AN EPIDEMIOLOGICAL SURVEY OF CARBOXYHEMOGLOBIN IN NONSMOKERS IN LOS ANGELES

INTERAGENCY AGREEMENT
ARB 847

FINAL REPORT
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ABSTRACT

The uptake of carbon monoxide in Los Angeles commuters was measured by determining carbon monoxide concentrations in expired air before and after commuting. Estimates of exposure were made by determining carbon monoxide concentrations in air collected within each car during each trip. There was a definite increase in expired air carbon monoxide concentration during the morning commuting trip, but not during the afternoon trip. No consistent differences by geographic area were noted. The regression of post-commuting expired air carbon monoxide values on ambient air values resulted in statistically significant regression and correlation coefficients. Multiple regression analysis showed that the post-commuting values depended more upon pre-commuting expired air values than upon ambient concentrations of carbon monoxide experienced during commuting. Smokers showed consistently higher values of carbon monoxide than nonsmokers, and the effect of smoking was several times greater than that of exposure to carbon monoxide levels experienced during commuting.

This report was submitted in fulfillment of Project No. 700-283-9 under Contract No. ARB-847 by the State Health Department under the sponsorship of the California Air Resources Board. Work was completed as of February 1, 1975.

The statements and conclusions in this report are those of the contractor and not necessarily those of the California Air Resources Board. The mention of commercial products, their source or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products.

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ORAL PRESENTATIONS BASED ON PRELIMINARY RESULTS

"Quantitative Uptake of Carbon Monoxide in Commuters", APHA Annual Meeting, San Francisco, November 8, 1973.

"Quantitative Uptake of Carbon Monoxide in Commuters", Sacramento Statistical Association, Sacramento, December 6, 1973.

"Effects of Carbon Monoxide on Los Angeles Commuters", University of Sussex, England, August 17, 1974.

"Health Effects of Carbon Monoxide Uptake in Los Angeles Commuters", Seventh Bay Area Biostatistics Colloquim, Berkeley, April 8, 1975.

The above presentations were given by Margaret Deane.

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Department of Health
ARB No. 7co-283-9

I. STATEMENT OF WORK**CONTRACT

Statement of Work

A. General

The contractor shall perform all work and services as outlined in the proposal to complete the study in evaluating the CO uptake in human subjects experienced during commuting in the Los Angeles area. The contractor shall also perform the following supplementary tasks:

B. Supplementary Tasks:

Task 1: The contractor shall obtain and select three groups of smoker and non-smoker subjects, with 20 in each. No less than 10 non-smoker subjects shall be included in each population group. Subjects will be selected among commuters coming from the Riverside-San Fernando area, Los Angeles County foothills area, and Los Angeles County-Orange County coastal area. Subjects will be screened to eliminate those with respiratory conditions.

Task 2: Subjects shall collect their own breath samples before and after commuting in the morning and in the evenings during 10 days between May and September, and during 20 days in December, January and February. Five percent of these breath samples will be validated with carboxyhemoglobin on blood specimens taken at the same time as the expired air specimens. The collection of the breath specimens will also be validated on a 10 percent sub-sample by collection of duplicate specimens by an experienced technician. Grab air samples for CO and NO_x analyses from the driver's compartment will be obtained during the commute trips. Some air samples inside the subjects' houses will also be collected for CO analysis. Conditions of commuting such as time, stopover and traffic flow will be logged by each subject. The number of samples to be collected and analyzed are listed in Table I.

Task 3: Sampling will be done during periods of high and low ambient CO levels. The selection of sampling days will be based on previous air monitoring data. Questionnaires will be used to collect data for evaluation of residential history, occupation, smoking, and commuting.

Task 4: Methemoglobin analysis of the blood specimens collected for COHb analysis and specimens collected by the Air Resources Board project on oxidant effects on school children will be made. Number of samples to be collected and analyzed are listed in Table I.

Task 5: In analyzing and interpreting data obtained in the proposed study, major efforts will be made to evaluate and compare CO body burden before and after commuting.

Revision - Epidemiological Study of
Carbon Monoxide Exposures in Los Angeles
February 29, 1972

At the request of the Air Resources Board, the amount of work to be completed in this study has been increased as shown in the attached table (Table I). In part, this represents an increase in the number of subjects in order to obtain better representation of the commuting population, including smokers. In addition, the number of study days will be doubled during the months of expected high concentrations of carbon monoxide. This will permit extending the period to include December, as well as allowing more intensive sampling during this period. Sampling of carbon monoxide will be done during each commuting trip.

Pilot work will be undertaken by contract to detect nitric oxide hemoglobin combinations using isotope dilution methods. It has been recommended that each participant in the study keep a record of any cigarettes smoked on study days and a log of any travel undertaken, including commuting, to provide a record of time-of-day, duration, and distance traveled. At least half of the participants will be nonsmokers who will be traveling in a car containing no smokers.

The total number of specimens handled has been increased, resulting in corresponding increases in personnel and operating expenses. Methemoglobin analysis of the blood specimens collected for COHb analysis has been added as well as methemoglobin analysis of additional specimens collected by the Air Resources Board Project on Effects of Air Pollution on Children. This can be done at relatively small additional cost and will provide information on the methemoglobin levels in adults in relationship to smoking and exposure to automobile exhaust. Practically no information is available in the literature concerning this potential problem.

Table I

Number of Samples to be Collected

	Total	Areas I	II	III
Number of Subjects	60	20	20	20
Specimens				
Expired Air				
December-February 20 days, 4/day	4800	1600	1600	1600
May-September 10 days, 4/day	2400	800	800	800
Duplicates for 10%	720	240	240	240
Blood COHb	360	120	120	120
Methemoglobin	1000*	120	120	120
Exposure Air Samples	3600	1200	1200	1200
Total Specimens	12,880			

*Includes an additional 640 specimens from the Air Resources Board Project on Effects of Air Pollution On Children.

II. Fulfillment of Contract Requirements

A. General

Field work, laboratory analysis, statistical analysis, and interpretation have been completed.

B. Supplementary Tasks

Task 1: Completed. A description of the subjects is shown in Table 1 (Section III F). A description of geographic areas is shown in Exhibit B.

Task 2: Completed with the following exceptions:

a. The total number of valid carbon monoxide samples is less than that required by the contract because of occasional equipment failure (pump failure for ambient air, bag failure for both ambient and expired air), illness or other failure of subjects to collect samples, or samples not large enough for laboratory analysis. A comparison of the target number of samples per week with the actual number of valid samples collected during each week is shown in Table 2 of the carbon monoxide study (Section III F). In the commuter study, 240 of the required 360 specimens for methemoglobin were analyzed. A chemist was not available for analysis of specimens in the field during the first week of the study and part of the second week. Furthermore, because of the instability of methemoglobin, specimens which could not be analyzed within one-half hour of drawing the blood were discarded. In the study of children, 398 of the 640 required specimens were collected and analyzed (Section IV).

b. Grab samples of air during commuting were replaced with intermittent samples collected by a battery-operated pump throughout each trip (Exhibit J).

c. It proved to be technically unfeasible to collect NO_x samples during commuting.

d. No CO samples were collected in subjects' homes.

The form used for logging commuting information is shown in Exhibit K (Section III).

Task 3: Completed. Sampling periods are defined in Exhibit H (last page). The questionnaire is shown in Exhibit D.

Task 4: Completed (Section IV).

Task 5: Completed (Section III).

Revision: Completed under appropriate tasks listed above. In addition, the pilot study of use of isotope dilution methods on determination of nitric oxide in blood was completed (Section V).

III. General Report - Carbon Monoxide Uptake in Los Angeles Commuters

A. INTRODUCTION

Experimental and epidemiological evidence is accumulating which suggests that carbon monoxide, as it occurs in relatively low levels in the ambient air, may have significant health effects. However, no attempt has previously been made to measure the exposure of commuters to carbon monoxide during their routine day-to-day commuting trips nor to estimate the effect in terms of elevation of carboxyhemoglobin measured either directly from blood specimens or indirectly from expired air obtained following twenty-second breath holding. Several considerations must be taken into account in designing and executing a study of this type.

1. Some system is needed to measure the carbon monoxide concentration to which the commuter is exposed within his automobile. This concentration will be a composite of carbon monoxide from several sources -- the ambient air in the general community through which the commuter travels; the air immediately adjacent to his automobile, including the elevated levels of carbon monoxide which result from surrounding traffic; and the air within his car, which may include carbon monoxide emitted by his own car or by cigarette smoking within the car.

   Regardless of the source, measurements taken within the car are assumed to be the best measure of exposure to carbon monoxide in ambient air during commuting. The term "ambient" is not used in this study in its usual sense of the air to which a community is exposed, but rather to mean the air in the specialized environment within each car.

2. Other possible sources of carbon monoxide must be taken into account; for example, exposure to carbon monoxide inhaled directly from the subject's own cigarette, and exposure to specialized sources of carbon monoxide in the home or at the place of work.

B. AIMS AND HYPOTHESES

The general aim of the study of the effects of carbon monoxide on commuters in Los Angeles is to determine whether the levels encountered in normal commuting are high enough to have a significant effect on health. Exposure to carbon monoxide was measured by sampling the air within each automobile during morning and afternoon commuting trips. The effect was measured by determining the carbon monoxide concentration in expired air

before and after each trip as an indirect measurement of carboxy-hemoglobin in the blood.

Collection of the air within the car and collection of the expired air specimens was done by each participant in the study, who was taught the appropriate techniques. Ten percent of the expired air specimens were validated by comparison with duplicate samples collected by an experienced technician. Five percent of the validation expired air samples were further validated by comparison with determinations of percent carboxyhemoglobin in corresponding blood specimens.

Seventy-five individuals participated in the study; most were employees of the Los Angeles County Department of Water and Power, the remainder were employed by the Los Angeles Police Department. The collection of samples was carried out during six one-week periods between August, 1972 and March, 1973. Two of these weeks occurred during the period of expected low carbon monoxide concentration in the Los Angeles area; four occurred during the period of expected high carbon monoxide concentration.

The original 60 participants were divided into three groups commuting from three general areas into downtown Los Angeles. These are the south coastal region, the San Fernando Valley, and the Los Angeles foothills. The remaining 15 participants were used as replacements for dropouts. Information on smoking, additional travel by car, and other possible exposures to carbon monoxide were obtained from each participant to aid in the evaluation of the role of exposure during commuting.

The analysis of the data consists primarily of examining 1) the relation between the original expired air samples and the validation expired air and blood samples, 2) the differences in mean carbon monoxide concentration in expired air taken before and after commuting, and 3) the relation between exposure and change in expired air carbon monoxide during commuting. Other factors which might represent individual exposures or differences in uptake and excretion of carbon monoxide were found to not differ significantly. Comparisons among the three geographic areas are also made for ambient and expired air carbon monoxide.

C. METHODS

1. Data Collection.

a. Subcontract with Environmental Measurements Incorporated.

In order to facilitate field work including obtaining the cooperation of employment groups, instructing

participants, and coordinating field operations and collection of specimens, arrangements were made to subcontract this portion of the study. Requests for proposals were mailed to several private firms, and competitive bidding was invited. Discussions were held with the low bidder, Environmental Measurements Incorporated (EMI), and the principal investigator and staff were satisfied that this bidder could satisfactorily carry out the work within the budget submitted. Close communication between EMI and the study staff insured that appropriate methods were being used, and a member of the Department staff was in the field with EMI personnel during at least part of each week of the study. The arrangement worked very well, and EMI is to be commended for their role in the study. A copy of the subcontract is appended (Exhibit A).

b. Selection of Geographic Areas.

The three geographic areas were selected to represent different ambient air and commuting conditions to downtown Los Angeles. They are the south coastal region, the San Fernando Valley, and the Los Angeles foothills. Cities included in each area are listed in Exhibit B.

c. The Los Angeles County Department of Water and Power was selected as the major participating employment group by the subcontractor with the approval of the study staff. This Department represents a work location in downtown Los Angeles and a large enough employment group to obtain suitable participants for the study. Officials who were initially contacted expressed considerable enthusiasm and interest in the study. The employees who served as participants proved to be reliable and interested. In order to meet quotas in certain smoking and commuting groups, several employees of the Los Angeles Police Department also participated. The Police Department is located close to the Department of Water and Power, representing similar commuting conditions and facilitating collection of specimens from the two locations.

d. Selection of Subjects.

A copy of the memorandum issued by the Department of Water and Power to solicit participants is appended (Exhibit C). This included a brief screening questionnaire. Individuals interested in participating were then interviewed in more detail, both to determine whether they would be suitable subjects and to obtain

additional data for use in interpreting and analyzing results.

Each participant was required to meet the following conditions: residence in one of the study areas, a one-way commuting time between 30 and 90 minutes, age between 18 and 65, no respiratory illness, no occupational driving, no use of motorcycle in driving to and from work, no smokers in a car being used by a nonsmoking participant.

Initially, 20 commuters from each of the three areas were enrolled in the study, with emphasis on nonsmokers. Although the original study design had limited the study to the effects of carbon monoxide on nonsmokers, it was agreed that inclusion of some smokers would be useful in estimating the relative effects of smoking and exposure to carbon monoxide. The period of the study was from August, 1972, through March, 1973. Replacement of some participants was necessary because of family emergencies, illness, and unwillingness to continue participating. However, no attempt was made to make replacements for lost time periods of less than a week. A description of the initial population sample and the replacements is given by sex and smoking habit in Table 1.

Missing values resulted from absence of the participant from work, failure of pumps to fill ambient air bags, leakage of collecting bags, or the participant forgetting to collect specimens.

e. Questionnaire Data.

The questionnaire administered by an interviewer to all participants is appended (Exhibit D). Information was sought on place of residence, age, occupation, distance to and from work and time required, driving pattern or speed, air conditioning in the car, commuting hours, smoking in the car, accustomed exercise, height, weight, present and past smoking history, type of housing, type of domestic heating, fuels used at home, and history of chronic respiratory conditions. An additional questionnaire was used to obtain information on the specific freeways used in commuting to and from work (Exhibit E). Instructions to interviewers are also appended (Exhibit F).

f. Consent Form.

In conformity to Federal and State practice, each participant was asked to sign an informed consent form (Exhibit G).

g. Instructions for Sampling by Participants.

Written instructions were given to each participant concerning collection of expired and ambient air samples, completion of the daily log (Exhibits H and I) and collection of expired air samples and blood samples.

The method used for collection of expired air was that requiring a deep inspiration followed by 20-second breath-holding, discarding the first portion, and blowing the remaining breath into a bag made from aluminized polyester film. During the five working days of each week of the study period, each participant was asked to collect expired air specimen before and after the morning and evening commuting trips. Results from twenty percent of the expired air samples which the participant collected after arriving at work in the morning and before leaving work in the afternoon (ten percent of the total expired air samples) were validated by the analysis of duplicate samples collected at work by a trained technician.

h. Pumps for Collection of Ambient Air.

Considerable experimentation was carried out to determine whether a commercially available pump was available which would be suitable for collecting ambient air in specimen bags during commuting trips. All pumps investigated were designed for other purposes and would have required some modification. After the data collection subcontract with EMI was negotiated, additional arrangements were made to buy a modified pump supplied by them. This proved to be satisfactory after some initial problems involving battery failure and occasional loose connections. These problems account for some of the "lost" measurements described elsewhere. An excerpt from the EMI newsletter describing the pump is appended (Exhibit J). Collection of air in the car throughout the commuting period was accomplished by an "on-off" pulsing mechanism rather than by continuous sampling. The frequency of the pulse was adjusted so that the collection bag is approximately filled over the average commuting time for each participant. Obvious disadvantages include the possibility of inadequate air samples during an unusually

short commute trip (unlikely), lack of sample [illegible] toward the end of an unusually long commute trip (also unlikely), and inability of the sample to represent rapid fluctuations in carbon monoxide concentration from time-to-time or place-to-place (possibly an advantage). Also, for short trips the air collected would represent a larger proportion of the air to which an individual was exposed than would air collected during long trips.

i. Commuting Log.

A sample of the commuting log is appended (Exhibit K). This was provided to obtain a more detailed represention of commuting conditions, including smoking in the car, than could be provided by the original questionnaire. Information from it was used to confirm the adequacy of the questionnaire data. The information collected on smoking during each commuting trip was not adequate to evaluate the effect of smoking on changes in expired air carbon monoxide concentration during individual commuting trips.

j. Blood Specimens.

The blood drawing schedule is appended (Exhibit L). For logistical reasons, no blood drawing was done on Monday mornings or Friday afternoons. Collection was scheduled on Tuesdays, Wednesdays, and Thursdays so that both morning and afternoon expired air samples on each day of the week could be validated by carboxyhemoglobin determination. Five percent of the total number of expired air specimens were validated in this way, but these represented only specimens taken at work. This aspect of the study was carried out by EMI under the supervision of Department staff. Phlebotomists from the Los Angeles County General Hospital were employed to draw blood specimens. Instructions for collection of the samples for carboxyhemoglobin determination are appended (Exhibit M). Specimens for methemoglobin analysis were also drawn for immediate analysis by a Department chemist in the field. If analysis was delayed for more than one-half hour, the specimen was discarded since methemoglobin is highly unstable.

2. Laboratory Analysis.

With the exception of the methemoglobin analysis, which was done in the field by a chemist from the Department's

With the exception of the methemoglobin analysis, which was done in the field by a chemist from the Department's Air and Industrial Hygiene Laboratory, all laboratory analyses were done in the Department's Berkeley laboratory. Descriptions of the laboratory methods are attached (Exhibit N). The laboratory analyses consisted of:

a. Determination of carbon monoxide concentration of expired and ambient air.

b. Determination of percent carboxyhemoglobin.

c. Determination of methemoglobin.

3. Statistical Analysis.

The basic statistical comparisons made are as follows:

a. Validation procedures are compared with data collection procedures by regression analysis.

b. Change in expired air carbon monoxide concentration during commuting is analyzed by comparison of group means before and after commuting.

c. Differences in effect of commuting from the three geographic areas are analyzed by comparison of group means.

d. The relation of concentration of carbon monoxide in ambient and expired air is explored by regression analysis.

D. Results

1. Number of Valid Samples

As mentioned earlier, it was not possible to obtain the number of valid laboratory results planned. However, there is no suggestion that bias has resulted, and the percent of adequate samples obtained should provide a guide for determining the number of possible samples needed in subsequent studies in order to obtain a desired number of valid results. The target number of valid laboratory samples per week is shown in Table 2, along with the actual number of valid samples obtained. Since analysis of the relation between ambient air carbon monoxide concentration and carbon monoxide levels in expired air before and after commuting is one of the analytical methods used, the lack of any one of these three measurements for a given commute trip limits the use of the other two.

2. Frequency Distributions

The distributions of concentration of carbon monoxide in expired air appear to be skewed and, in the case of smokers, bimodal. This is shown in Figures I-VIII separately for smokers and nonsmokers for each of the four samples collected during the day. The bimodal distribution in smokers appears to be related to the questionnaire responses as to the amount of smoking and whether the respondent smoked while commuting.

Ambient carbon monoxide distributions are also somewhat skewed (Figures IX and X). The use of normal theory is justified by the large numbers of observations.

3. Validation of Expired Air Technique

Regression analysis was carried out to estimate the relation between the laboratory results on expired air samples collected by the participants themselves and the results on validation samples collected by a trained observer. Differences observed could be due to faulty technique by the participant, such as rebreathing or not holding the breath for the required 20 seconds, or to a change in exposure to expired air between collection of the two samples. Smokers were asked not to smoke between their own collection of samples and collection of the validation samples.

The scatter diagrams in Figures XI-XVI show the relation between carbon monoxide concentration in self-collected samples and concentration in validation samples for all participants, for smokers, and for nonsmokers. Morning and afternoon data were analyzed separately. Nonsmokers are shown on an expanded scale since they are represented by smaller values. To aid comparison, the regression equations are summarized in Table 3. Correlation and regression coefficients are lower among nonsmokers than among smokers, especially in the afternoon. This may be because the carbon monoxide concentrations are at the lower end of the scale for nonsmokers. No explanation can be offered for the very low coefficients among nonsmokers in the afternoon. In the morning the self-collected samples were taken before the validation samples, in the afternoon the order was reversed. No information is available on the time elapsed between the two samples, but validation samples were taken in the medical unit just after arrival in the morning and before departure in the afternoon. If there is any significant difference between time elapsed in the morning and afternoon, one would expect that it would be greater in the morning since there could have been a short wait for the validation sample, adding to the time interval between the two. In the afternoon this would not effect the time interval, since the subject would proceed directly to his own car to collect his own sample. The regression coefficients were all less than 1.00, showing that, in general, the carbon monoxide concentrations in the self-collected samples were lower than those in the validation samples. This could reflect inadequate breath holding or rebreathing during self-collection of samples.

It was found that some of the pairs of samples with large differences represent a few individuals, raising the question of consistent faulty technique in the self-collected specimens. These observations, however, were not eliminated before analysis of the data.

4. Validation of Expired Air Carbon Monoxide Concentration as an Indicator of Percent Carboxyhemoglobin

Similarly, regression analysis was used to estimate the relation between expired air carbon monoxide concentration and carboxyhemoglobin. Validation expired air samples were used rather than self-collected samples because both the validation samples and the blood specimens were taken in the medical unit with a very short time interval between. Scatter diagrams are shown in Figures XVII and XVIII. The regression equations and correlation coefficients are shown in Table 4. The correlations are high, and the regression coefficients are close to those found by other investigators.

5. Comparisons of Mean Carbon Monoxide Values by Season and Sex

Expired air carbon monoxide concentrations were cross-classified by season (weeks 1-2, weeks 3-6), by smoking (non-smokers, smokers), time of day (morning, afternoon), relation to commuting (before, after), and sex. Means were calculated for each of the thirty two resulting cells shown in Table 5. Differences between means were tested for statistical significance for each variable in turn, within each cell of the classification by the remaining variables.

6. Sex Differences

The results of tests of differences between mean values in men and women show that two of the eight t-values for nonsmokers were significant at the 5% level, one of these indicating that men had the higher mean, the other indicating a lower mean. Of the six differences which were not statistically significant, half the means were higher for men, half were lower. Among smokers, men had lower means in all groups; two differences were significant at the 5% level, two at the 1% level. This could be a reflection of differences in smoking habits. The results suggest that no intrinsic sex difference exists of detectable magnitude.

7. Seasonal Differences

Seasonal differences were significant at the 5% level (most at the 1% level) for all but three of the 16 differences tested, supporting the assumption that the last four weeks of the study represent relatively high ambient carbon monoxide levels compared to the first two weeks.

8. After - Before Commuting Differences

The effect of commuting on expired air carbon monoxide concentration was measured by comparing the mean values before and after commuting. In the morning all but one of the eight differences tested were statistic-

ally significant at the 5% level. All differences, including the one not statistically significant, represent an increase in expired air carbon monoxide concentration during commuting. The afternoon differences were inconsistent; three of the eight were statistically significant and represent an increase during commuting. Four of the five non-significant differences represent a small decrease in carbon monoxide concentration.

The percent of observations showing an increase in expired air carbon monoxide concentration during commuting is shown by smoking, sex, and geographic area in Table 6. Between 70% and 90% of the observations in each group showed an increase in the morning; corresponding percents were considerably smaller in the afternoon.

