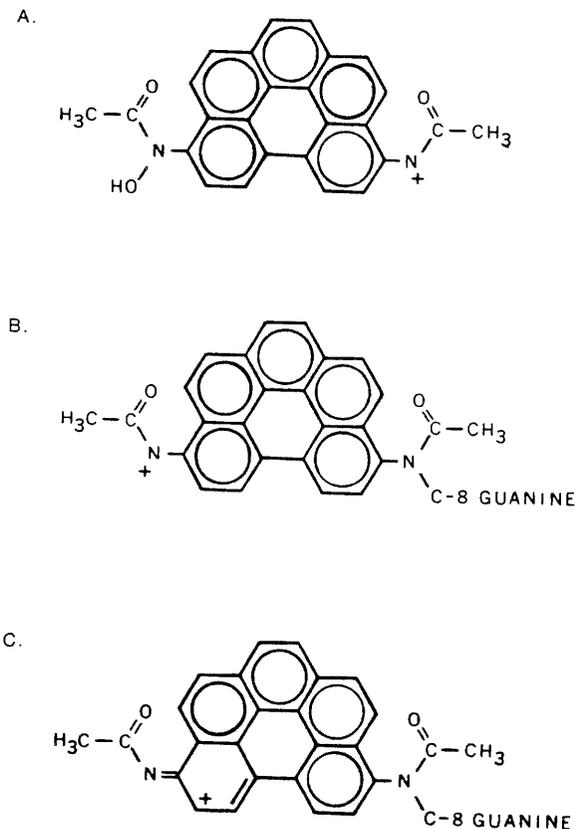


Fig. 4. Proposed steps in the formation of a bifunctional electrophile from a metabolically activated dinitroarene. The initial nitrenium ion (A) may be formed by loss of an acetate ion or a protonated hydroxyl group. After addition to a nucleophile, such as the C-8 of guanine, a second nitrenium ion can form (B) which can be resonance stabilized to form a carbocation (C). The resulting acetimido group would be planar out to the carboxyl carbon but free to rotate about the carboxyl C-N bond. If the acetyl group is replaced by a H, then the imine group would be planar with the aromatic rings.

increase in response over that of the 3,6- and tentatively identified 3,7-dinitro isomers. It is useful to note that 3,10-dinitroperylene is homologous to 5,10-dinitrobenzo(ghi)perylene with respect to position and orientation of nitrogroups. We expect that intramolecular distances between reactive centers as well as bonding angles to a reactive center are the key to understanding the potent mutagenicity of dinitroarenes in the Ames test.

The direct-acting mutagenicity observed for the diNO₂B(ghi)Per is also informative regarding substrate specificities of bacterial nitroreductases. For example, apparently 5-NO₂B(ghi)Per is not activated by the enzyme(s) that activate 5,10-diNO₂B(ghi)Per in TA98 (Fig. 1). Löfroth [1983] observed similar results using 3-nitroperylene and a mixture of 3,9- and 3,10-dinitroperylene (1:2) in TA98. In both cases there is "bilateral" homology of the dinitroarenes with respect to the mononitroarenes. We interpret these findings as indicating a very high degree of substrate

specificity in the enzymes which activate dinitroarenes if the mode of activation is similar to that of the mononitroarenes. It is also interesting to note that the dinitrobenzo(ghi)perylene and dinitroperylene [Löfroth, 1983] have markedly reduced mutagenicity in TA98-1,8DNP. What metabolic activation step is common to dinitro derivatives of pyrene, perylene, and benzo(ghi)perylene that make them weak mutagens in this particular strain? It has been suggested that the putative lesion in TA98-1,8DNP is an enzyme involved in esterification of hydroxylamines [McCoy et al, 1982]. However, Mermelstein et al [1983] have tested this strain with 1,8-dinitropyrene and Zn/NH₄Cl, which presumably would generate hydroxylamines in situ [Karpinsky et al, 1982], and restored mutagenicity to 45% of that observed in the parent strain, TA98. Perhaps esterification is not required for mutagenic activity. It should be noted that it has not as yet been clearly demonstrated that both nitro groups are reduced in vivo. In fact, Quilliam et al [1982] identified the principal metabolite of 1,8-dinitropyrene in TA98 as 1-amino-8-nitropyrene. Obviously many experiments remain to be done with the dinitroarenes to determine how the bacterial nitroreductases metabolically change on this class of compounds.

Our results with the new tester strain, TA97, reported in Table I, are preliminary. This strain grows to only half the titer of TA 98 and TA98NR in overnight cultures. Consequently one is limited to concluding that the diNO₂B(ghi)Per's are active as frameshift mutagens and cannot say whether or not they are more potent in +1 or -1 frameshift mutants. TA97 is also known to be a "slow grower" in colony formation [Levin et al, 1982], which may make interstrain comparisons difficult.

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