9. Comparison by Area

For nonsmokers, mean carbon monoxide concentrations in expired and ambient air were analyzed by analysis of variance. Area differences in expired air were statistally significant for the post-commuting samples for both men and women. Differences in pre-commuting means were significant only for men and only in the morning (Table 7). The change in expired air carbon monoxide concentration showed significant area differences only among men. Area differences for ambient air were small, but all were statistically significant (Table 8). In spite of the statistical significance of area comparisons, however, the lack of consistency in the direction of the differences between areas suggests that commuters from any one area as defined in this study do not experience consistently greater uptake of carbon monoxide than commuters from any other area.

No analysis was attempted of area differences among smokers since it was not possible to evaluate exposure to cigarette smoking during commuting.

10. Smoker-Nonsmoker Differences

For comparable sets of observations, smokers show two to four times as great a concentration of mean carbon monoxide as do nonsmokers.

The morning post-commuting mean carbon monoxide concentration in nonsmokers is almost twice that of the pre-commuting values. The relative increase in smokers is smaller because of their generally higher pre-commuting values. Consistent increases in mean values were not observed during the afternoon in either smokers or nonsmokers.

11. Relation Between Expired Air and Ambient Air Carbon Monoxide Concentration

Regression analysis was carried out on several subsets of observations. The most desirable method would be to regress change in expired air carbon monoxide on change in ambient carbon monoxide. However, initial ambient values at the start of the trip were not available. Lacking these, three models were tried using the available data. These were regression of post-commuting expired air carbon monoxide concentration (1) on ambient concentration, (2) on time-weighted ambient concentration, and (3) on pre-commuting expired air carbon monoxide concentration and ambient concentration.

If commuting time is long enough for equilibrium between ambient carbon monoxide and carboxyhemoglobin to have occurred, then the post-commuting expired air carbon monoxide should be approximately linearly related to the ambient carbon monoxide. This assumes, however, that the ambient concentration has remained constant over the entire commuting period, which it probably has not. The measurement actually used to represent exposure was ambient air concentration integrated over time. However, if commuting time is relatively short in terms of the time required for equilibrium, then use of a time-weighted ambient concentration and, in addition, limiting the analysis to those individuals who showed an increase in expired air CO concentration might give a better fit. In fact, results of both these analyses were quite similar (Table 9). For morning values, the analysis using unweighted ambient concentrations resulted in regression coefficients of 0.16 for nonsmokers and 0.24 for smokers. The corresponding values for regression on the time-weighted ambient concentrations were 0.13 and 0.22. The afternoon values for nonsmokers and smokers were, respectively, 0.11 and 0.31 for unweighted ambient concentrations and 0.10 and 0.34 for weighted ambient concentrations. Correlation coefficients for unweighted ambient concentrations were, for nonsmokers and smokers respectively, in the morning, 0.32 and 0.15; and in the afternoon 0.27 and 0.15. Corresponding coefficients for weighted ambient concentrations were, in the morning, 0.30 and 0.18, and, in the afternoon, 0.34 and 0.22. Thus weighting the ambient concentrations by time of exposure appears to have little effect on the results of regression analysis.

A third analysis was carried out using post-commuting expired air values as the dependent variable and both the unweighted ambient values and the pre-commuting expired air value as independent variables. For both smokers and nonsmokers the regression on pre-commuting expired air values was greater than that on ambient values. Corresponding partial correlation coefficients were also greater for pre-commuting expired air values than for ambient values except for nonsmokers in the afternoon.

The relation between post-commuting and pre-commuting expired air values was stronger for smokers than for nonsmokers, while the relation between post-commuting values and ambient values was generally weaker for smokers than for nonsmokers.

The multiple regressions were carried out with the pre-commuting variable entered first since the object was to estimate the additional effect of the ambient exposure. The addition of ambient exposure did increase the overall correlation in both smokers and non-smokers, but the increase in smokers was very slight.

In summary the post-commuting value depends mainly on the pre-commuting value for smokers, but mainly on the ambient value for non-smokers.

E. Summary and Conclusions

Based on comparisons of group means, there appears to be a definite increase in expired air carbon monoxide concentration during the morning commuting trip. No consistent change occurs during the afternoon trip.

Neither expired air carbon monoxide values nor ambient values show consistent area differences.

The regression of post-commuting expired air carbon monoxide values on ambient air values resulted in statistically significant regression and correlation coefficients. The results are virtually the same whether unweighted or time-weighted ambient values were used.

Multiple regression of post-commuting expired air values on pre-commuting values and on ambient air values shows that the post-commuting values depend more upon pre-commuting values than upon ambient concentrations of carbon monoxide experienced during commuting.

Smokers show consistently higher values of carbon monoxide than nonsmokers, and the effect of smoking is obviously greater than that of exposure to air during commuting at the carbon monoxide levels experienced in this study.

An evaluation of the health effects of the carbon monoxide levels reported here must await clarification of the role of the behavioral and physiological effects of low level exposure reported in the literature.

F. Tables

Table 1

Number of Participants by Area, Sex, and Smoking

Description	Area		
	I	II	III
Original Sample, Total	20	20	20
Male			
Nonsmokers	11	7	8
Smokers	3	5	6
Female			
Nonsmokers	5	6	4
Smokers	1	2	2
Replacements, Total	6	6	3
Male			
Nonsmokers	4	3	1
Smokers	1	1	-
Female			
Nonsmokers	-	-	2
Smokers	1	2	-

Table 2

Numbers of Observations
Compared with Target Numbers

Type of Observation	Target Number	Suitable for Analysis	
		Number	Percent
Expired Air			
Single Observations	7200	6348	88
Differences (After-Before)	3600	2913	81
Ambient Air	3600	3066	85
Validation Expired Air	720	633	88
Bloods for COHb	360	318	88
Comparisons			
Expired Air vs Validation Expired Air	720	606	84
Validation Expired Air vs Blood COHb	360	303	84
Expired Air Differences vs Ambient Air	3600	2907	81

Table 3

Validation of Expired Air Technique
by Collection of Duplicate Samples

Description	Regression Equation	Correlation Coefficient	N
Morning			
Total	Y = 5.07 + 0.72 X	0.69	323
Nonsmokers	Y = 6.65 + 0.46 X	0.36	223
Smokers	Y = 9.54 + 0.61 X	0.54	100
Afternoon			
Total	Y = 3.63 + 0.75 X	0.80	283
Nonsmokers	Y = 7.64 + 0.13 X	0.11	203
Smokers	Y = 9.18 + 0.64 X	0.68	80

Note:
Y represents CO concentration, self-collected sample.
X represents CO concentration, validation sample.

Table 4

Validation of Carbon Monoxide Concentration in Expired Air as an Indicator of Percent Carboxyhemoglobin

Description	Regression Equation	Correlation Coefficient	N
Morning	Y = 0.02 + 4.34 X	0.90	163
Afternoon	Y = 1.04 + 3.91 X	0.88	140

Note:
Y represents CO concentration, validation sample.
X represents percent carboxyhemoglobin.

Table 5

Mean Carbon Monoxide Concentration in Expired Air
by Sex, Smoking, Season, Time of Day
(ppm)

Description	Male				Female			
	Weeks 1-2		Weeks 3-6		Weeks 1-2		Weeks 3-6	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
Nonsmokers								
Morning								
Before Commuting	5.46	0.16	6.33	0.13	5.17	0.18	6.37	0.22
After Commuting	9.88	0.26	10.71	0.21	10.77	0.37	11.29	0.30
Afternoon								
Before Commuting	8.34	0.27	8.96	0.16	7.94	0.24	9.39	0.33
After Commuting	8.25	0.21	9.72	0.15	7.52	0.24	9.34	0.27
Smokers								
Morning								
Before Commuting	14.73	0.83	17.79	0.71	15.68	1.77	21.65	1.48
After Commuting	22.00	1.08	24.23	0.99	25.88	2.19	29.27	2.06
Afternoon								
Before Commuting	26.24	1.55	32.09	1.29	32.00	2.62	32.28	2.25
After Commuting	24.76	1.45	29.42	1.24	26.67	2.47	32.81	2.09

Table 6

Percent of Observations With Increase in Expired Air Carbon Monoxide Concentration During Commuting

Description	All Areas N	All Areas % With Increase	Area I N	Area I % With Increase	Area II N	Area II % With Increase	Area III N	Area III % With Increase
Morning								
Nonsmokers	1015	85.8	415	84.1	337	84.6	263	90.1
Male	711	85.8	291	89.0	237	81.0	183	86.9
Female	304	85.9	124	72.6	100	93.0	80	97.5
Smokers	425	82.1	104	76.9	142	85.9	179	82.1
Male	326	82.8	59	89.8	114	83.3	153	79.7
Female	99	79.8	45	60.0	28	96.4	26	96.2
Afternoon								
Nonsmokers	1043	50.9	402	51.2	351	48.7	290	53.1
Male	740	53.0	299	51.2	243	48.1	198	61.6
Female	303	45.9	103	51.5	108	50.0	92	34.8
Smokers	430	39.1	95	46.3	151	41.1	184	33.7
Male	326	39.3	59	45.8	115	40.0	152	36.2
Female	104	38.5	36	47.2	36	44.4	32	21.9

Table 7

Area Comparisons of Mean Carbon Monoxide Concentration
in Expired Air
Nonsmokers
(ppm)

Description	Male					Female				
	Area I	Area II	Area III	F	d.f.	Area I	Area II	Area III	F	d.f.
Morning										
Before Commuting										
Mean	5.66	6.17	6.56	6.06**	2/789	6.16	5.96	5.56	1.28	2/323
Standard error	0.15	0.15	0.25			0.29	0.23	0.27		
After Commuting										
Mean	10.54	9.52	11.45	10.10**	2/794	12.06	10.40	10.87	4.62*	2/325
Standard error	0.28	0.24	0.35			0.41	0.38	0.42		
Afternoon										
Before Commuting										
Mean	9.08	8.44	8.68	1.93	2/791	8.91	9.39	8.10	2.84	2/321
Standard error	0.26	0.19	0.25			0.35	0.44	0.34		
After Commuting										
Mean	9.21	8.87	9.83	4.58*	2/791	8.64	9.50	7.59	8.45**	2/320
Standard error	0.19	0.19	0.26			0.32	0.36	0.29		

*Area differences significant at 5% level.
**Area differences significant at 1% level.

Table 8

Area Comparisons of Mean Carbon Monoxide Concentration
in Ambient Air and Change in Expired Air
Nonsmokers
(ppm)

	Male					Female				
	Area I	Area II	Area III	F	d.f.	Area I	Area II	Area III	F	d.f.
Ambient Air										
Morning										
Mean	18.43	16.94	19.59	4.30*	2/747	20.14	17.32	17.13	3.83*	2/309
Standard error	0.64	0.48	0.65			0.99	0.79	0.77		
Afternoon										
Mean	17.18	18.82	16.90	3.40*	2/783	18.67	19.49	16.57	3.50*	2/315
Standard error	0.51	0.52	0.64			0.77	0.74	0.89		
Expired Air Change[1]										
Morning										
Mean	4.78	3.35	4.96	8.85**	2/708	5.79	4.43	5.36	2.49	2/301
Standard error	0.25	0.23	0.42			0.47	0.40	0.50		
Afternoon										
Mean	0.13	0.47	1.24	3.25*	2/737	-0.35	0.37	-0.47	1.01	2/300
Standard error	0.32	0.32	0.33			0.47	0.46	0.42		

1 "After commuting" minus "before commuting".

*Area differences significant at 5% level.
**Area differences significant at 1% level.

Table 9

Simple Regression Analysis of Expired Air Carbon Monoxide Concentration
On Unweighted or Weighted Ambient Air Concentrations

Independent Variable	Regression Coefficient	S.E.	t	N	Correlation Coefficient	Proportion of Variance Cumulated
Ambient CO						
Morning						
Nonsmokers	0.16**	0.015	10.69	1033	0.32**	0.100
Smokers	0.24**	0.077	3.12	430	0.15**	0.022
Afternoon						
Nonsmokers	0.11**	0.012	9.18	1066	0.27**	0.073
Smokers	0.31**	0.097	3.19	437	0.15**	0.023
Time-weighted Ambient CO[1]						
Morning						
Nonsmokers	0.13**	0.014	9.47	904	0.30**	0.091
Smokers	0.22**	0.066	3.39	353	0.18**	0.032
Afternoon						
Nonsmokers	0.10**	0.010	9.31	679	0.34**	0.113
Smokers	0.34**	0.106	3.18	193	0.22**	0.050

1 Analysis using time-weight ambient values was limited to subjects showing an increase or no change in expired air carbon monoxide concentration.

*Significant at 5% level.
**Significant at 1% level.

Table 10

Multiple Regression Analysis of Post-Commuting Expired Air Carbon Monoxide On Pre-Commuting and Ambient Air Concentrations (ppm)

Independent Variables	Regression Coefficient	S.E.	t	N	Partial Correlation Coefficient	Proportion of Variance Cumulated	Multiple Correlation Coefficient
Morning							
Nonsmokers							
Before Commuting Expired Air	0.46**	0.046	10.00	1015	0.30**	0.110	0.41**
Ambient CO	0.12**	0.015	8.09	1015	0.25**	0.054	
Smokers							
Before Commuting Expired Air	0.80**	0.053	15.10	425	0.59**	0.345	0.61**
Ambient CO	0.23**	0.061	3.74	425	0.18**	0.021	
Afternoon							
Nonsmokers							
Before Commuting Expired Air	0.12**	0.027	4.50	1043	0.14**	0.042	0.31**
Ambient CO	0.10**	0.012	8.07	1043	0.24**	0.056	
Smokers							
Before Commuting Expired Air	0.59**	0.036	16.60	430	0.62**	0.407	0.64**
Ambient CO	0.11	0.068	1.55	430	0.08	0.003	

*Significant at 5% level.
**Significant at 1% level.

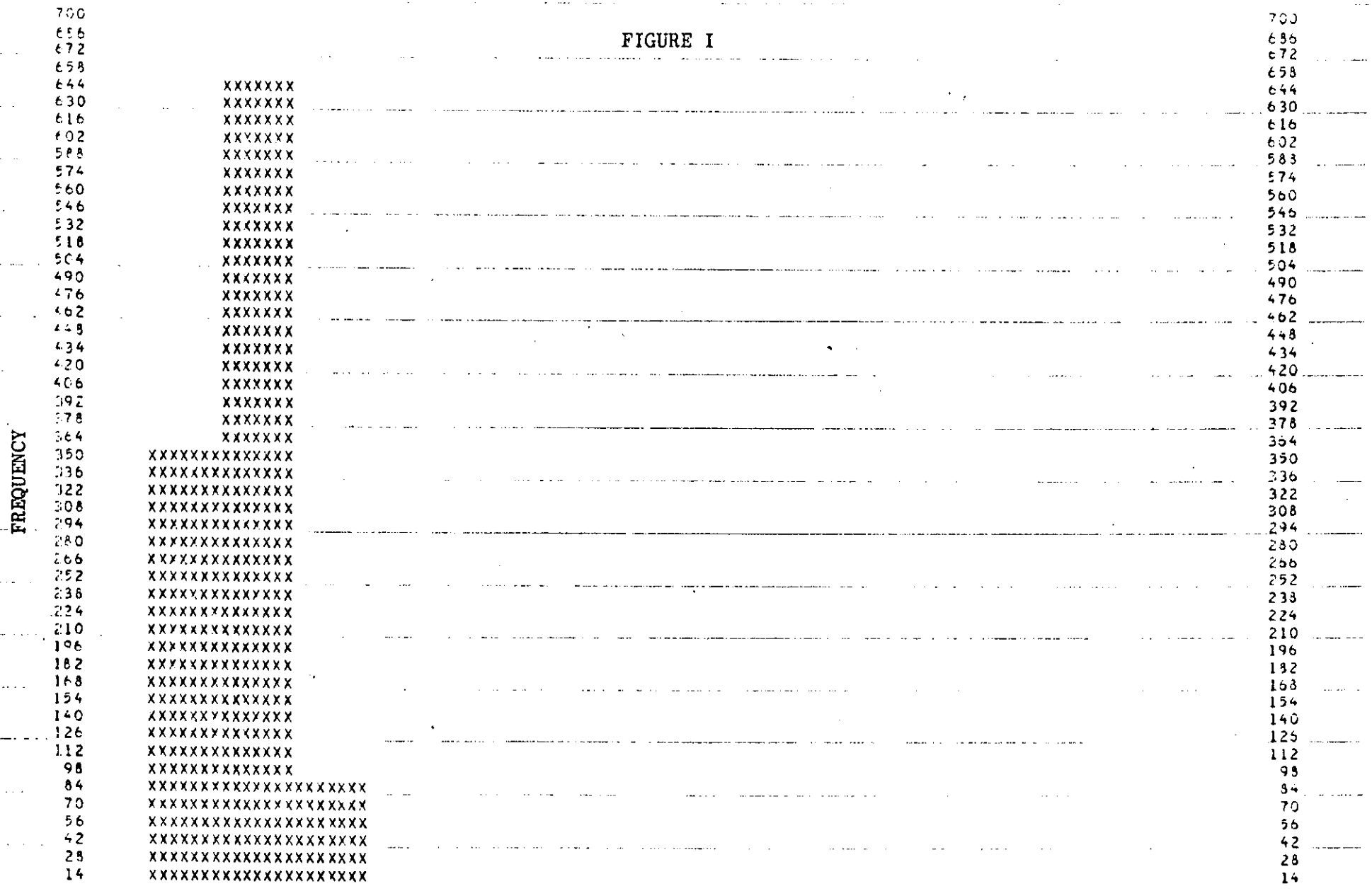
G. Figures

Figure XVI: Regression of Carbon Monoxide Concentration in Afternoon Self-Collected Expired Air on Concentration in Validation Samples, Smokers.

Figure XVII: Regression of Carbon Monoxide Concentration in Validation Expired Air Samples on Percent Carboxyhemoglobin, Morning.

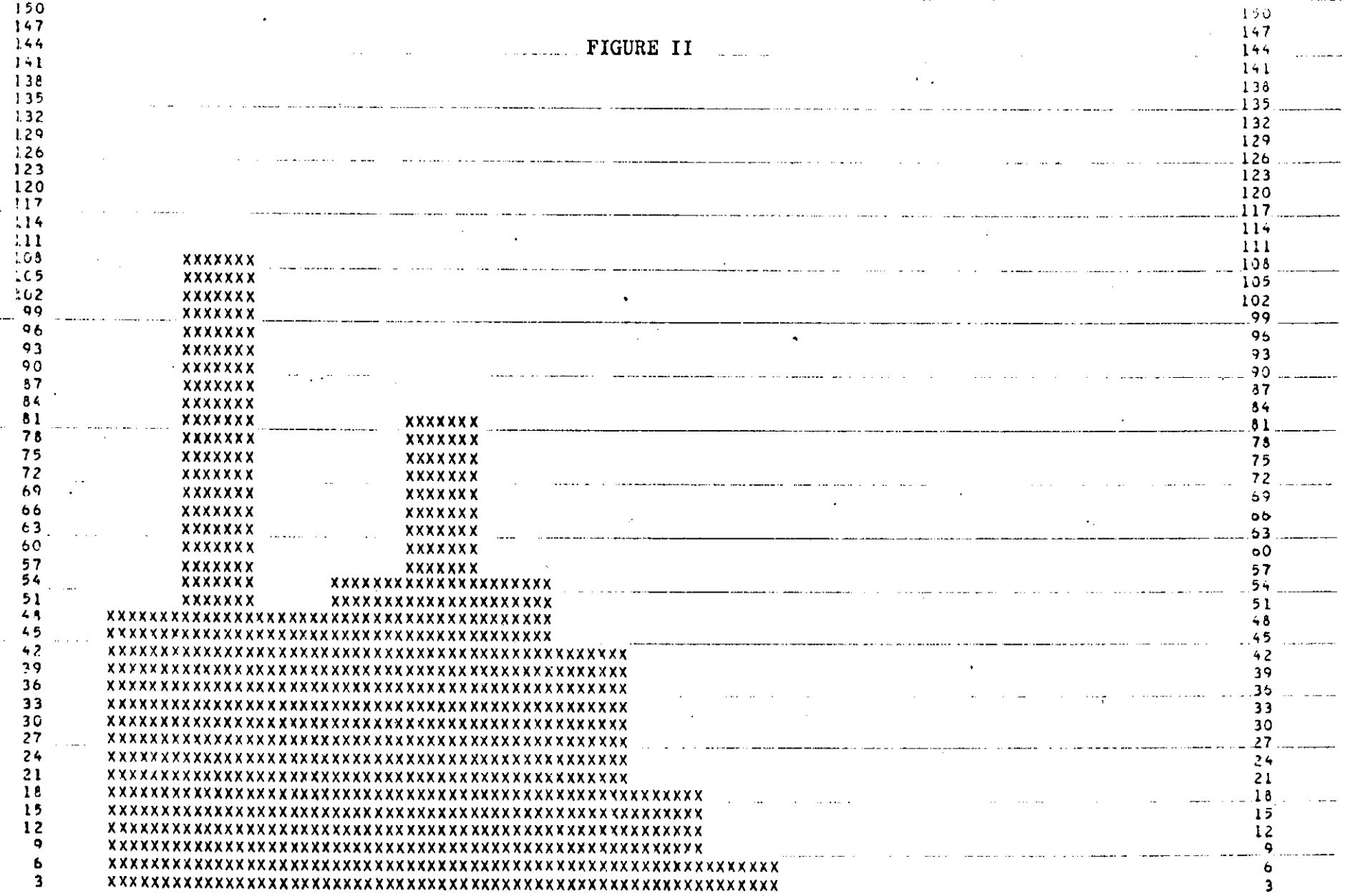
Figure XVIII: Regression of Carbon Monoxide Concentration in Validation Expired Air Samples on Percent Carboxyhemoglobin, Afternoon.

FIGURE I



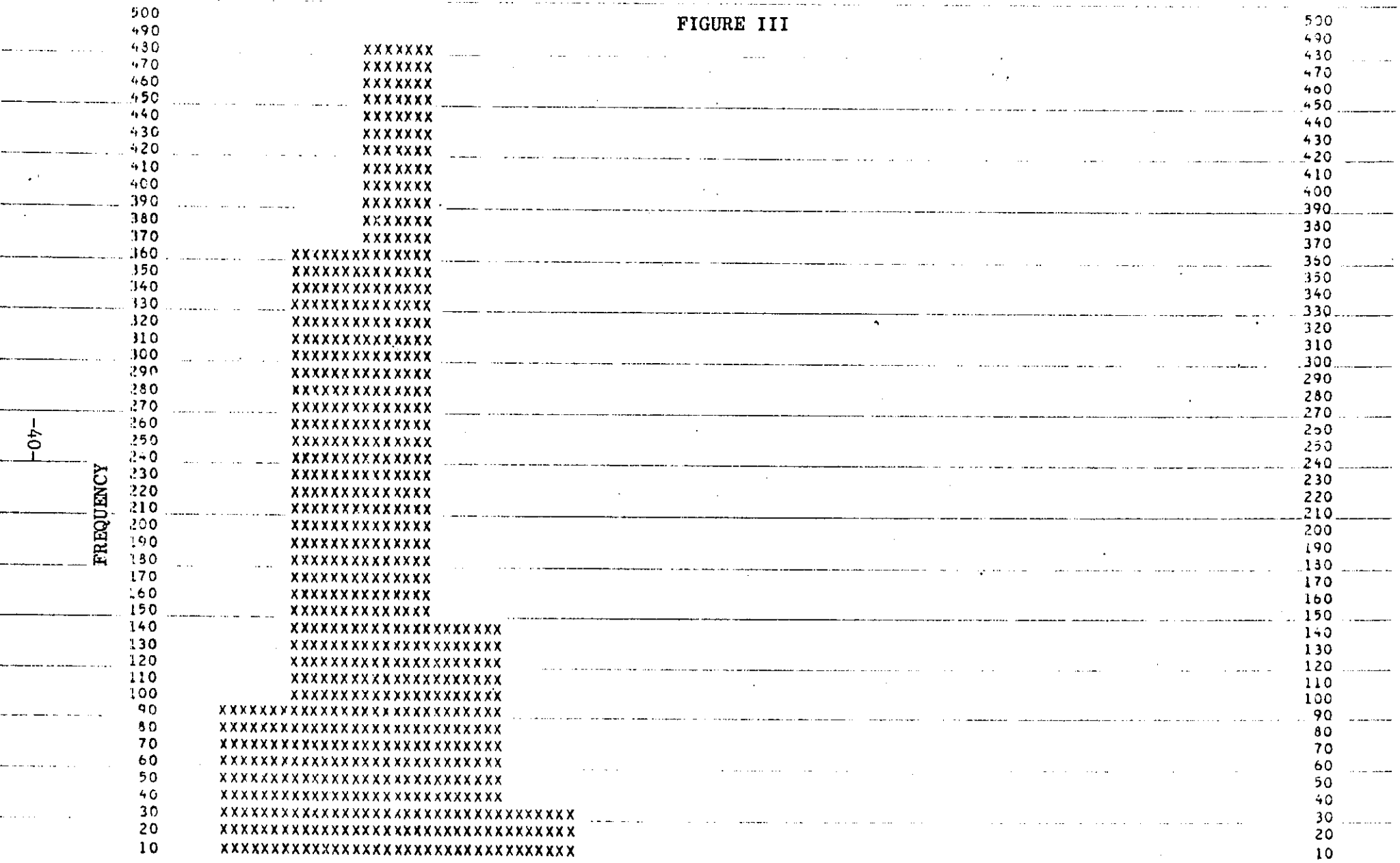
AM EXPIRED AIR AT HOME ALL NON SMOKERS

FIGURE II



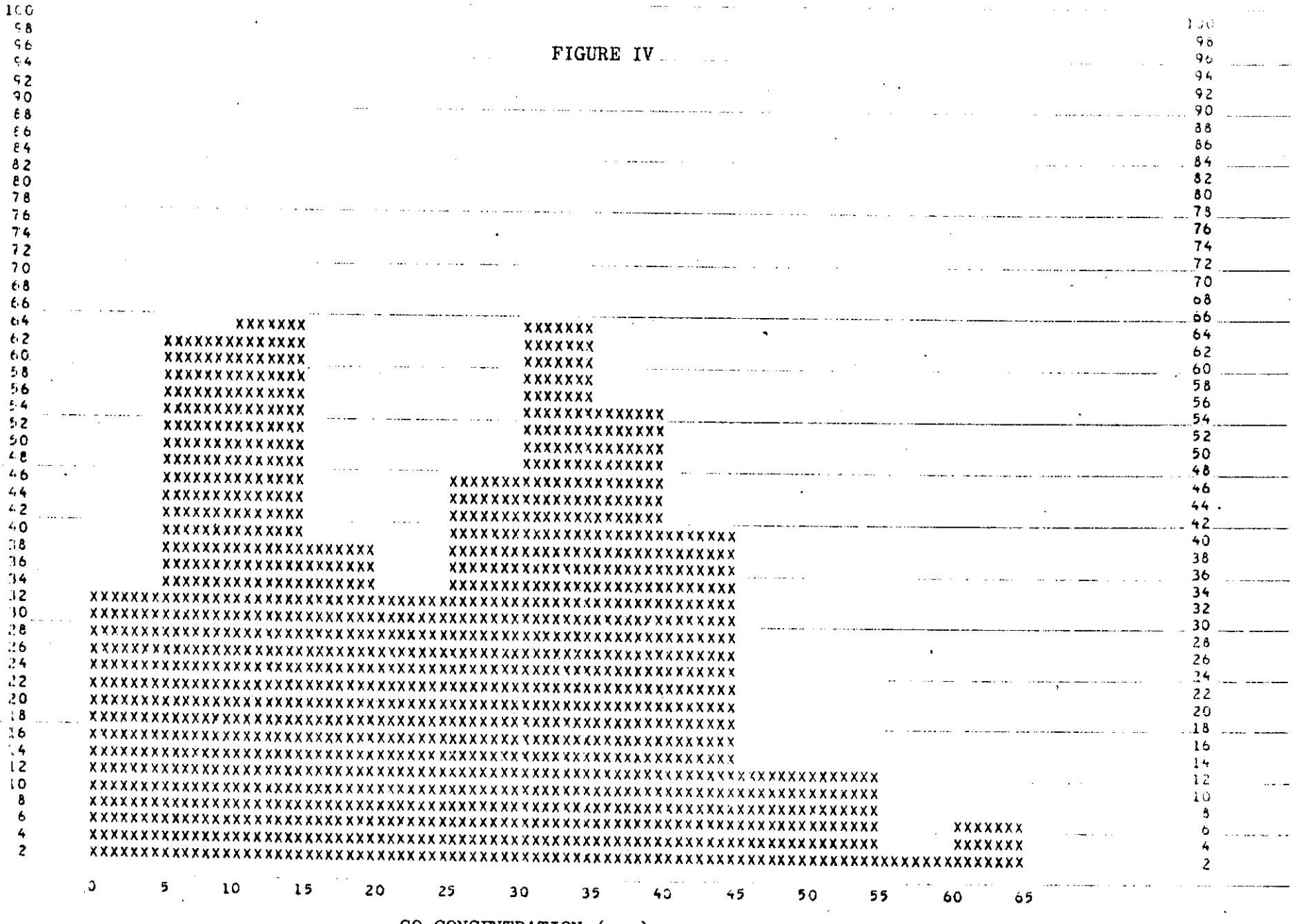
AM EXPIRED AIR AT HOME ALL SMOKERS

FIGURE III



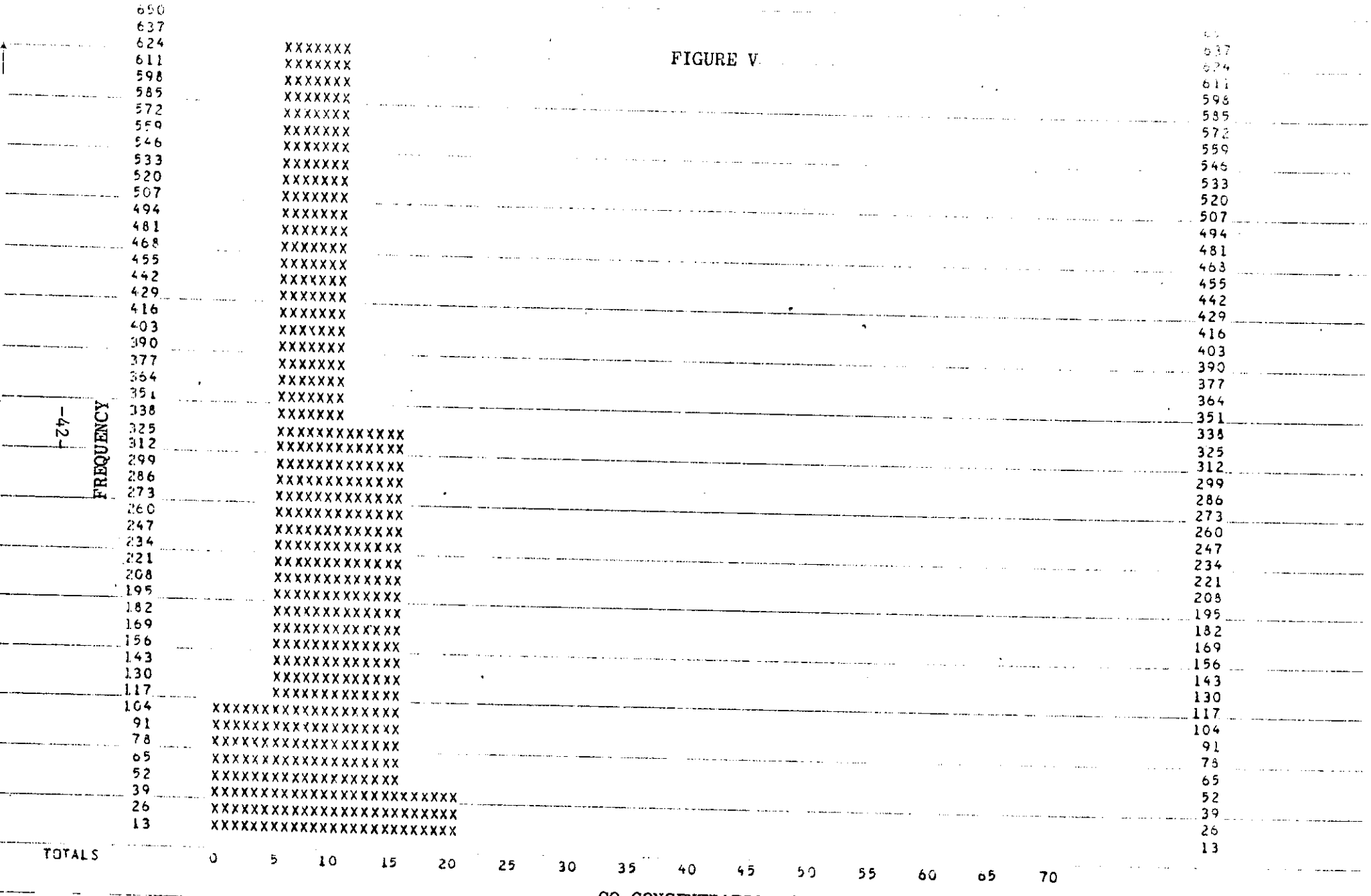
AM EXPIRED AIR AT WORK ALL NON SMOKERS

FIGURE IV



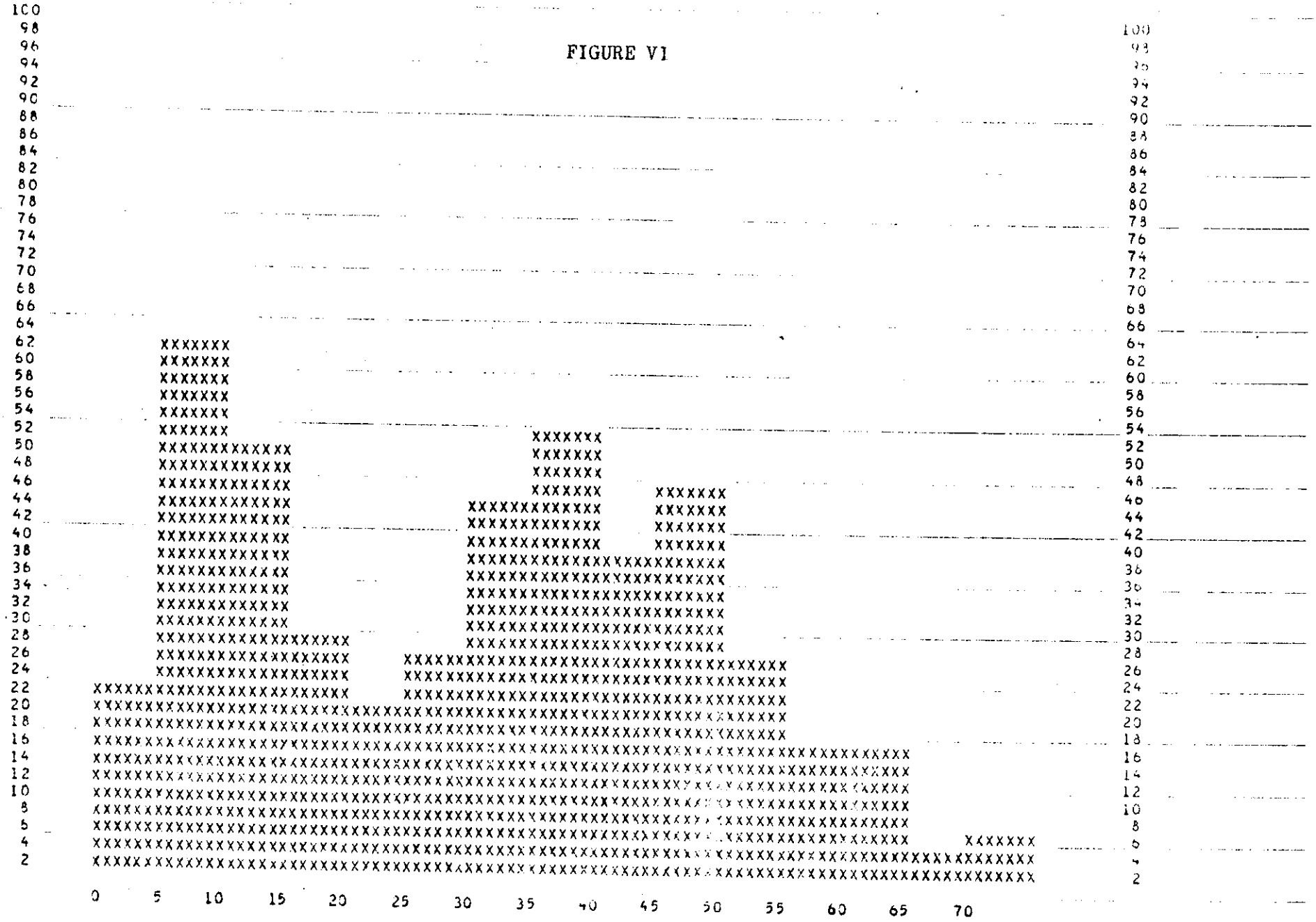
AM EXPIRED AIR AT WORK ALL SMOKERS

FIGURE V



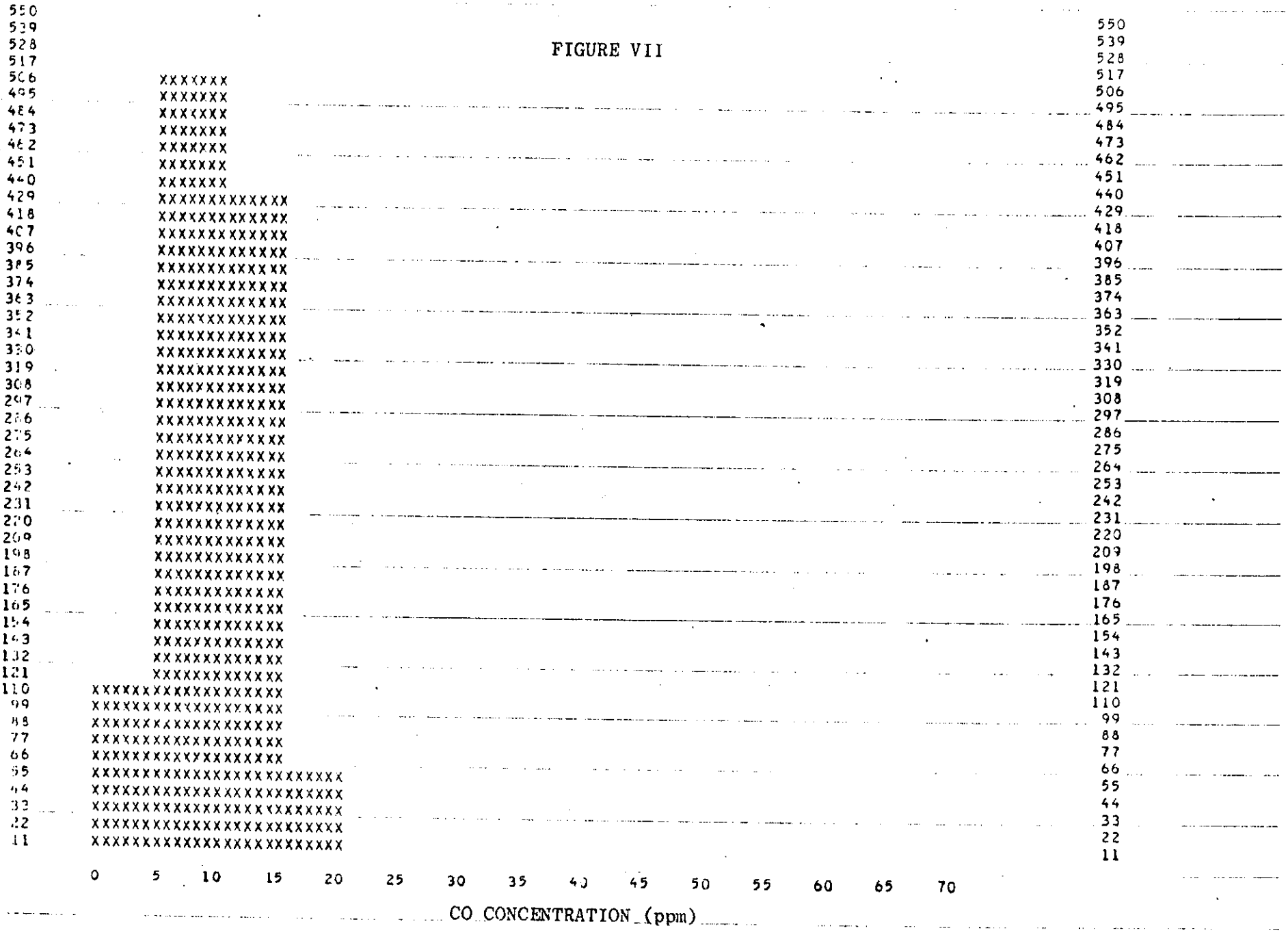
PM EXPIRED AIR AT WORK ALL NON SMOKERS

FIGURE V1



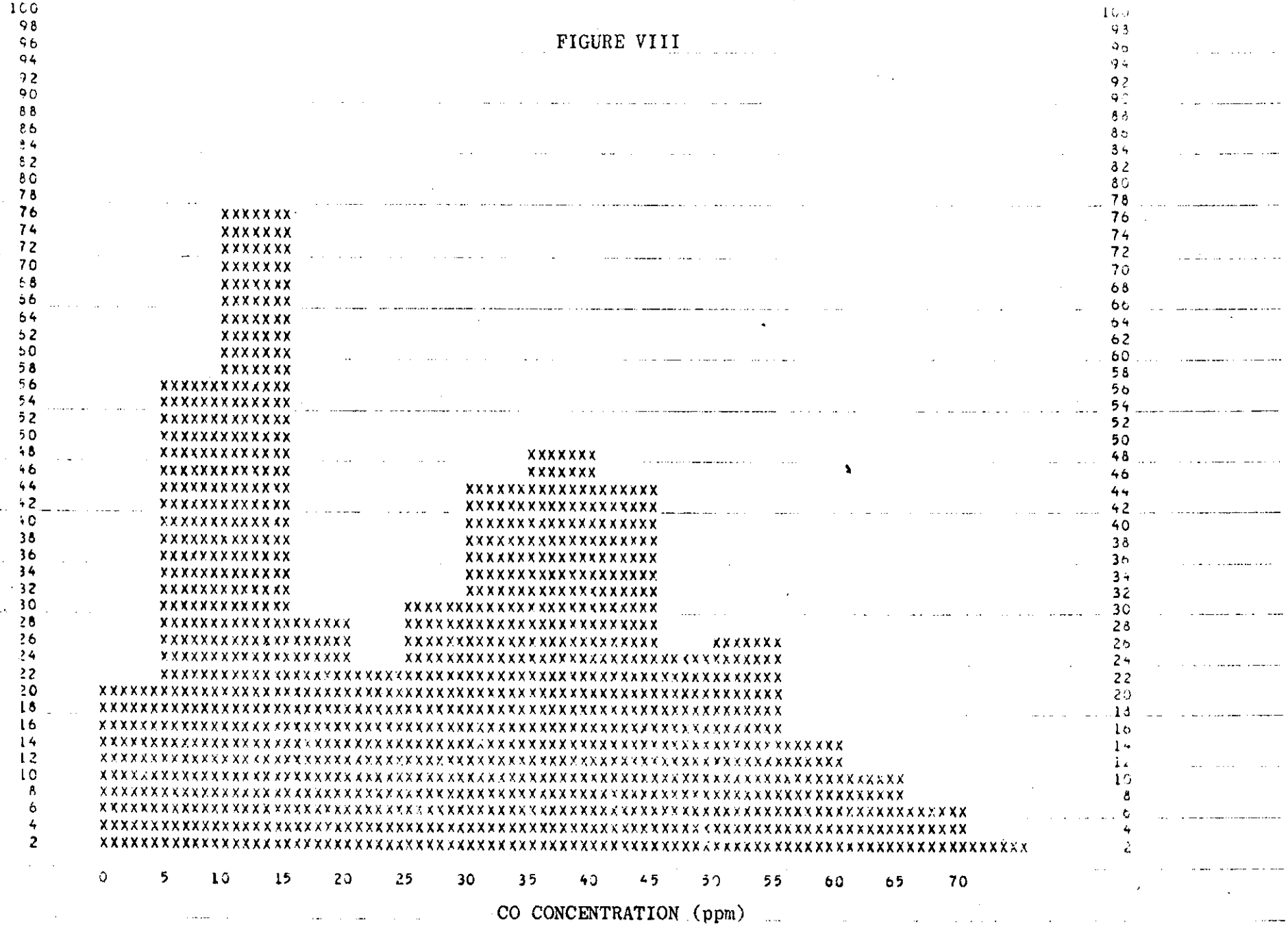
HISTOGRAM 3 PM EXPIRED AIR AT WORK ALL SMOKERS

FIGURE VII



HISTOGRAM 4 PM EXPIRED AIR AT HOME ALL NON SMOKERS

FIGURE VIII



HISTOGRAM 4 PM EXPIRED AIR AT HOME ALL SMOKERS

FIGURE IX

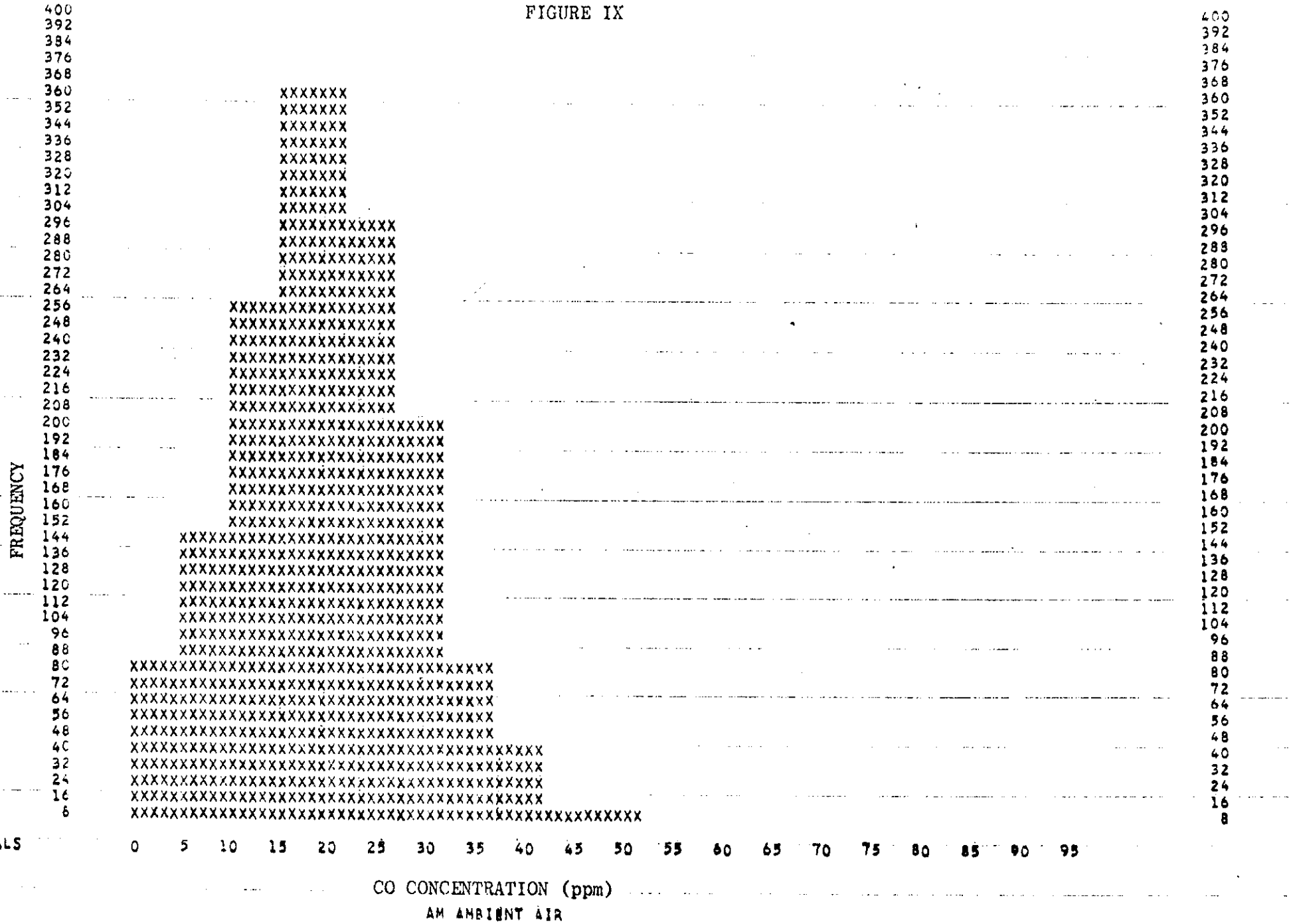

FREQUENCY
400
392
384
376
368
360
352
344
336
328
320
312
304
296
288
280
272
264
256
248
240
232
224
216
208
200
192
184
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
TOTALS
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95
CO CONCENTRATION (ppm)
AM AMBIENT AIR

FIGURE X

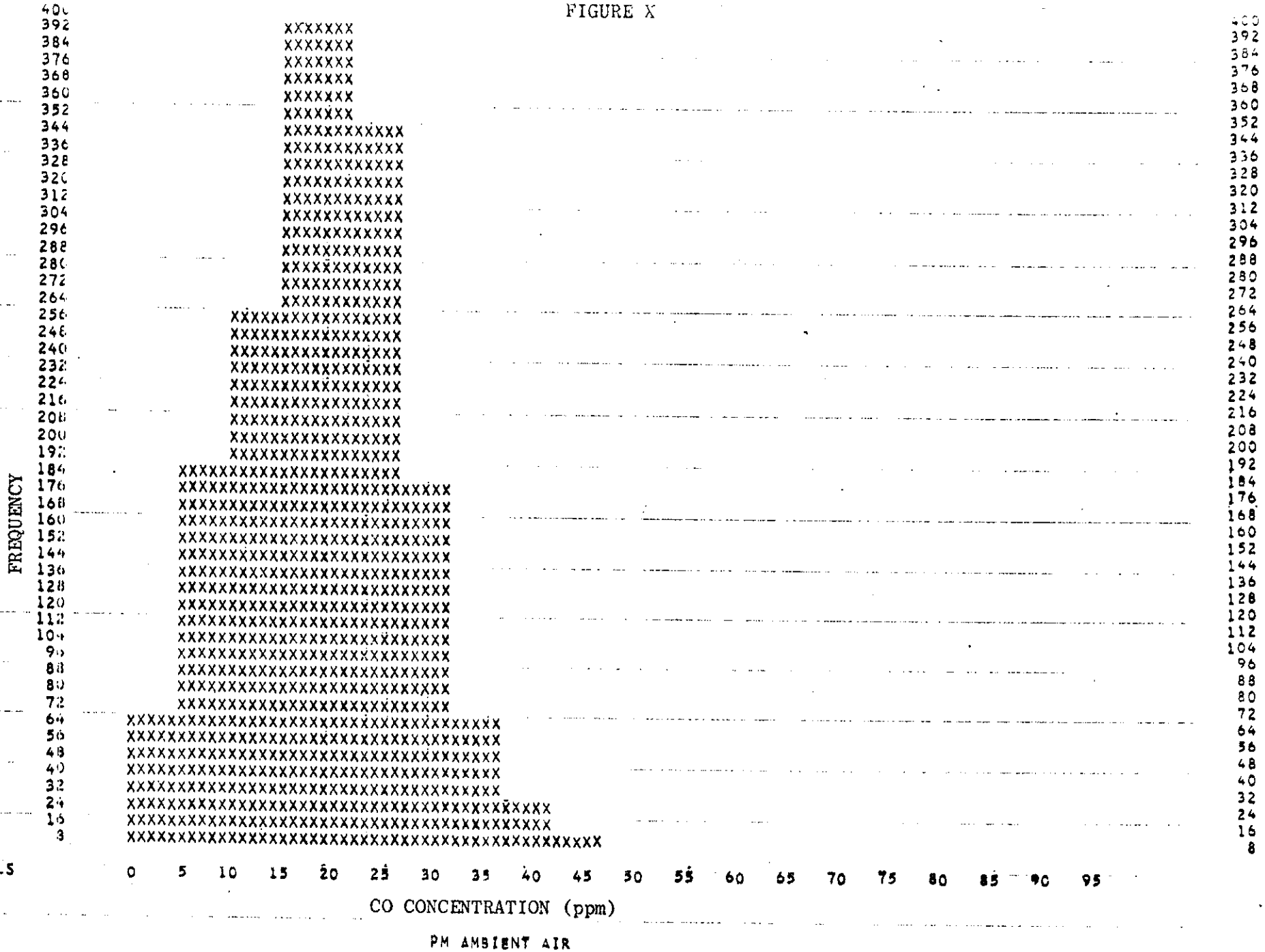

FREQUENCY
TOTALS
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95
CO CONCENTRATION (ppm)
PM AMBIENT AIR

FIGURE XI

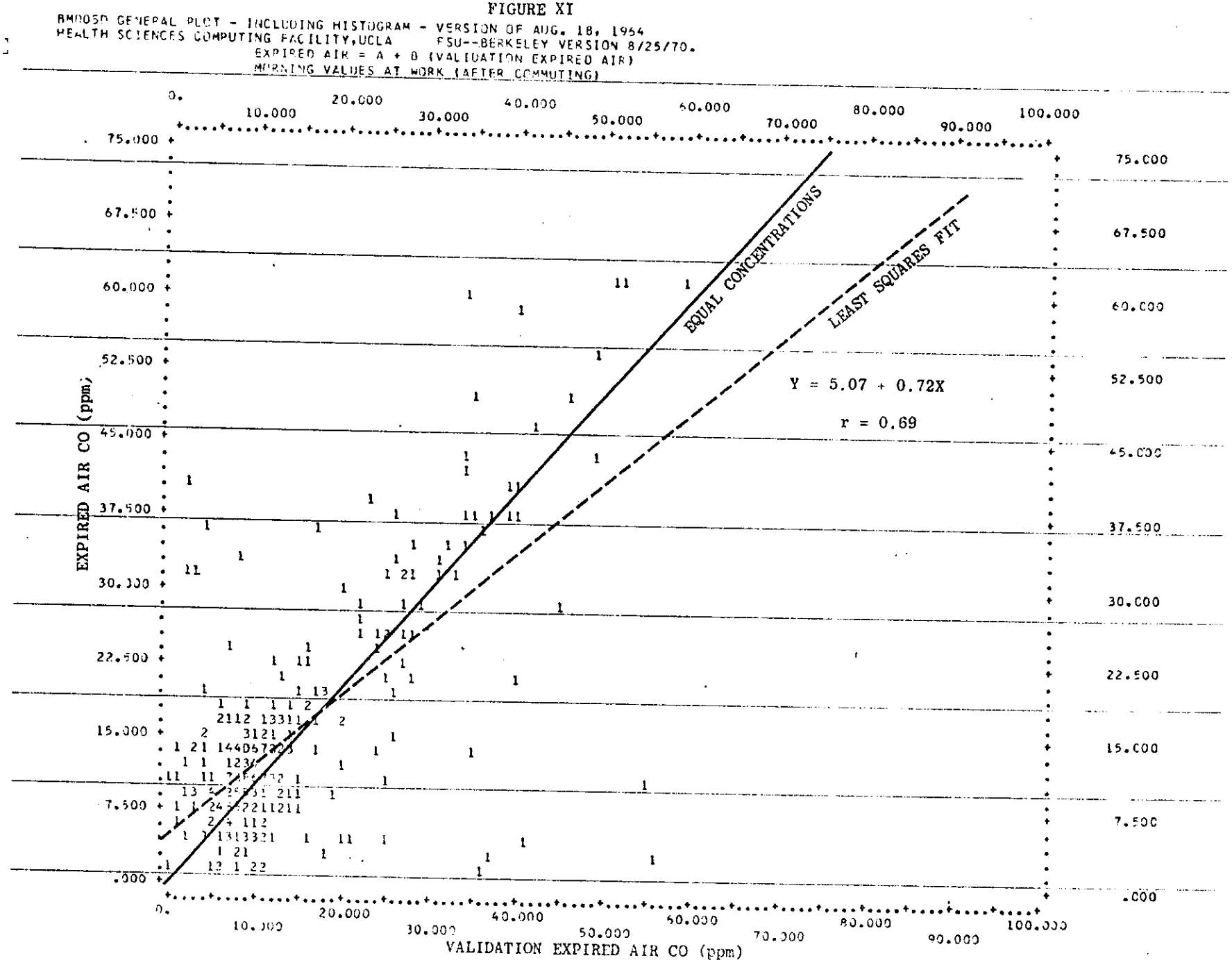

BMDO5D GENERAL PLOT - INCLUDING HISTOGRAM - VERSION OF AUG. 18, 1964
HEALTH SCIENCES COMPUTING FACILITY,UCLA FSU--BERKELEY VERSION 8/25/70.
EXPIRED AIR = A + B (VALIDATION EXPIRED AIR)
MORNING VALUES AT WORK (AFTER COMMUTING)
EQUAL CONCENTRATIONS
LEAST SQUARES FIT
Y = 5.07 + 0.72X
r = 0.69
EXPIRED AIR CO (ppm)
VALIDATION EXPIRED AIR CO (ppm)
0.
10.000
20.000
30.000
40.000
50.000
60.000
70.000
80.000
90.000
100.000
75.000
67.500
60.000
52.500
45.000
37.500
30.000
22.500
15.000
7.500
.000

FIGURE XII

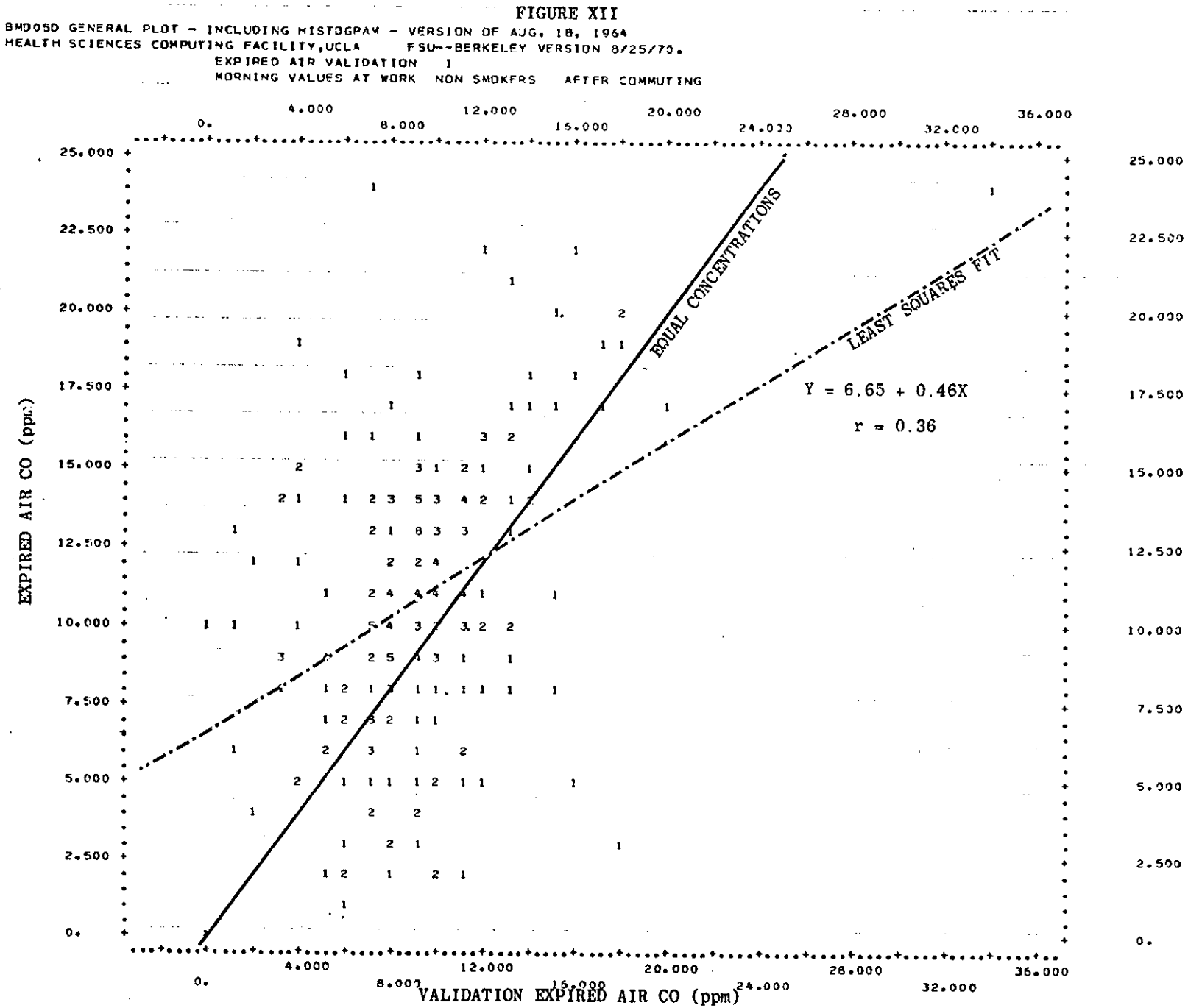

BMD05D GENERAL PLOT - INCLUDING HISTOGRAM - VERSION OF AUG. 18, 1964
HEALTH SCIENCES COMPUTING FACILITY, UCLA FSU--BERKELEY VERSION 8/25/70.
EXPIRED AIR VALIDATION I
MORNING VALUES AT WORK NON SMOKERS AFTER COMMUTING
EQUAL CONCENTRATIONS
LEAST SQUARES FIT
Y = 6.65 + 0.46X
r = 0.36
EXPIRED AIR CO (ppm)
VALIDATION EXPIRED AIR CO (ppm)
0.
2.500
5.000
7.500
10.000
12.500
15.000
17.500
20.000
22.500
25.000
4.000
8.000
12.000
16.000
20.000
24.000
28.000
32.000
36.000

FIGURE XIII

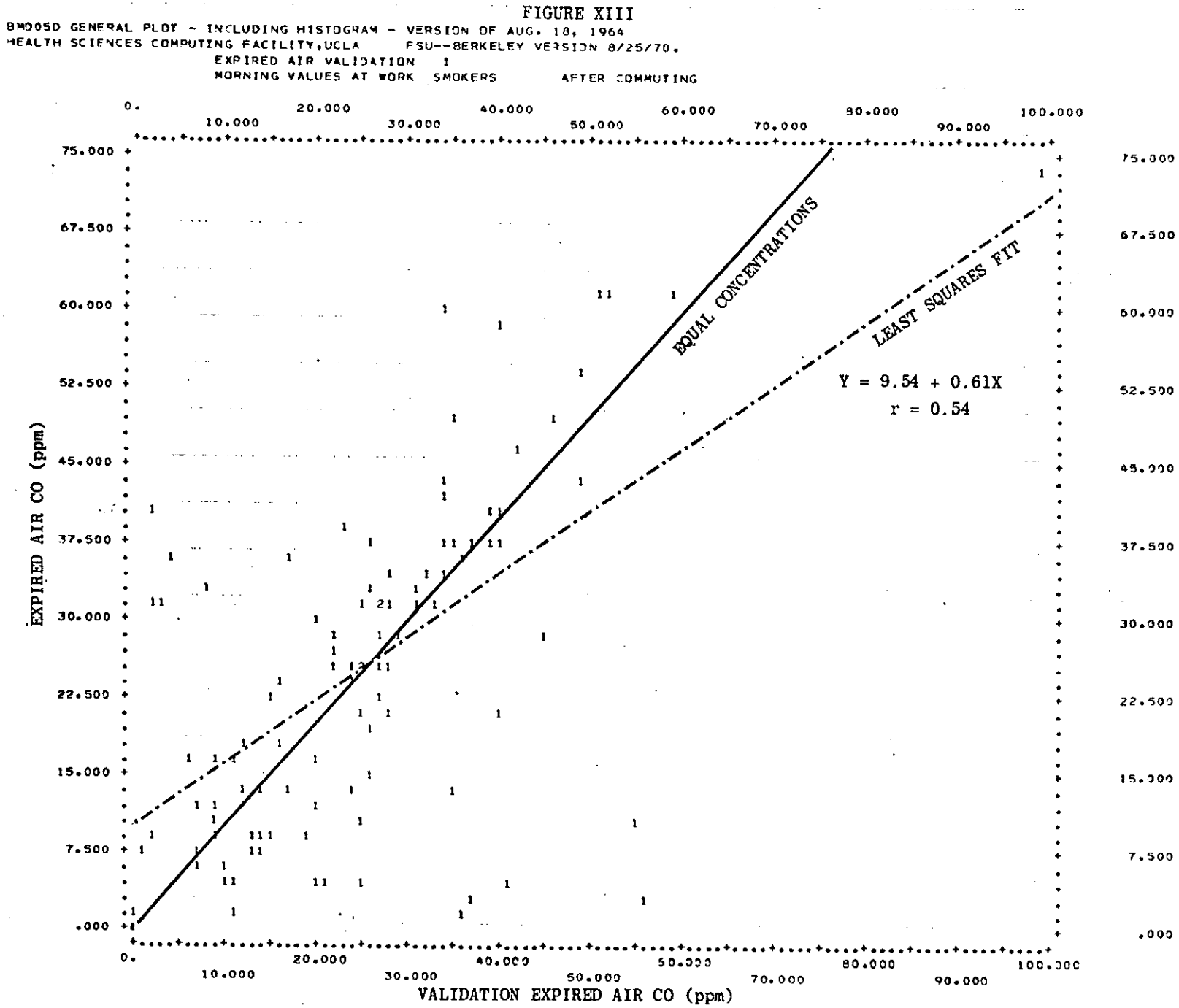

BMD05D GENERAL PLOT - INCLUDING HISTOGRAM - VERSION OF AUG. 18, 1964
HEALTH SCIENCES COMPUTING FACILITY,UCLA FSU--BERKELEY VERSION 8/25/70.
EXPIRED AIR VALIDATION 1
MORNING VALUES AT WORK SMOKERS AFTER COMMUTING
EQUAL CONCENTRATIONS
LEAST SQUARES FIT
Y = 9.54 + 0.61X
r = 0.54
EXPIRED AIR CO (ppm)
VALIDATION EXPIRED AIR CO (ppm)
0.
10.000
20.000
30.000
40.000
50.000
60.000
70.000
80.000
90.000
100.000
75.000
67.500
60.000
52.500
45.000
37.500
30.000
22.500
15.000
7.500
.000

FIGURE XIV

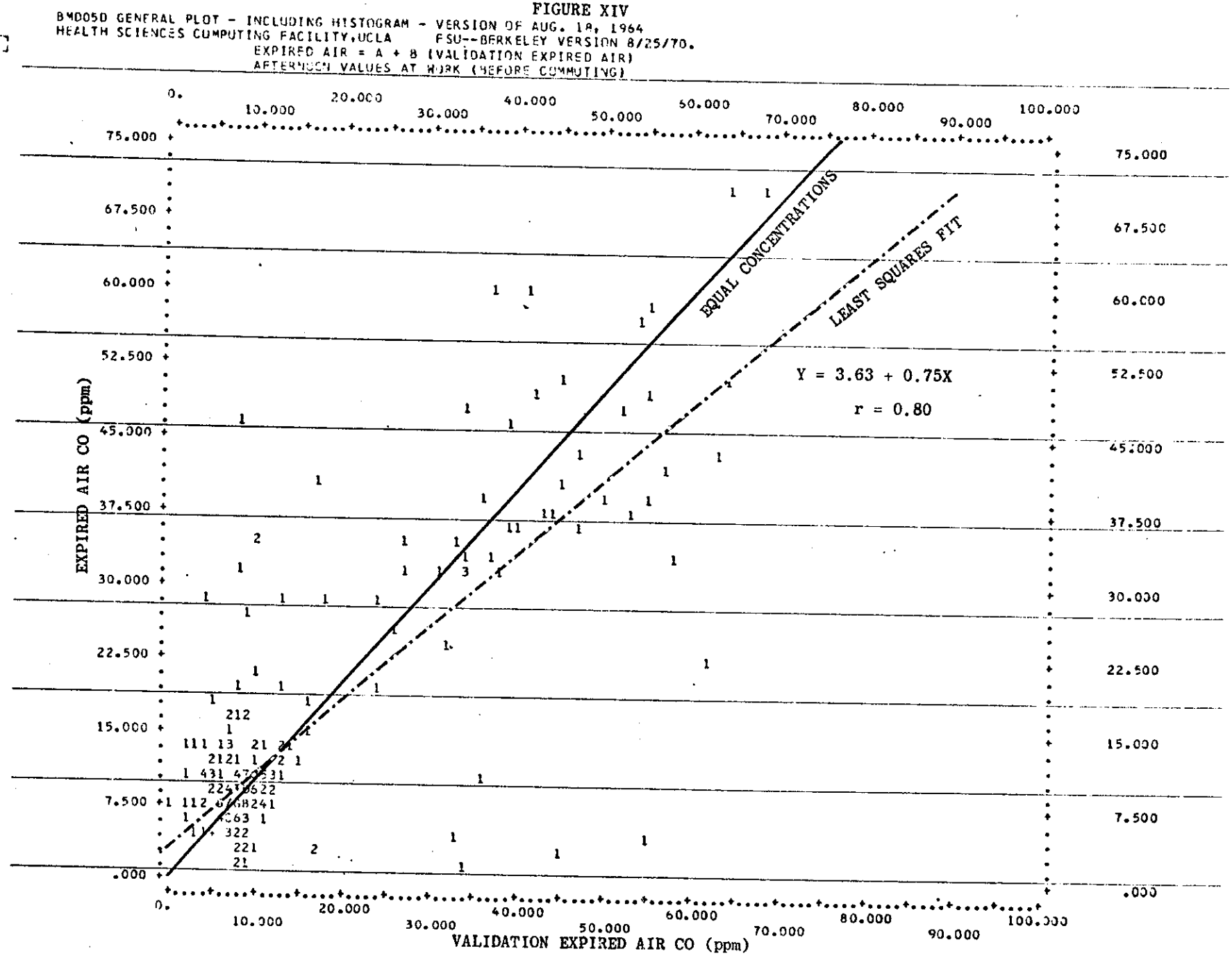
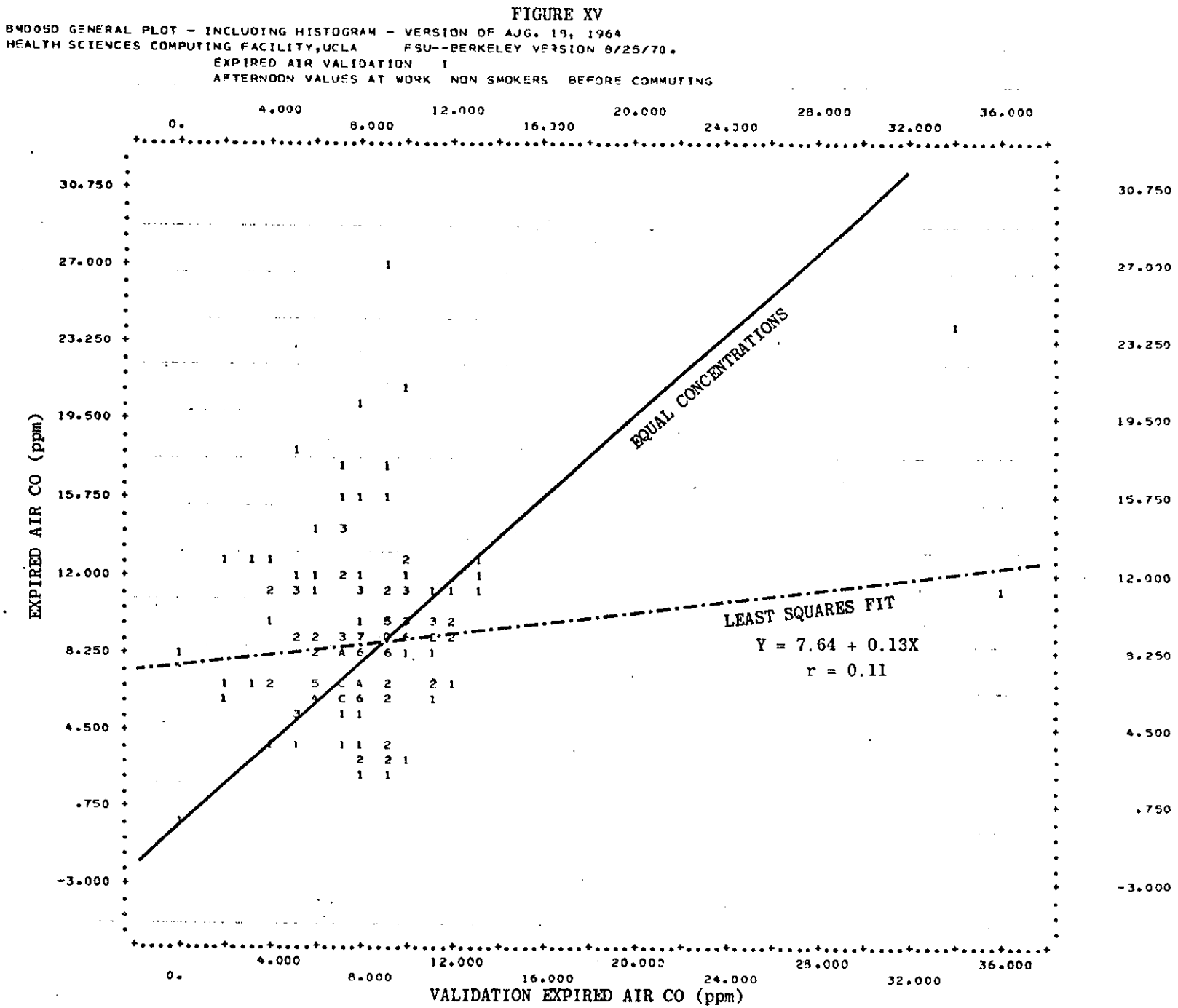

BMD05D GENERAL PLOT - INCLUDING HISTOGRAM - VERSION OF AUG. 18, 1964
HEALTH SCIENCES COMPUTING FACILITY,UCLA FSU--BERKELEY VERSION 8/25/70.
EXPIRED AIR = A + B (VALIDATION EXPIRED AIR)
AFTERNOON VALUES AT WORK (BEFORE COMMUTING)
EQUAL CONCENTRATIONS
LEAST SQUARES FIT
Y = 3.63 + 0.75X
r = 0.80
EXPIRED AIR CO (ppm)
VALIDATION EXPIRED AIR CO (ppm)
0.
10.000
20.000
30.000
40.000
50.000
60.000
70.000
80.000
90.000
100.000
75.000
67.500
60.000
52.500
45.000
37.500
30.000
22.500
15.000
7.500
.000

FIGURE XV


BMDO5D GENERAL PLOT - INCLUDING HISTOGRAM - VERSION OF AUG. 19, 1964
HEALTH SCIENCES COMPUTING FACILITY,UCLA FSU--BERKELEY VERSION 8/25/70.
EXPIRED AIR VALIDATION 1
AFTERNOON VALUES AT WORK NON SMOKERS BEFORE COMMUTING
EXPIRED AIR CO (ppm)
VALIDATION EXPIRED AIR CO (ppm)
EQUAL CONCENTRATIONS
LEAST SQUARES FIT
Y = 7.64 + 0.13X
r = 0.11
0.
4.000
8.000
12.000
16.000
20.000
24.000
28.000
32.000
36.000
30.750
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23.250
19.500
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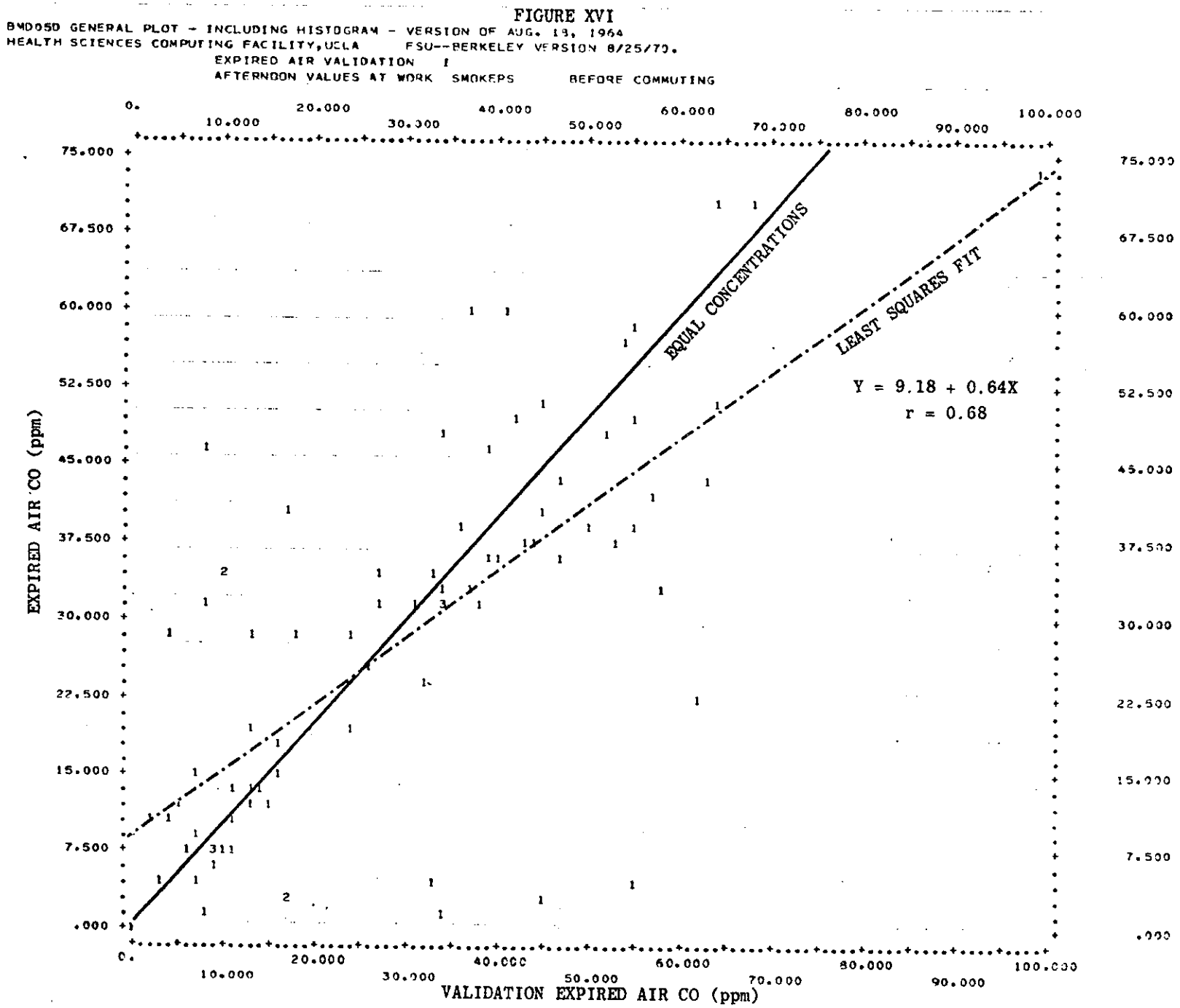
12.000
8.250
4.500
.750
-3.000

FIGURE XVI


BMD05D GENERAL PLOT - INCLUDING HISTOGRAM - VERSION OF AUG. 13, 1964
HEALTH SCIENCES COMPUTING FACILITY, UCLA FSU--BERKELEY VERSION 8/25/70.
EXPIRED AIR VALIDATION 1
AFTERNOON VALUES AT WORK SMOKERS BEFORE COMMUTING
EQUAL CONCENTRATIONS
LEAST SQUARES FIT
Y = 9.18 + 0.64X
r = 0.68
EXPIRED AIR CO (ppm)
VALIDATION EXPIRED AIR CO (ppm)
0.
10.000
20.000
30.000
40.000
50.000
60.000
70.000
80.000
90.000
100.000
75.000
67.500
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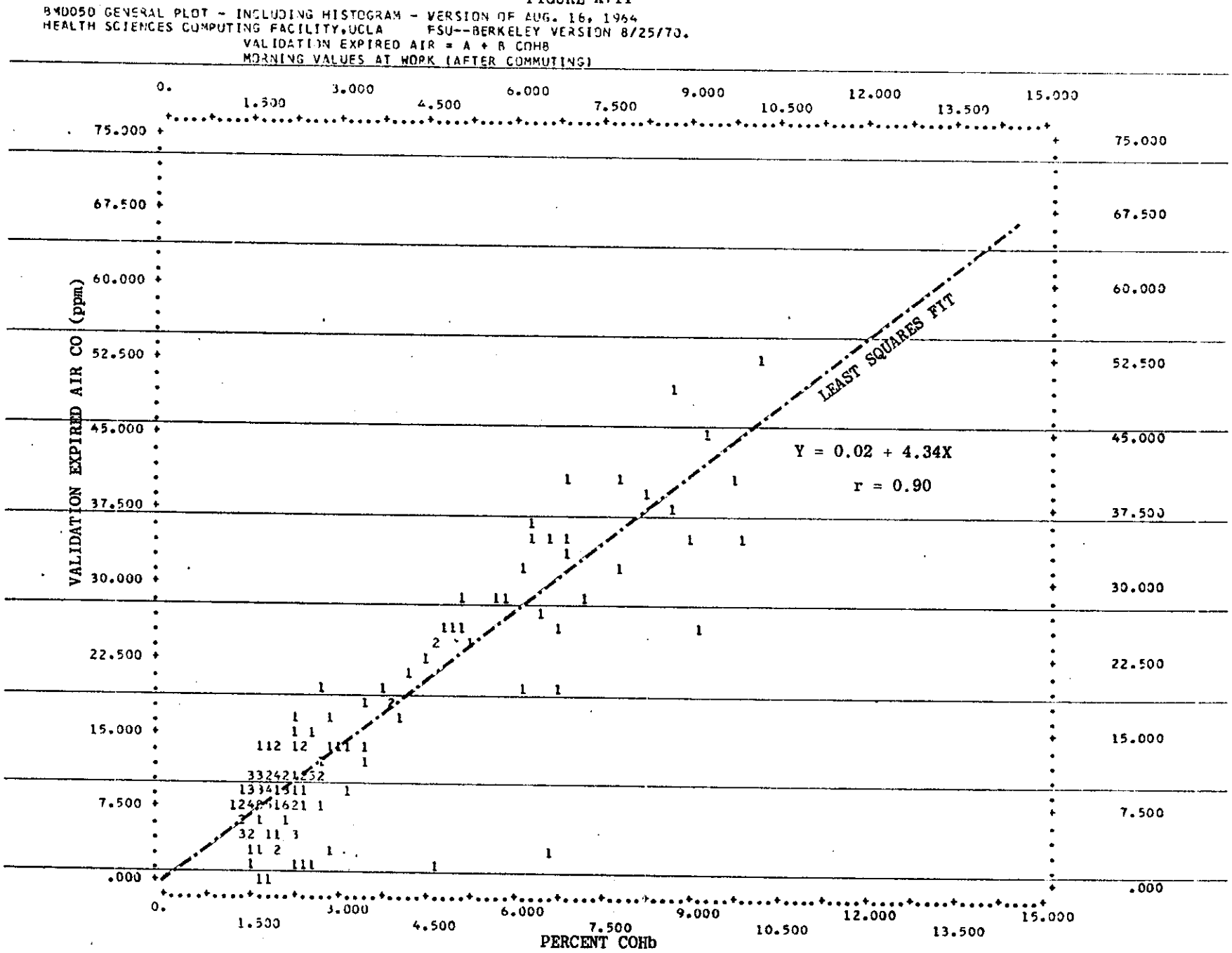
52.500
45.000
37.500
30.000
22.500
15.000
7.500
.000

FIGURE XVII


BMD05D GENERAL PLOT - INCLUDING HISTOGRAM - VERSION OF AUG. 16, 1964
HEALTH SCIENCES COMPUTING FACILITY,UCLA FSU--BERKELEY VERSION 8/25/70.
VALIDATION EXPIRED AIR = A + B COHB
MORNING VALUES AT WORK (AFTER COMMUTING)
VALIDATION EXPIRED AIR CO (ppm)
PERCENT COHb
LEAST SQUARES FIT
Y = 0.02 + 4.34X
r = 0.90
0.
1.500
3.000
4.500
6.000
7.500
9.000
10.500
12.000
13.500
15.000
75.000
67.500
60.000
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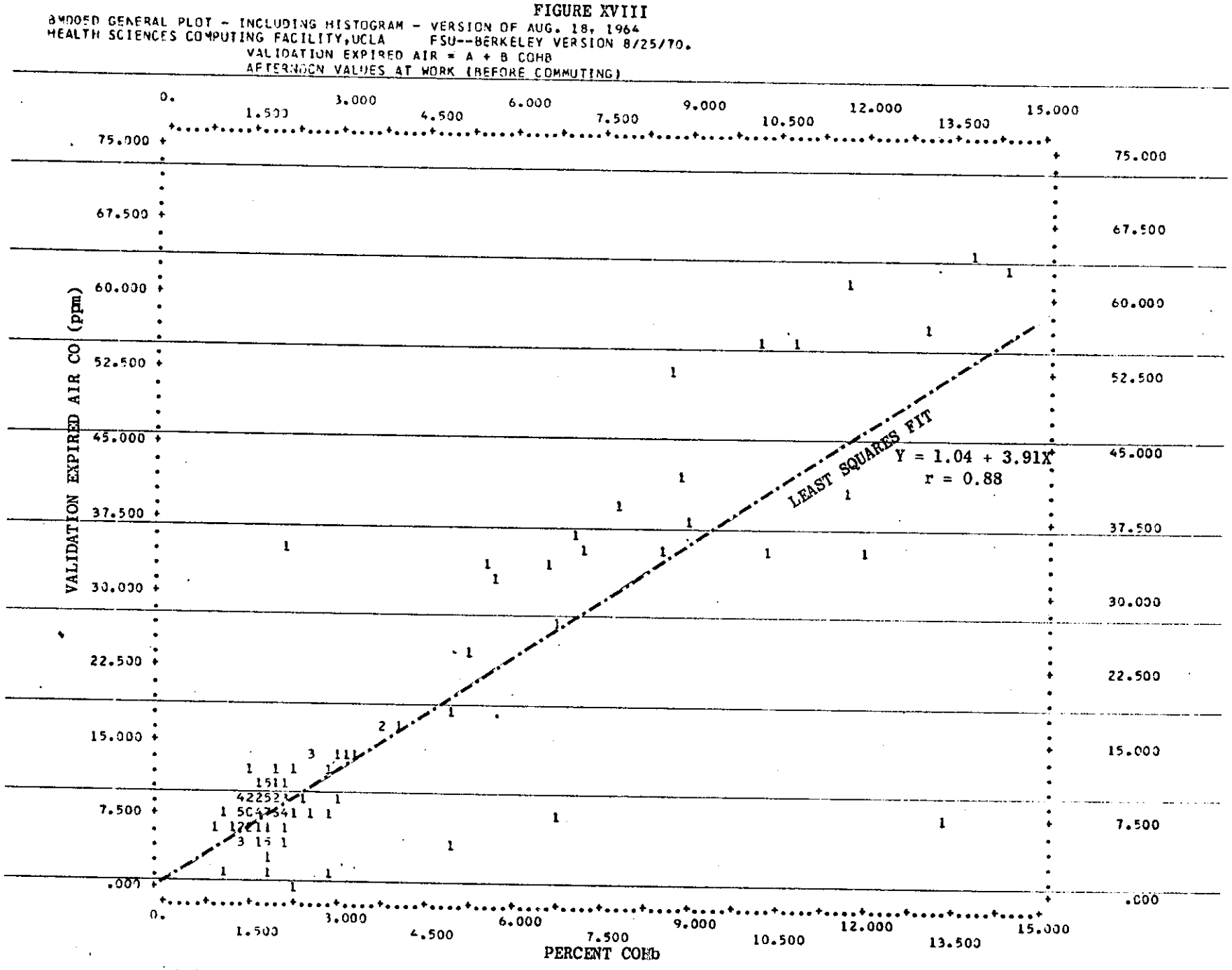
45.000
37.500
30.000
22.500
15.000
7.500
.000

FIGURE XVIII



H. Exhibits

Exhibit A: Subcontract With Environmental Measurements Incorporated.

Exhibit B: Cities Included Within Each Area.

Exhibit C: Memorandum to Employees of Department of Water and Power.

Exhibit D: LA CO Commuter Study Questionnaire.

Exhibit E: Use of Freeways.

Exhibit F: Interviewers' Special Instructions.

Exhibit G: Informed Consent Form.

Exhibit H: Instructions for Participants (A).

Exhibit I: Instructions for Participants (B).

Exhibit J: Description of Pulse Pump.

Exhibit K: Automobile Daily Log.

Exhibit L: Blood Drawing Schedule.

Exhibit M: Blood Drawing Instructions.

Exhibit N: Ringold, A.; Goldsmith, J.R.; Helwig, H.L.; Finn, R.; and Schuette, F.: Estimating Recent Carbon Monoxide Exposures, Arch. Env. Health 5:308, 1962.

EXHIBIT A

SUBCONTRACT WITH ENVIRONMENTAL MEASUREMENTS INCORPORATED

I. NATURE OF THE SURVEY

The body burden of COHb associated with motor vehicular air pollution among commuting populations of Los Angeles will be estimated by the measurement of expired air carbon monoxide, and in a sub-sample, by determination of COHb. Estimations will be made of the contribution of carbon monoxide from morning and evening commuting exposures. Data will be collected from three groups of commuters who are not occupationally exposed to motor vehicular exhaust. Measurements will be made at two different times of year and on days and in locations with a variety of expected levels of carbon monoxide based on past monitoring experience.

II. FIELD OPERATION

The study population will consist of three volunteer groups of non-occupationally exposed commuting persons. There will be 20 persons in each group; a total of 60 persons. The non-occupationally exposed groups will be selected from commuters using major freeways from the Riverside-San Fernando Valley area, the Los Angeles County foothills, and from the Los Angeles County coastal area. The groups will be composed of smokers as well as non-smokers.

III. PERIOD OF STUDY

The two study periods will be:

(a) ten days in the interval May, 1972 - September, 1972

(b) twenty days in the interval December, 1972 - February, 1973

(Contractor will be advised of exact dates by the State upon not less than 15 days written notice.)

IV. EXPIRED AIR SAMPLES

Contractor shall obtain four expired air samples per day which will be provided daily by each of the non-occupationally exposed persons - one before commuting to work in the morning and one after arrival at work; one before leaving work at the end of the working day, and one after arrival at home. For the combined study periods there will be 120 specimens per person or a total of 7200 specimens for the 60 persons for the study population. Each person shall be instructed by the Contractor how to take his own expired air sample. The collection of these self-taken samples will be validated on a 10% sub-sample of persons by collection of duplicate samples taken by an experienced technician. Exposure samples of ambient air along the commute route will be collected twice daily by means of a battery-operated air sampling pump that will be provided by the State Department of Public Health for each person's vehicle. The ambient samples will total 3600.

V. COLLECTION OF BLOOD SPECIMENS

Approximately 5% of the 7200 determinations of expired carbon monoxide made on the study subjects will be validated with COHb determinations made on blood specimens taken at the same time by the Contractor as the expired air samples (this amounts of 360 COHb determinations).

VI. COLLECTION OF OTHER PERTINENT DATA

A questionnaire, provided by the State Department of Public Health, will be used to collect data for the evaluation of residential history, occupation, smoking, and commuting. These will be completed by trained interviewers, provided by the Contractor, following initial screening and selection of the study population. In addition, each study subject shall maintain a log in which he enters the number of cigarettes smoked daily, if he is a cigarette smoker, and also records any other daily travel performed in addition to his daily commuting. The log provided each subject will be returned to the Contractor at the end of each study period. All questionnaires and logs shall be mailed to the State Department of Public Health, Environmental Epidemiology Unit, Room 333, 2151 Berkeley Way, Berkeley, California 94704, attention: Norman Perkins.

VII. The total number of specimens enumerated in items 4, 5 and 6 above equals 11,880. All laboratory analysis of specimens collected will be accomplished in the Air & Industrial Hygiene Laboratory, State Department of Public Health, 2151 Berkeley Way, Berkeley, California.

The Contractor shall be responsible for:

1. Acquiring the voluntary services of non-occupationally exposed persons from those segments of the community that are most relevant, i.e., commuters using major freeways from the Riverside-San Fernando Valley area, the Los Angeles County foothills area, and the Los Angeles County coastal area.

2. Managing the logistics of the collection of specimens and their transport to State Department of Public Health laboratories. This system shall include:

   (a) distributing expired air sampling containers to the subjects to be sampled (each subject will take expired air samples before and after commuting), and providing mailing containers for the specimens.

   (b) providing the central communication link between the State Department of Public Health and the subjects.

(c) arranging for the timely collection of alveolar breath samples from the participants (some will be duplicate samples), and delivery of same to State Department of Public Health for analysis. With the exception of mailing containers, breath sample bags and other apparatus and materials are to be supplied by State Department of Public Health.

(d) arranging for collection of air samples along the commute route.

3. Training the subjects in the techniques of taking their own expired air samples and in the operation of the air sampling pumps that will be carried inside the subject's cars for the purpose of collecting integrated air samples along the subjects commute route. (Technicians from State Department of Public Health will train the Contractor and its personnel how to take expired air samples and the operation of the pumps).

4. Obtaining a standardized smoking, commuting and occupational history of each subject for maintaining a close surveillance over the subjects performance in taking his own expired air sample, the maintenance of a log in which is entered the number of cigarettes smoked (if he is a cigarette smoker) in the course of the day and other travel in addition to commuting, and for arranging for blood samples to be taken from the subject.

5. Maintaining a close liaison with State Department of Public Health as to the progress of the field operation.

6. Contractor shall submit ambient and expired air samples and blood specimens to the State Department of Public Health, Air & Industrial Hygiene Laboratory at 2151 Berkeley Way, Berkeley, California 94704. No blood specimen or air sample may be held by the Contractor for a period exceeding 24 hours. All samples and specimens shall be mailed, on a daily basis, in suitable containers properly constructed so as to prevent loss of contents. Blood specimens must be shipped frozen in dry ice. All containers shall be provided by the Contractor.

EXHIBIT B

ENVIRONMENTAL
MEASUREMENTS, INC.
215 LEIDESDORFF STREET
SAN FRANCISCO, CA 94111
415/398-7664

REGION 1

Balboa
Barber City
Belmont Shore
Brentwood
Compton
Costa Mesa
Dominguez
El Segundo
Gardena
Hawthorne
Hermosa Beach
Huntington Beach
Lawndale
Lomita
Long Beach
Malibu
Manhattan Beach
Naples
Newport Beach
North Long Beach
Ocean Park
Pacific Palisades
Palos Verdes Estates
Redondo Beach
Rolling Hills
Rolling Hills Estates
Rossmoor
San Pedro
Santa Monica
Seal Beach
Signal Hill
Sunset Beach
Surfside
Topanga Beach
Torrance
Venice
Westchester
West Los Angeles
Wilmington

REGION 2

Agoura
Altadena
Arcadia
Azusa
Baldwin Park
Calabasas
Canoga Park
Chatsworth
Duarte
Encino
Grandada Hills
Hidden Hills
Highway Highlands
La Crescenta
Mission Hills
Monrovia
Northridge
Pacioma
Panorama City
Pasadena
Reseda
San Fernando
Santa Susana
Sepulveda
Sherman Oaks
Sierra Madre
Sunland
Sun Valley
Sylmar
Tarzana
Thousand Oaks
Tujunga
Van Nuys
Winnetka
Woodland Hills

REGION 3

Alta Loma
Arlanca Village
Arlington
Belvedere Heights
Bloomington
Casa Blanca
Charter Oak
Chino
Chrestmore
Claremont
Colton
Corona
Covina
Cucamonga
Etiwanda
Fontana
Glen Avon Heights
Glendora
Grand Terrace
Highgrove
Home Gardens
La Puente
La Sierra
La Verne
Loma Linda
Magnolia Center
Mira Loma
Montclair
Ontario
Pedley
Pomona
Rialto
Riverside
Rubidoux
San Bernardino
San Dimas
Sunnyslope
Upland
West Covina
West Riverside

EXHIBIT C

CODE 01146

MEMORANDUM

MEMO BY H. P. Gluckman TO All Concerned DATE July 11, 1972

FILE TITLE California Dept. of Public Health COHB Study

The Department is cooperating with the Calif. Dept. of Public Health in a study program associated with motor vehicular air pollution among commuting employees of the Department who work in the General Office Building.

The study population will consist of volunteer employees of non-occupationally exposed commuting persons. These persons will be selected from commuters using major freeways from the Riverside-San Fernando Valley area, the Los Angeles County foothills, and from the Los Angeles County coastal area. The groups will be composed of smokers as well as non-smokers.

PERIOD OF STUDY

The two study periods will be:

(a) Ten days in the interval May, 1972 - September, 1972.

(b) Twenty days in the interval December, 1972 - February, 1973.

REQUIREMENTS OF PARTICIPANTS

1. Work in the General Office Building and commute from one of the three geographic regions - 30 to 90 minutes one-way commuting time.
2. Must use freeway in commuting (should try to use same route each day).
3. No occupational driving, no motorcyclists.
4. Sex distribution - no requirements.
5. Age distribution - 18 to 65 years.
6. No smoking allowed for passengers in non-smoking car.
7. Health - No respiratory illness.
8. Blood specimens will periodically be taken.

The three geographic regions are attached.

Employees interested in volunteering for this study please fill in the form below, detach and send to H H Burris, Room 1010-B G.O.B. For further information about the program you can call Mr. Burris on extension 5552.

H.P. Gluckman

HHB

1 Attachment

To: H. H. Burris, Room 1010-B G.O.B.

NAME:

HOME ADDRESS:

WORKING HOURS:

AGE: SMOKER ☐ NON-SMOKER ☐

ROOM NO: TELEPHONE NO:

ENVIRONMENTAL
MEASUREMENTS, INC.
215 LEIDESDORFF STREET
SAN FRANCISCO, CA 94111
415/398-7664

CALIFORNIA DEPARTMENT OF PUBLIC HEALTH COHb STUDY

GEOGRAPHIC REGIONS

REGION 1	REGION 2	REGION 3
Balboa	Agoura	Alta Loma
Barber City	Altadena	Arlanca Village
Belmont Shore	Arcadia	Arlington
Brentwood	Azusa	Belvedere Heights
Compton	Baldwin Park	Bloomington
Costa Mesa	Calabasas	Casa Blanca
Dominguez	Canoga Park	Charter Oak
El Segundo	Chatsworth	Chino
Gardena	Duarte	Chrestmore
Hawthorne	Encino	Claremont
Hermosa Beach	Grandada Hills	Colton
Huntington Beach	Hidden Hills	Corona
Lawndale	Highway Highlands	Covina
Lomita	La Crescenta	Cucamonga
Long Beach	Mission Hills	Etiwanda
Malibu	Monrovia	Fontana
Manhattan Beach	Northridge	Glen Avon Heights
Naples	Pacioma	Glendora
Newport Beach	Panorama City	Grand Terrace
North Long Beach	Pasadena	Highgrove
Ocean Park	Reseda	Home Gardens
Pacific Palisades	San Fernando	La Puente
Palos Verdes Estates	Santa Susana	La Sierra
Redondo Beach	Sepulveda	La Verne
Rolling Hills	Sherman Oaks	Loma Linda
Rolling Hills Estates	Sierra Madre	Magnolia Center
Rossmoor	Sunland	Mira Loma
San Pedro	Sun Valley	Montclair
Santa Monica	Sylmar	Ontario
Seal Beach	Tarzana	Pedley
Signal Hill	Thousand Oaks	Pomona
Sunset Beach	Tujunga	Rialto
Surfside	Van Nuys	Riverside
Topanga Beach	Winnetka	Rubidoux
Torrance	Woodland Hills	San Bernardino
Venice		San Dimas
Westchester		Sunnyslope
West Los Angeles		Upland
Wilmington		West Covina
		West Riverside

EXHIBIT D

State of California
Department of Public Health

LA CO COMMUTER STUDY QUESTIONNAIRE

The information contained in this questionnaire is confidential.

PERSONAL DATA

1. NAME — Last _____ First _____ Initial _____ I.D. # _____ (1-3)

2. How long have you lived in the Los Angeles area? _____ (4-5) years _____ (6-7) months

3. Present home address: Number _____ Street _____ City _____ State _____ Zip _____

4. How long have you lived at this address? _____ (8-9) years _____ (10-11) months

5. What is your telephone number? _____ _____ (12-14)

6. Sex (15) 1☐ Male 2☐ Female

7. Age last birthday _____ (16-17) years

8. Occupation (18) 1☐ Office (Clerical) 2☐ Non-clerical (Inside blue collar) 3☐ Inside managerial or supervisory

9. How long in occupation? _____ (19-20) years _____ (21-22) months *If less than one year,*

10. What was your last occupation? _____ (23)

11. How long? _____ (24-25) yrs _____ (26-27) mos

(If NA, code "9" for question 10, and "99" "99" for question 11)

COMMUTING DATA

12. What is the approximate distance from your home to work? _____ (28-29) miles

13. How long does it usually take you to commute from home to work? _____ (30-31) minutes

14. Approximately, how much of this time is freeway driving? _____ (32-33) minutes

15. Do you usually use the same commute route returning home from work? (34) 1☐ Yes 2☐ No
(If "Yes", skip to question 19; if "No", ask questions 16-18)

16. Using the alternate route, what is the distance from work to home? _____ (35-36) miles

17. How long does it take you to commute from work to home, using the alternate route? _____ (37-38) minutes

18. Approximately, how much of this time is freeway driving? _____ (39-40) minutes

19. What are your usual commuting hours in the morning going to work? From _____ to _____ (41)

20. What are your usual commuting hours in the evening returning home from work? From _____ to _____ (42)

21. What is your usual commute driving pattern or speed? (43)
1☐ Mostly Stop & Go
2☐ Less than 25 mph
3☐ 25 - 45 mph
4☐ Over 45 mph

22. Is your car air conditioned? (44) 1☐ Yes 2☐ No

23. For drivers who are smokers: Do you smoke in the car during the period you are commuting? (45)
1☐ Yes 2☐ No 3☐ Not applicable, driver is a nonsmoker

PHYSICAL EXERCTION

24. During your free time away from work, how much physical exercise have you usually taken? (46)
1☐ Hardly any
2☐ Light exercise, e.g., walks, family sports, gardening, etc.
3☐ Regular participation in sports, e.g., golf, tennis, swimming
4☐ Strenuous training, e.g., bowling, baseball, basketball, football

25. How tall are you? _____ (47-48) inches

26. How much do you weigh? _____ (49-51) pounds

27. When did you weigh yourself last? (52)
1☐ Today
2☐ Within the past week
3☐ About a month ago
4☐ More than a month ago

TOBACCO SMOKING

28a Do you smoke? ☐ Yes ☐ No

Record 'Yes' if regular smoker (as defined in question 28b) up to one month ago.

If 'No' to question 28a, ask question 28b

If 'Yes' to question 28a

Do you inhale the smoke? ☐ Yes ☐ No

Would you say you inhale the smoke slightly (S), moderately (M), deeply (D)? ☐ S ☐ M ☐ D

How old were you when you started smoking regularly? _____ years old

How many manufactured cigarettes do you usually smoke per day? _____ per working day

_____ at weekends

How much tobacco (oz/g) do you usually smoke per week in hand-rolled cigarettes? _____

How much pipe tobacco (oz/g) do you usually smoke per week? _____

How many cigars do you usually smoke per week? _____

Specify large (L) or small (S).

28b Have you ever smoked as much as one cigarette a day [or one ounce of tobacco a month] for as long as a year? ☐ Yes ☐ No

If 'No' to question 28b, go to question 29

If 'Yes' to question 28b

How old were you when you started smoking regularly? _____ years old

How old were you when you last gave up smoking? _____ years old

How many manufactured cigarettes per day were you smoking before you gave up? _____ per working day

_____ at weekends

How much tobacco (oz/g) per week were you smoking in hand-rolled cigarettes before you gave up? _____

How much pipe tobacco (oz/g) per week were you smoking before you gave up? _____

How many cigars per week were you smoking before you gave up? _____

Specify large (L) or small (S).

CODING FOR SMOKING HISTORY

Before coding refer to instructions.

Smoking history

Never smoked	☐ 1	(53)
Ex-smoker	☐ 2	
Present smoker - does not inhale	☐ 3	
Present smoker - inhales slightly	☐ 4	
Present smoker - inhales moderately	☐ 5	
Present smoker - inhales deeply	☐ 6	

Type of smoker

Cigarettes only	☐ 1	(54)
Pipe only	☐ 2	
Cigars only	☐ 3	
Cigarettes and pipe/cigars	☐ 4	
Cigars and pipe	☐ 5	
Non-smoker	☐ 6	

Amount smoked per day* (average including weekends)

Cigarette tobacco:

Nil	☐ 1	(55)
1-4 g	☐ 2	
5-14 g	☐ 3	
15-24 g	☐ 4	
25-34 g	☐ 5	
35 g or more	☐ 6	

Pipe/cigar tobacco:

Nil	☐ 1	(56)
1-4 g	☐ 2	
5-14 g	☐ 3	
15-24 g	☐ 4	
25-34 g	☐ 5	
35 g or more	☐ 6	

** 1 oz of pipe tobacco = 28 cigarettes = 28 g*
1 small cigar = 2 cigarettes
1 large cigar = 5 cigarettes

Age started (years)	Code 00 if a non-smoker	(57) ☐	(58) ☐
Age stopped (years)	Code 00 if a present smoker	(59) ☐	(60) ☐

HOUSING

29. What type housing unit do you live in? (61)

1☐ House
2☐ Apartment
3☐ Flat
4☐ Trailer, on wheels, or can easily be put on wheels
5☐ Trailer, on a permanent foundation

FUELS USED IN HOME FOR VARIOUS PURPOSES

30. How is your house heated? (62)

1☐ Steam or hot water
2☐ Warm air furnace with individual room regulator
3☐ Floor, wall or pipeless furnace
4☐ Electric unit(s)
5☐ Room heater(s) connected to chimney or flue
6☐ Room heater(s) not connected to chimney or flue
7☐ Other Method
8☐ Not heated

31. What kind of fuel do you use for:

	House Heating (63) Yes	Cooking (64) Yes	Water Heating (65) Yes
1 Utility gas (from underground pipes serving the community)	☐	☐	☐
2 Bottled, tank, or LP gas	☐	☐	☐
3 Fuel oil, kerosene, etc.	☐	☐	☐
4 Electricity	☐	☐	☐
5 Other fuel, or no fuel used	☐	☐	☐

32. Do you have an electric or gas clothes dryer (not shared with other households)? (66)

1☐ Electric
2☐ Gas, not vented
3☐ Gas, vented
4☐ Not applicable, does not have clothes dryer

CHRONIC CONDITIONS

33. Has a medical doctor ever told you that you have a chronic respiratory condition? (67)

1☐ Yes 2☐ No 3☐ Does not recall having been told

34. *If "Yes" to question 33,* What did he call it? _____ (68-70)

If "No" to question 33, code "000" in space provided.

EXHIBIT E

1972/73 L.A. CO. COMMUTER STUDY

Name_____

(Please do not write in space below)

_____

We would like to know the freeway(s) you most regularly use in commuting to and from work. The major freeways are listed below. Please place an "X" in the appropriate box(es) under the applicable heading that best describes your freeway commute routes. (If more than one freeway is used in either going to or from work, place an "X" in the appropriate boxes).

Name of Freeway	Same Route To and From Work	Home to Work	Work to Home
Golden State	☐	☐	☐
Harbor	☐	☐	☐
Hollywood	☐	☐	☐
Long Beach	☐	☐	☐
Pomona	☐	☐	☐
Riverside	☐	☐	☐
San Bernardino	☐	☐	☐
San Diego	☐	☐	☐
Santa Ana	☐	☐	☐
Santa Monica	☐	☐	☐
Ventura	☐	☐	☐
Other (Write In)	☐	☐	☐

EXHIBIT F

INTERVIEWERS' SPECIAL INSTRUCTIONS

Questions 2, 4, 9, and 11 require double entries: If respondent gives only a "year" answer to any of these, record "00" in the space provided for a month response. For example, if the answer given is "10 years", record "10" in the space provided for years, and "00" in the space provided for months. If, on the other hand, the response was 01, 02,...11 months only, record "00" in the space provided for years, and record the given number of months in the space provided for months.

Question 9: If the response is less than a year, then ask question 10, and enter appropriate response. If the answer to question 9 is a year or more, do not ask question 10; instead, enter "9" in the space labeled (23), and "99" in each of the spaces labeled (24-25) and (26-27).

Question 29: The distinction between a "flat" and an "apartment" usually is considered to be - a "flat" is a floor or story in a building, especially a floor or suite of rooms all on one floor, and used as a residence; an "apartment" is considered to be a suite or set of rooms (even a single room) occupied as a residence, and two or more of these units may be on one floor. In either case, whether "flat" or "apartment", each unit must be self-contained having its own cooking and bathing facilities not shared with other tenants. If the respondent occupies only a room in a living unit and does not eat his meals with others living in the unit, code this person to the type living unit in which his room is located.

Question 31: This question must not be construed to mean that the respondent answers only if he provides fuel for heating, cooking and water heating. It is not uncommon in some types of multiple housing for the landlord to provide fuel for heating the house, and for heating the water. Since we are interested only in what type of fuel is provided for these purposes, the respondent should answer regardless of whether he himself provides these fuels.

EXHIBIT G

I hereby agree to participate in a study of carbon monoxide exposures and carboxyhemoglobin levels in commuters in Los Angeles, California and understand that this includes giving expired air and blood specimens to be analyzed by the State Department of Public Health laboratories. The procedures to be used have been explained to me, and I understand what is involved and agree to cooperate.

_____
Signature

_____
Date

ENVIRONMENTAL
MEASUREMENTS, INC.
215 LEIDESDORFF STREET
SAN FRANCISCO, CA 94111
415/398-7664

CALIFORNIA DEPARTMENT OF PUBLIC HEALTH LA CO COMMUTER STUDY

INSTRUCTIONS FOR PARTICIPANTS

Each study period will begin on Monday morning before your leave for work and end on Friday evening after you arrive at home at the end of the working day. The following paragraphs describe the procedures you are to use during the study periods.

COLLECTING THE EXHALED AIR SPECIMENS

1. Open valve on small bag by turning it to your left (counter-clockwise) as far as it will go.

2. Attach "Y" tube to valve so it fits snugly.

3. Blow out all the air in your lungs, then take a deep breath and hold it twenty seconds.

4. At the end of twenty seconds, put the mouthpiece of the "Y" tube in your mouth and exhale steadily, allowing approximately the first half second's worth of air to escape through the open leg of the "Y" tube. Then bend the open leg of the "Y" tube firmly to prevent any further escape of air and continue exhaling steadily until the bag is full (or you run out of air). Never take another breath during this procedure. Fill the bag only with the air you have held for 20 seconds.

5. Remove the tubing from the bag and close the valve snugly by turning it to your right (clockwise) as far as it will go. Recheck the label to be sure you have filled the correct bag.

COLLECTING THE "AIR IN CAR" SPECIMENS

1. Open the valve on the large bag labelled "Air In Car" (check to be sure you have the right day and time). By turning it to your left (counterclockwise) as far as it will go.

2. Attach the tube from you air pump to the valve so it fits snugly and be sure the tube is not crimped.

3. When you get in your car to leave for work (or home), turn on the pump by flipping the switch towards the silver screw on the top of the pump. Because the pump is designed to work on an intermittent basis, it may take up to 30-45 seconds for the first pumping cycle to begin. Listen for

this first cycle so you can be sure it is on. Be sure bottom of pump (end with the serial no. plate) is exposed while the pump is on.

4. Leave the pump on until you reach your destination and park your car.

5. After you have parked your car, turn off the pump by flipping the switch away from the silver screw, remove the tubing from the bag, and close the valve by turning it to your right (clockwise) until it is snugly closed.

FILLING OUT THE "AUTOMOBILE DAILY LOG"

Be sure to fill out the log each day during the study period and include all automobile usage, whether for commuting to and from work, or for shopping, entertainment, social engagements, and so on.

The total number of cigarettes you smoke during each day of the study period should also be entered in the log.

EACH MORNING

1. Just before you leave for work, exhale into the bag labelled "(MONDAY-FRIDAY) MORNING EXHALED - HOME."

2. Place your air pump in the car, attach the large bag labelled "(MONDAY-FRIDAY) MORNING AIR IN CAR." Switch pump on when you leave.

3. When you have parked, turn off the pump, remove the bag, and close the valve.

4. When you enter the building, exhale into the bag labelled "(MONDAY-FRIDAY) MORNING EXHALED - WORK,"

5. Turn in box of bags from previous day at the designated pick-up point.

6. Report to Room 531 if you are scheduled to give a blood sample.

EACH AFTERNOON

1. Just before you leave for home, exhale into bag labelled "(MONDAY-FRIDAY) AFTERNOON EXHALED - WORK,"

2. Attach bag labelled "(MONDAY-FRIDAY) AFTERNOON - AIR IN CAR" to your air pump in the car and switch on pump when you leave.

3. When you arrive at home, turn off the pump, remove the bag and close the valve.

4. As soon as you enter your home, exhale into the bag labelled "(MONDAY-FRIDAY) AFTERNOON EXHALED - HOME."

TENTATIVE STUDY SCHEDULE

Study Period	
1	August 21-25, 1972
2	September 18-22, 1972
3	December 11-15, 1972
4	January 8-12, 1973
5	January 29-February 2, 1973
6	February 26-March 3, 1973

EXHIBIT I

INSTRUCTIONS

Before leaving for work in the morning, check to see that

(1) Plastic tubing is firmly attached to both the bag and the pump; and

(2) Bag valve is open. (To open bag valve, grasp the serrated valve extension and turn to the left as far as it will go).

As you move off, flip the toggle switch (on top of pump assembly) to the right position. After about twenty seconds, pump will start going on and off at about two second intervals. Let the pump operate during entire trip.

When you reach work, turn pump off by flipping toggle switch to the left position.

Detach plastic tubing from bag, and close bag valve by turning serrated valve extension to the right as far as it will go. Bring bags into office.

In the course of each day, I shall collect from you two filled bags, and shall give you two empty ones; one will be labeled "PM", the other "AM".

Before starting for home at the end of the day, attach plastic tubing to the bag labeled "PM", and repeat above operation to cover your trip home.

EXHIBIT J

PULSE PUMP

The Pulse Pump is self-contained and is used to fill plastic sample bags at remote sites.

A client needed a pump, an inexpensive, self-contained pump to fill air sample bags. The collected air would contain representative proportions of important air contaminants. The twist: each bag was to represent a different length sampling period.

After a search for available hardware, which did not satisfy the convenience and price requirements of the project, EMI produced the *Pulse Pump*.

Its uniqueness is that the electronic control circuit may be adjusted so that a fixed volume of air can be pumped over a variable length of time. By changing the number of seconds the pump is *on* and *off*, its fixed pumping rate fills the bag in a preselectable time. Typically, a six-liter sample can be obtained in five minutes, five hours, or 24 hours. For monitoring inert or stable gases, this is a convenient means for gathering time-averaged samples.

The Pulse Pump is the Company's first product. (As an accessory, EMI also has available a convenient-sized, aluminized mylar sampling bag.) It is small -- only about the size of a medium-sized transistor radio -- and operates continually for several days on a single set of dry batteries (one 1.5-volt and one 9-volt cell). The scuff-resistant plastic case clips to a belt or convenient structure, and a single switch is the only control. The simple diaphragm pump is closed when not operating to retain the sample. A piece of inert plastic connects the pump to the sampling bag.

Since the Pulse Pump's availability in late 1972, it has been used in several sampling modes. In one interesting project, under contract to the California State Department of Public Health, samples of air were gathered inside commuters' automobiles. Later analysis of these air samples determined the average amount of carbon monoxide breathed dur-

Pulse Pumps are produced in batch lots. Printed circuit boards, shown here being assembled, are used in the pump's timing control.

ing the course of each day's drive. Another program involved the collection of CO samples along and adjacent to a major highway. Elsewhere, a city is gathering samples at sites of suspected high CO concentrations; garages and locations of traffic congestion are being monitored.

The uses are many and as broad as the needs for measuring the air. Obviously, some air contaminants are reactive and change their nature when stored. They cannot be reliably studied in this fashion; yet, other important gases may be. Samples that remain stable can be measured later in the laboratory under ideal conditions for the analytical instruments; many sites may be measured without committing an expensive sampler to each.

The Pulse Pump is an inexpensive, nearly disposable accessory. It facilitates increasing the density of data grids, an important need in the analysis of any pollutant problem.

Pulse Pumps and sample bags for commuter carbon monoxide study. Every pump was individually timed for each subject's normal driving time.

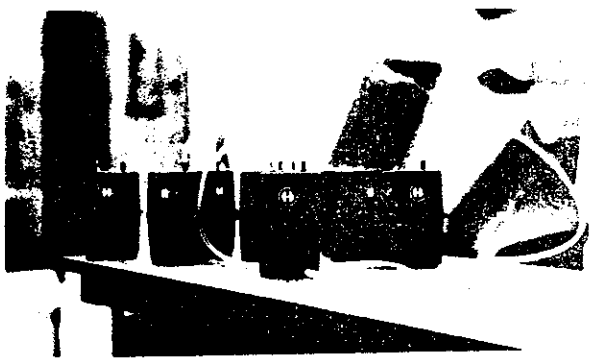


EXHIBIT K

1972/73 LA CO Commuter Study

AUTOMOBILE DAILY LOG

(Record all daily use of car whether for commuting to and from work, or for shopping, entertainment, social engagements, etc.)

[illegible] ____________________

ADDRESS ____________________

I.D. ____________________

DAY/DATE	HOUR OF DEPARTURE FROM	HOUR OF ARRIVAL AT	TRAFFIC FLOW		ANYONE IN CAR SMOKING?	WINDOWS OPENED OR CLOSED	AIR CONDITIONER ON or OFF?	FREEWAYS OR CITY STREETS?	NUMBER OF CIGARETTES PER TRIP	TOTAL CIGARETTES FOR DAY
			FAST	SLOW						

EXHIBIT L

BLOOD DRAWING SCHEDULE

LOS ANGELES COMMUTING STUDY

	Mon.	Tues.	Wed.	Thurs.	Fri.
WEEK I					
AM		x		x	x
PM	x		x		
WEEK II					
AM			x		x
PM	x	x		x	
WEEK III					
AM		x	x		x
PM	x			x	
WEEK IV					
AM				x	x
PM	x	x	x		
WEEK V					
AM		x			x
PM	x		x	x	
WEEK VI					
AM			x	x	x
PM	x	x			

EXHIBIT M

Collection-COHb Study

Collect blood in the heparinized tube (BD L-3200) using the vacutainer holder and Monoject 210 20 GA 1½ inch needle. Invert tube gently 6 times to mix blood thoroughly with heparin to prevent clotting. Label tube. Place tubes in styrofoam blood container. Place rubber band around container. Keep bloods cold at all times but do not freeze.

Use frozen gel-packs to keep Bio-mailers cold.

EXHIBIT N

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Estimating Recent Carbon Monoxide Exposures

A Rapid Method

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Introduction

In studying the health effects of community air pollution exposures, it is customary to measure contaminant levels at a few fixed outdoor points and to make certain assumptions concerning the persons who might be exposed to these levels and then to develop inferences concerning what the expected effects might be.

Carbon monoxide is a major pollutant emitted from automobiles. The source is in motion, the atmospheric air which dilutes the emitted carbon monoxide is in motion, and the exposed population also is moving simultaneously from place to place; therefore continuous sampling at fixed points together with population exposure estimates based thereon may not provide an adequate basis for estimating the effects on a population whose members are exposed to a variety of concentrations and locations in the course of a single day.

Because most of the carbon monoxide absorbed by the body is bound to circulating hemoglobin to form carboxyhemoglobin, we were attracted to the idea that the pool of hemoglobin which circulates in the blood may represent an integrated sample of a person's recent exposures to carbon monoxide. All that would be required to use this system would be a satisfactory "readout" technique by which the amount of carboxyhemoglobin could be indicated from time to time.

This paper presents the experience with using expired air as an indicator of the per cent of hemoglobin bound as carboxyhemoglobin. The precision of the method is estimated, and by measurement of carboxyhemoglobin, the validity is assessed. Experience with the use of this method in population samples is presented.

Basis for Using Expired Air Tension to Estimate Recent Exposures

Carbon monoxide present in inspired air is readily absorbed by the blood at a rate depending upon the ventilation rate and pattern, the concentration of carboxyhemoglobin, the blood volume, and, under some circumstances, on the diffusing capacity of the lung. For exposures to relatively high concentrations Pace et al.[1] estimate this fractional absorption in healthy young men at about 42% of the amount of carbon monoxide in the inspired air, but this estimate is valid for values of per cent carboxyhemoglobin up to one-third the equilibrium value for the carbon monoxide concentration of air under consideration. Carbon monoxide is excreted by the lung. Studies of the possible utilization of carbon monoxide in the body's metabolism have not succeeded in demonstrating the utilization of the material nor its excretion by other routes.[2] Hence it is

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reasonable to estimate carboxyhemoglobin from expired air which has had an opportunity to equilibrate with blood in the pulmonary circulation.

It has been shown recently, by Sjostrand and his colleagues [3] that carbon monoxide is produced both physiologically and pathologically by the breakdown of hemoglobin. Studies of Engstedt [4] further showed that the expired air carbon monoxide reflected the rate of hemoglobin destruction. Thus, excretion of carbon monoxide in expired air has been shown to be a physiological phenomenon. Indeed, the affinity of carbon monoxide and hemoglobin plays a role in the excretion of this waste product similar to that of the several hepatic and renal conjugation mechanisms in urinary excretions of wastes.

If we assume a fixed level of carbon monoxide from endogenous sources we may utilize this relationship of expired air carbon monoxide to carboxyhemoglobin for estimating carboxyhemoglobin levels due to exogenous sources of carbon monoxide.

Instrumentation

Development of the infrared absorption method [5] for analysis of carbon monoxide in air has permitted more precise measurements than the Hopcalite method, available to Sjostrand.[3] Gaensler et al.[6] have adapted commercially available infrared carbon monoxide analysis equipment so that as little as 0.5 ppm could be detected with gas samples of 150 ml.

A Liston-Becker Model 15A nondispersive infrared analyzer * was used throughout our study for the quantitative determination of the carbon monoxide in expired air and blood samples. The analyzer was equipped with a 10 in. sample cell, 15¼ in. reference cell, and 5 in. filter cell containing water vapor and carbon dioxide. The purpose of the filter cell is to attenuate the infrared radiation at the wavelengths absorbed by water vapor and carbon dioxide so the effect of their presence in the sample gas is minimized. Nevertheless, to avoid all interference due to water vapor and carbon dioxide, a drying tube containing Anhydrone and Ascarite was also attached to the inlet of the sample cell. A glass flowmeter connected to the outlet of the sample cell was used to meter the flow of gas between 300 and 500 ml. per minute and to detect or prevent pressure build-up in the sample cell or clogging of the drying tube. An Esterline-Angus Graphic Ammeter (0-5.0 ma.) connected to the amplifier output of the infrared analyzer provided a continuous record of response to sample gases.

The response of the analyzer and recording ammeter was calibrated by introducing standard dilutions of carbon monoxide in nitrogen. The gas dilutions were obtained commercially † and standardized in the laboratory by the iodine pentoxide method.[7] The analyzer was calibrated and generally operated at maximum sensitivity (maximum gain) to provide a working range of 0 to 100 ppm carbon monoxide with a sensitivity of 1 ppm. The sensitivity attenuator of the amplifier is linear and may be used for analyzing samples having a concentration greater than 100 ppm of carbon monoxide.

The equilibrium relationship between the partial pressures of carbon monoxide and oxygen and between the carboxyhemoglobin level and oxyhemoglobin levels are those given by the well-known equation attributed to Haldane:

$$M\frac{(CO)}{(O_2)} = \frac{HgbCO\%}{HgbO_2\%}$$

in which the values of CO and O_2 are in units of concentration.

This relationship has also been utilized by Hackney, Kaufman, Lashier, and Lynn in rebreathing techniques starting with 100% oxygen in a 5-liter bag,[8] and previously by others.[3,5] In the methods they have developed it is necessary to measure the equilibrium oxygen tension as well as that of carbon monoxide.

The methods of sample collection to be presented here are somewhat more rapid and are based primarily on the breath-holding techniques first suggested by Jones et al.[9] Our initial work was undertaken by collection of the terminal volume of gas in forced expiration. Subsequently, following the recommendation of Jones et al., the data were collected from persons who had held their breath for 20 seconds.

Sample Collecting Equipment

The collection apparatus for breath samples was a collapsible bag which could be shipped conveniently and which was relatively impermeable to carbon monoxide and would retain samples without change of composition over several days. Rubber, polyethylene, and polyvinyl bags were tested. Losses of 30% of the contained carbon monoxide occurred in 24 hours from rubber and polyethylene bags, whereas losses were not detectable from polyvinyl bags of 0.012 in. thickness.‡ No difficulty has ever been noticed with the valves failing. The gas is easily expressed by external pressure or suction.

* Manufactured by Beckman Instruments, Inc., Fullerton, Calif.

† The Matheson Company, East Rutherford, N.J. A certified analysis of the carbon monoxide dilutions is available at extra cost from the manufacturer.

‡ Obtained from the House of More, 1475 Folsom, San Francisco.

The Breath-Holding Technique

In the procedure we utilized, the subject exhales completely, fills his lungs rapidly, and holds his breath for 20 seconds while being timed by the technician. He then exhales rapidly and without hesitation into one limb of a Y tube. The first few hundred milliliters are discarded, since this represents unequilibrated gas from the pulmonary dead space, and the remaining portion of expired air is admitted to the previously evacuated bag. In our experience this maneuver is easy for untrained subjects to perform correctly. The equipment is simple and inexpensive.

Gas Analysis

The infrared analyzer is "zeroed" on pure oxygen before the analysis of each breath sample. The breath sample is then transferred to the sample cell of the analyzer by application of pressure to the polyvinyl bag allowing the gas to flow through the drying tube and analyzer at a rate between 300-400 ml. per minute until a maximum and stable reading has been obtained on the stripchart recorder. The net recorder reading is referred to the previously obtained calibration chart for the determination of carbon monoxide concentration in the sample.

The readings for pure nitrogen and for nitrogen containing 8% carbon dioxide were compared and no differences were observed; therefore, no error is anticipated in the determinations because of the presence of any carbon dioxide which may not have been removed by the Ascarite. This appears to represent an improvement over the method reported by Jones et al.[9]

Collection and Analysis of Blood Samples

For comparing carboxyhemoglobin with the estimates based on expired air analysis, blood samples were obtained by venipuncture using 7 ml. vacutainers § containing 7 mg. disodium EDTA. The samples were stored in unopened vacutainers at 4 C until analyzed. Storage studies were conducted, and it was determined that blood samples collected and stored in this way did not show significant change in their carbon monoxide content.

The method of analysis for carbon monoxide in blood is a modification of the methods described by Lawther and Apthrop [5] and by Gaensler et al.[6] Carbon monoxide is extracted under vacuum, diluted quantitatively with oxygen, and analyzed in the infrared analyzer.

A measured volume of blood is pipetted into a reaction cup. The pipette is rinsed with a few drops of distilled water, and the surface of the blood in the cup is covered with approximately 8 drops of *n*-octyl alcohol. A half-inch Teflon-coated magnetic stirring bar is added to the cup. The reaction cup is joined to the head of the extraction assembly using vacuum grease to complete the seal, and the side-arm connected to the vacuum is opened momentarily to remove room air. Three milliliters of a solution containing 92 volumes of 32% potassium ferricyanide (32 gm. $K_3Fe[CN]_6$ in 100 ml. H_2O) and 8 volumes of concentrated lactic acid are added to the reagent dropping cup. The reagent is allowed to drop *slowly* into the blood sample, retaining the last drop or two in the cup to prevent admission of air to the extraction chamber. Throughout the remainder of the extraction procedure, the blood sample is agitated by means of the magnetic stirrer. The collecting bulb, the volume of which must be known accurately, is filled with mercury, the stopcock at its top is closed, the mercury lowered to evacuate the bulb, and then the stopcock is turned to join the bulb to the extraction chamber. The mercury is evacuated to the lower stopcock which is then closed, and the extraction of the carbon monoxide from the blood sample is allowed to proceed under vacuum for 5 minutes. Following this, all the extracted gas containing the carbon monoxide is flushed into the collecting bulb with oxygen until the contents of the bulb are at atmospheric pressure. Finally, the stopcock is turned to join the collecting bulb and infrared analyzer which was just previously "zeroed" on pure oxygen. The concentration of carbon mon-

§ Manufactured by Becton-Dickinson Products, Rutherford, N.J.

oxide in this first extraction is read from the analyzer recorder and the analyzer is again "zeroed." The extraction procedure is repeated a second time. The final extraction should show less than 2 ppm carbon monoxide which is equivalent to 0.02 vol. % CO when the volume of blood sample used is 3 ml. and the volume of the gas collecting bulb is 300 ml. Two extractions are generally adequate if the carbon monoxide content of the blood sample is below 1 vol. %. The volume per cent carbon monoxide is calculated as given at the bottom of the page in equation (1).

Using 3 ml. of blood, a 300 ml. collecting bulb, and the infrared analyzer with a sensitivity of 1 ppm carbon monoxide, we are able to determine blood carbon monoxide concentration with a sensitivity of 0.01 vol. %.

Per Cent COHgb Saturation.—Hemoglobin content of the blood sample was determined by the cyanmethemoglobin method.[10] In calculating the per cent saturation, we assumed that the total gas combining capacity of a blood sample containing 15 gm. of hemoglobin per 100 ml. of blood was 20 vol. %. Then, the per cent of saturation is determined as shown at the bottom of the page as equation (2).

Results

Table 1 shows the stability of the carbon monoxide concentration taken from the same

TABLE 1.—*Stability of Carbon Monoxide Levels in Specimens Taken Periodically from Gas Stored in Polyvinyl Bags*

Day	Concentration, ppm	
	Sample I	Sample II
0	11	
		28
8		30
11		30
18		28
25		27
29	11	26
39	11	
49	11	

TABLE 2.—*Stability of Carbon Monoxide Levels in Specimens Stored in Polyvinyl Bags Mailed from Berkeley to Los Angeles and Returned to Berkeley*

	Specimen		
	1	2	3
Before mailing	11	30	13
After round trip by mail (6 days later)	10	28	13

2 bags during a protracted period of laboratory storage. The readings are the average of 2 determinations.

In order to obtain data concerning the possibility of mailing and shipping the bags, 2 bags were filled in the laboratory and mailed from Berkeley to Los Angeles and returned by mail. The data are shown in Table 2.

The reproducibility of samples taken by the breath-holding method is shown in Table 3.

Validity

In order to study the validity of this method, 4 male subjects, 2 smokers and 2 nonsmokers, received experimental exposures to carbon monoxide. The amount administered was calculated to be that which would

TABLE 3.—*Variability of Carbon Monoxide Measurements in Expired Air Samples, Equilibrated by Twenty-Second Breath-Holding (ppm)*

Subject	A	B_1	B_2	C	D
Trial					
1	3	40	26	46	75
2	3	44	25	43	73
3	3	43	26	42	71
4	3	41	25	43	75
Mean	3	42	25.5	43.5	73.5
S.D.	0	1.6	0.5	1.5	1.7
Coeff. variat., %	0	3.8	2.0	3.5	2.3

A: male subject—twice before and twice after smoking one pipeful, at 3-minute intervals. B_1: a male smoker performing maneuver at 3-minute intervals starting 10 minutes after smoking a cigarette. B_2: same subject tested in same way 7 hours after smoking last cigarette. C: data from a female cigarette smoker, the first specimen being taken 5 minutes after smoking one cigarette. Trials at about 3 minute intervals. D: a male cigarette smoker; the first specimen being obtained 5 minutes after smoking a cigarette. Trials at about 3-minute intervals.

$$\text{vol. \% CO} = \frac{(\text{ppm CO in collection gas})}{(\text{volume of blood sample in ml.})} \frac{(\text{volume of collection bulb in liters})}{(10)} \qquad (1)$$

$$\text{COHgb\%} = 100 \frac{(\text{measured vol. \% CO})}{(\quad 20 \quad)} \left(\frac{15}{\quad}\right) (\text{gm. Hgb. per 100 ml. blood}) \qquad (2)$$

produce approximately a 5% increase in carboxyhemoglobin. Three such doses in succession were given each subject. Blood and expired air specimens were taken before exposure, after each dose, and at 2 hours and 4 hours subsequent to the last exposure. The expired air specimens were obtained in duplicate, one taken before and one taken after the blood sample was drawn. Duplicate blood analyses were performed on a single specimen.

Figure 1 and Table 4 show the results. The regression relationship shown in Figure 1 may be approximated by

$$COHgb\% = 0.5 + \frac{CO \text{ in ppm}}{5.}$$

These results agree with those of Jones et al.[9]; the comparison is shown in Figure 2.

Experience with Application of the Method

During the summer of 1960, this method was used in a study of expired air carbon monoxide levels among a group of persons having routine medical examinations in Oakland and Los Angeles. Composite specimens for the estimation of ambient air exposure were obtained on each day of the study by using a rubber "squeeze bulb" of about 100 ml. volume. One squeeze was added to a

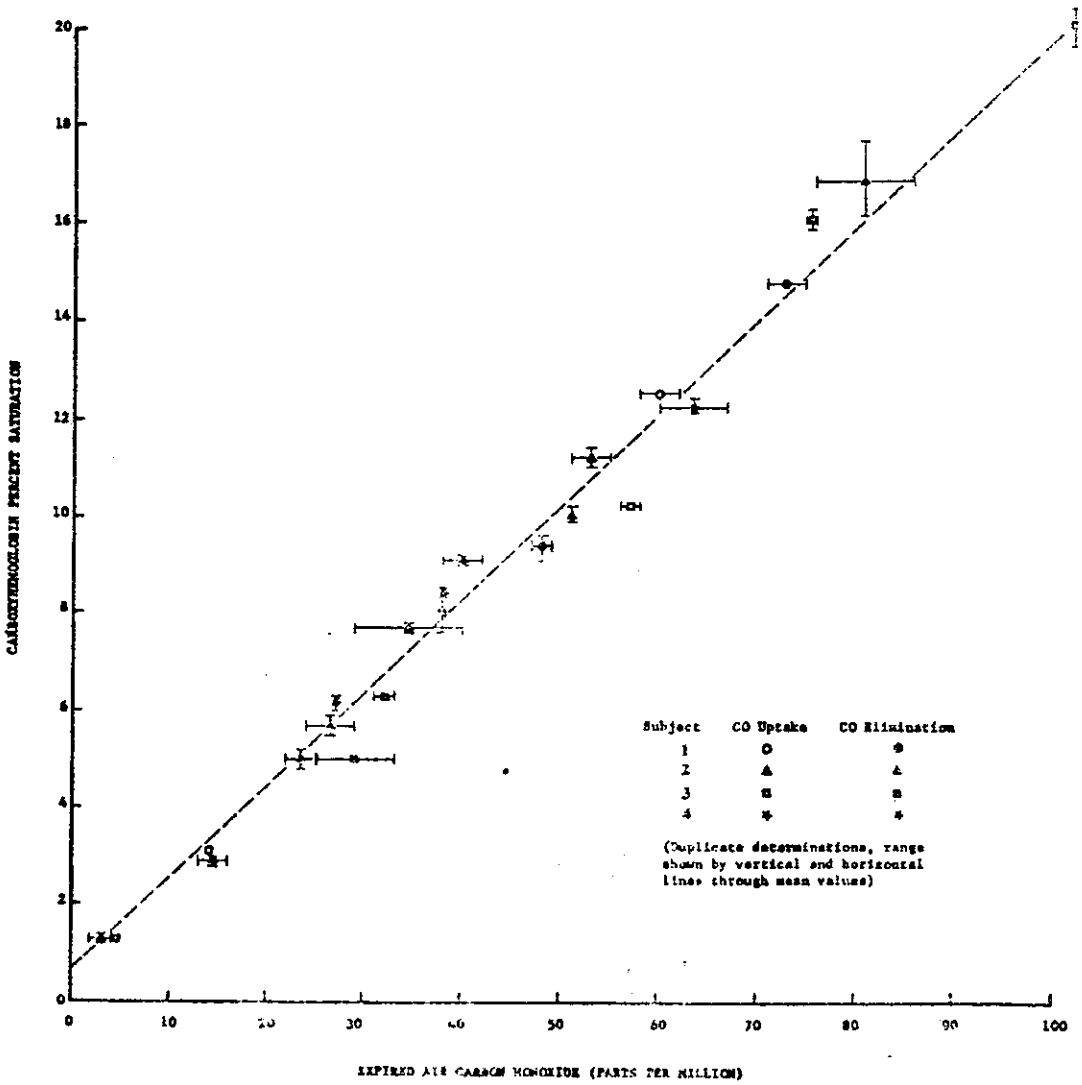


Fig. 1.—Correlation graph of expired air carbon monoxide and carboxyhemoglobin. Experimental exposures (20-second breath holding method).

TABLE 4.—*Relationship of Expired Air Carbon Monoxide and Carboxy Hemoglobin in Experimental Exposures (20-Second Breath-Holding)*

SUBJECT	PHASE OF EXPOSURE	EXPIRED AIR CARBON MONOXIDE IN PPM		BLOOD CARBON MONOXIDE			
		Observations	Mean	Volume Percent	Hemoglobin Gram Per 100 ml	Carboxy Hemoglobin Percent	Carboxy Hemoglobin Mean Percent
1	Pre-Exposure	46 60	53.0	a a	16.2	-	-
	Carbon Monoxide Uptake Exposure I	58 62	60.0	2.70 -	16.2	12.3 -	12.5
	Exposure II	75 76	75.5	3.49 3.42	16.2	15.2 15.8	16.0
	Exposure III	102 102	102.0	4.38 4.21	16.2	20.3 19.5	19.9
	Carbon Monoxide Elimination Two Hour Post-Exposure	71 75	73.0	3.18 3.18	16.2	14.7 14.7	14.7
	Four Hour Post- Exposure	47 49	48.0	1.97 2.07	16.2	9.1 9.6	9.4
2	Pre-Exposure	2 4	3.0	0.24 0.22	13.15	1.8 1.7	1.8
	Carbon Monoxide Uptake Exposure I	24 29	26.5	0.96 1.03	13.5	5.2 5.9	5.7
	Exposure II	51 51	51.0	1.78 1.74	13.5	9.2 9.9	10.0
	Exposure III	86 76	81.0	2.82 3.08	13.15	16.1 17.6	16.8
	Carbon Monoxide Elimination Two Hour Post-Exposure	55 51	53.0	1.93 2.00	13.15	11.0 11.4	11.2
	Four Hour Post-Exposure	29 40	34.5	1.33 1.37	13.15	7.6 7.8	7.7
3	Pre-Exposure	4 5	4.5	0.27 0.27	15.3	1.3 1.3	1.3
	Carbon Monoxide Uptake Exposure I	14 14	14.0	0.64 0.64	15.3	3.1 3.1	3.1
	Exposure II	33 31	32.0	1.28 1.30	15.3	6.3 6.4	6.3
	Exposure III	58 56	57.0	2.09 2.07	15.3	10.2 10.2	10.2
	Carbon Monoxide Elimination Two Hour Post-Exposure	38 38	38.0	1.56 1.69	15.3	7.6 8.3	8.0
	Four Hour Post-Exposure	33 25	29.0	1.03 1.03	15.3	5.0 5.0	5.0
4	Pre-Exposure	16 13	14.5	0.53 0.56	14.0	2.8 3.0	2.9
	Carbon Monoxide Uptake Exposure I	25 22	23.5	0.91 0.96	14.0	4.8 5.1	5.0
	Exposure II	38 38	38.0	1.59 1.56	14.0	8.5 8.4	8.4
	Exposure III	67 60	63.5	2.31 2.26	14.0	12.4 12.1	12.2
	Carbon Monoxide Elimination Two Hour Post-Exposure	38 42	40.0	1.69 1.71	14.0	9.0 9.2	9.1
	Four Hour Post-Exposure	27 27	27.0	1.18 1.13	14.0	6.3 6.0	6.2

a Blood sample clotted.

Source: State of California, Department of Public Health, Chronic Diseases Air Pollution Medical Studies.

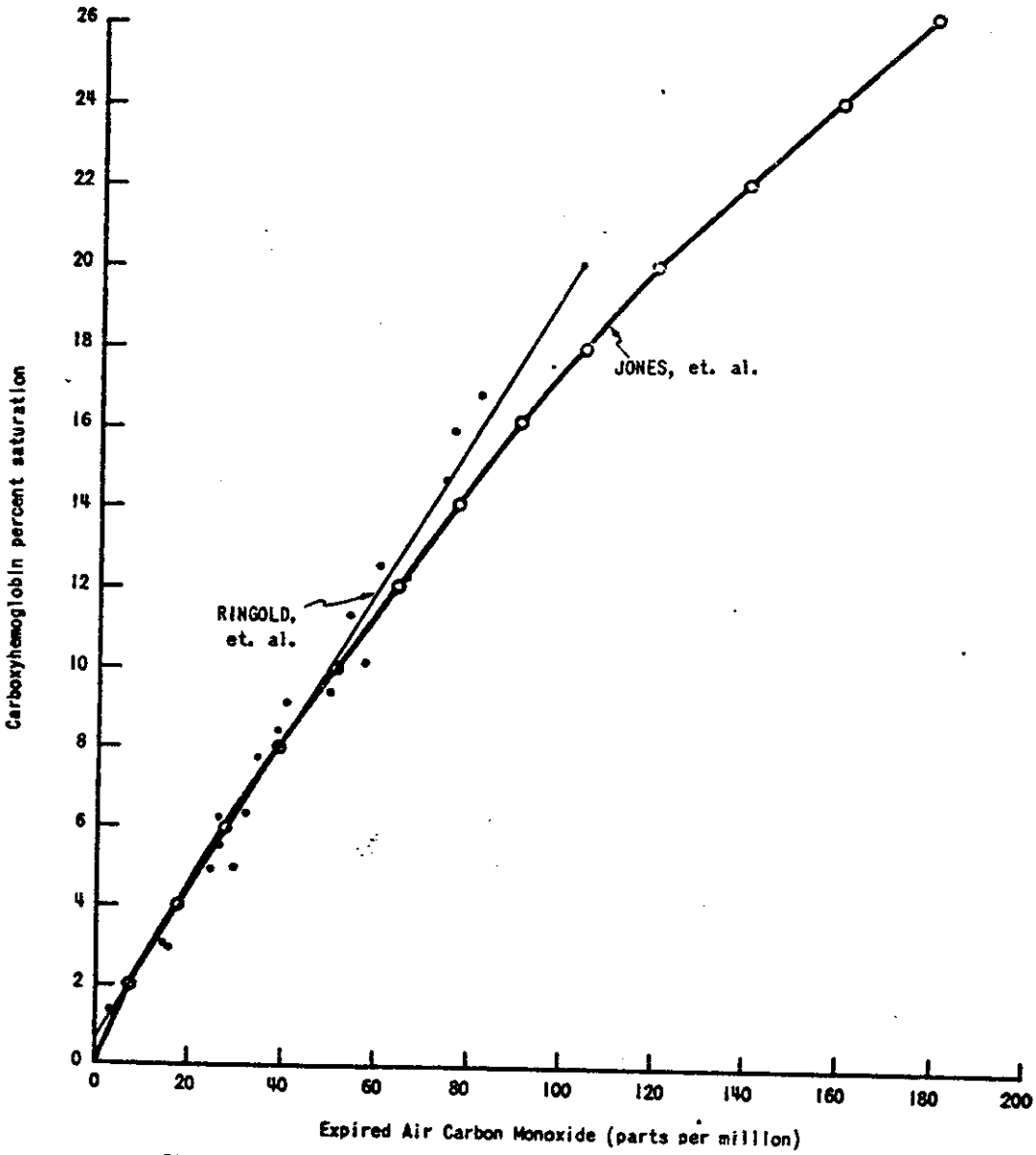


Fig. 2.—Comparison of results with 20-second breath-holding method.

polyvinyl bag when each person's specimen was collected.

The data related to cigarette smoking are shown in Figures 3, 4, and 5. The time course of expired air carbon monoxide in a cigarette smoker is shown in Figure 6. The data presented are corrected for the estimated ambient air levels by subtracting the tension found in the composite sample for that day from the tension found in the expired air samples.

Figure 7 shows the relationship of the composite ambient air specimens to the equilibrated expired air specimens for nonsmokers.

Comment on the Findings

In Figure 3, note that only one specimen from a nonsmoker was found to exceed 12.5 parts per million. This man was reinterviewed, and additional specimens were taken. He works as a paint crew supervisor in an

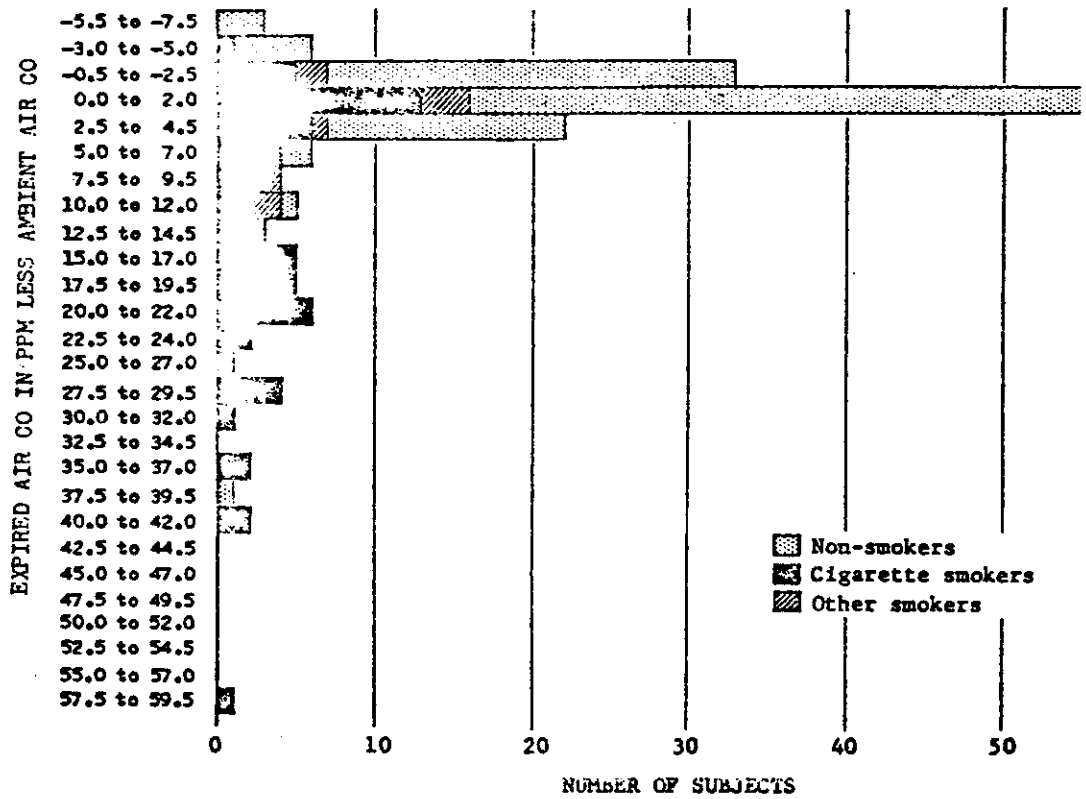


Fig. 3.—The distribution, according to smoking history, of expired air carbon monoxide tension, corrected for the ambient air carbon monoxide.

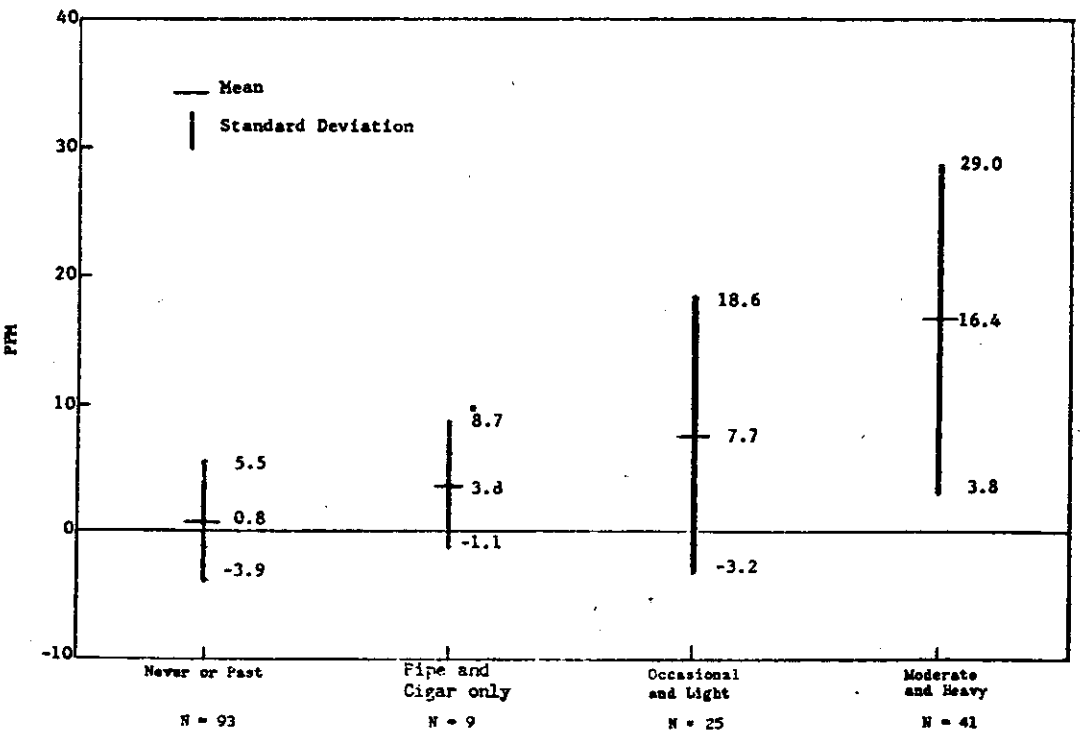


Fig. 4.—Alveolar air CO less ambient air CO by normal smoking behavior.

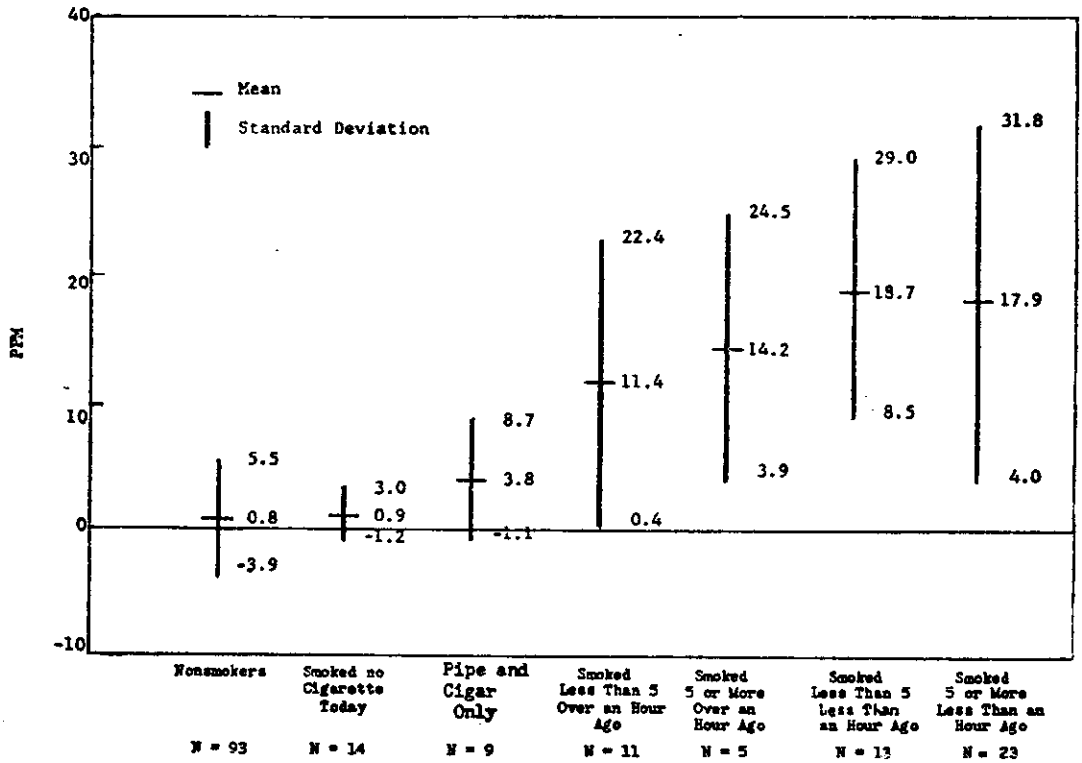


Fig. 5.—Alveolar air CO less ambient air CO by smoking experience on day of interview.

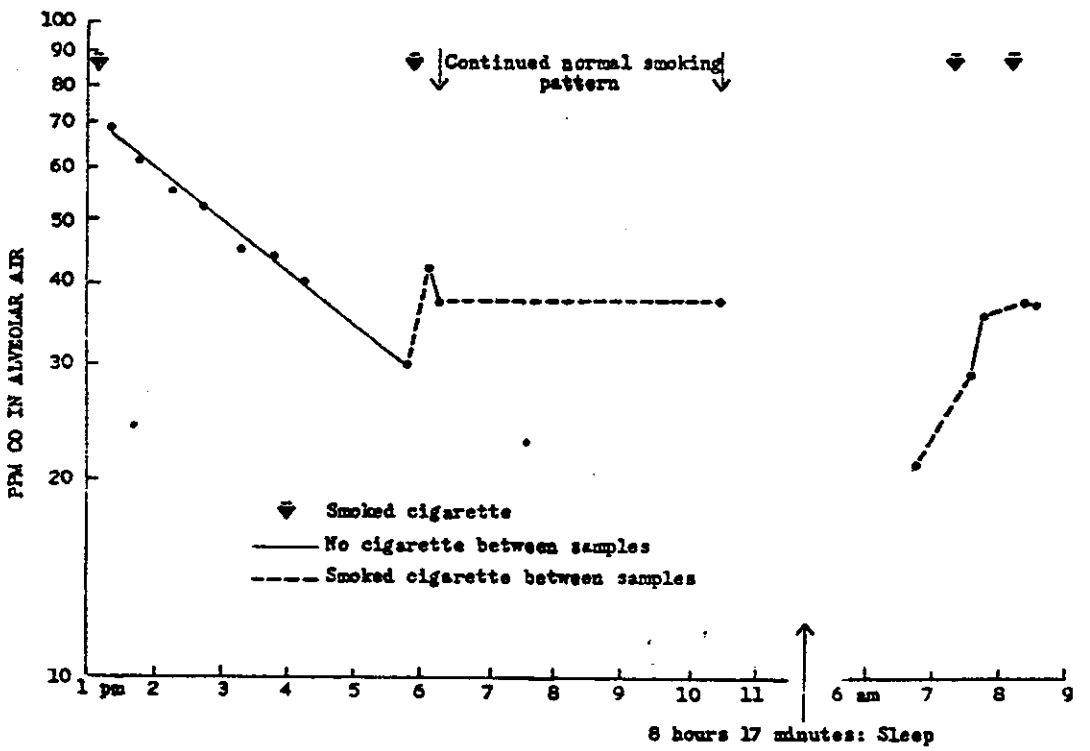
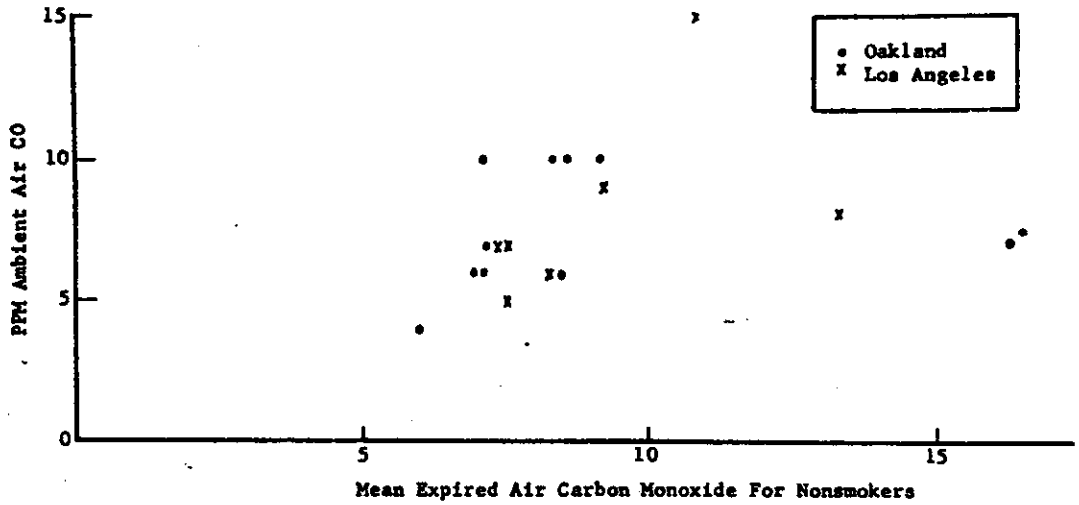


Fig. 6.—Relationship of alveolar air CO to cigarette smoking. Subject male, Aug. 25-26, 1960.



VALUES PLOTTED

OAKLAND N=46				LOS ANGELES N=45			
	Carbon Monoxide in PPM				Carbon Monoxide in PPM		
Date	Ambient Air	Expired Air Mean	N	Date	Ambient Air	Expired Air Mean	N
July 18	10	7.00	12	July 26	15	10.83	3
19				27	9	8.88	8
21				28	8	13.33	3
27	10	9.00	2				
28	10	8.50	5	August 8	7	7.50	7
29	10	9.33	3	9	7	7.50	4
				10	5	7.91	11
August 1	6	7.10	5	11	6	8.17	9
2	6	7.10	5				
4	6	8.50	4				
11	7	7.25	6				
25	7	16.25*	4				
29	4	6.00	2				

* This mean includes one extreme observation 45.0; without this observation, the mean would be 6.7.

Fig. 7.—The relationship between composite ambient air specimens (at test sites) and the mean expired carbon monoxide for nonsmokers.

office adjacent to an auxiliary gasoline power plant. His second set of tests showed values below 12.5 ppm, but he did not recall whether the gasoline engine was running on the day of his original examination.

In Figure 4, the low values observed for pipe and cigar smokers are noteworthy. In Figure 5, the recency of smoking and the amount of smoking are seen to have a detectable effect on the population mean carbon monoxide gradient, although the values overlap substantially. The absorption of carbon monoxide by cigarette smokers was originally noted by Baumberger [11] and also by many subsequent observers.

Figure 6 shows that cessation of smoking causes a drop in expired air carbon monoxide, while the regular smoking pattern appears to produce a more or less constant carbon monoxide level.

Compared to the effect of cigarette smoking, the effect on nonsmokers of estimated ambient air levels in the examining room is less clear-cut, as seen in Figure 7. This particular set of studies was not well suited for demonstrating an effect of community air

pollution for several reasons. The amount of exposure to ambient carbon monoxide was low, the maximum being 15 parts per million. The ambient measurements were made in only one of the many locations in which the subjects had been exposed, and at these low levels it would take many hours for equilibrium to be reached.

Summary and Conclusions

A valid method for determining the carboxyhemoglobin level in the body has been developed. The method is based on the 20-second breath-holding method first described by Jones et al.,[9] using an infrared analyser first reported by Lawther and Apthrop.[5] Our work shows that a polyvinyl bag can be used for collection of samples in the field.

An application of this method is reported. This confirms the fact that cigarette smoking is a major factor in determining the carboxyhemoglobin level in a population. Pipe and cigar smokers appear not to have much increase in carboxyhemoglobin.

The method should be suitable for studying the relationship of carboxyhemoglobin to occupational and to ambient air pollution exposures.

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IV

DISTRIBUTIONS OF PERCENT METHEMOGLOBIN IN SEVERAL POPULATION GROUPS IN CALIFORNIA

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For The 7th International Scientific Meeting
International Epidemiological Association
Session VIII C August 19, 1974
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DISTRIBUTIONS OF PERCENT METHEMOGLOBIN IN SEVERAL POPULATION GROUPS IN CALIFORNIA

ABSTRACT

Several epidemiological studies in California have yielded data on percent methemoglobin in healthy individuals. The population groups represented include infants, elementary and high school children, and adults. The distributions of values in each group are described, as well as the differences between groups. Factors affecting the distributions are discussed.

In the study of infants, the factors assessed include respiratory and gastrointestinal disease, and food and water intake. In school children, the effect of age and location of residence within Southern California are evaluated, and in adults, smoking, gender and time of day.

Among infants in the 31 to 60 day age range, 33 percent had methemoglobin levels of 3 percent or above, while 8 percent had methemoglobin levels of 4 percent or above. Among adults, 15 percent had levels of 3 percent or above, while 2 percent had levels of 4 percent or above. Among both elementary and high school students, 3 percent had methemoglobin levels of 3 percent or above, while less than 1 percent had levels of 4 percent or greater.

DISTRIBUTIONS OF PERCENT METHEMOGLOBIN IN SEVERAL POPULATION GROUPS IN CALIFORNIA

Introduction

Elevated levels of methemoglobin imply an impairment of the oxygen transport function of the blood. To quantify this impairment requires a valid way to measure the methemoglobin and an understanding of the variation which may occur in population groups. The importance to health of low level impairment of oxygen transport has recently been defined for exposures to carbon monoxide with resulting levels of carboxy-hemoglobin being used as an index of the probable impairment[1]. Since exposures to nitrogen oxides in air and to nitrates and nitrite in food and water can produce methemoglobin in the body, the analogy has even greater interest. We shall report measurements of methemoglobin levels in infants, school children and commuting adults, comparing results from the latter studies with measurements of carboxyhemoglobin.

Quantitative relationships are well established for resting adults, between levels of carbon monoxide in air, duration of exposure, and levels of carboxy-hemoglobin[2]. When inactivation of hemoglobin to the extent of 5 percent as carboxyhemoglobin occurs, there is thought to be sufficient hazard to require air pollution control[3]. Recent studies of subjects with atherosclerotic cardio-vascular disease[4,5] have shown that increases of an amount averaging 1.5% COHb will impair exercise capacity.

Methemoglobin is a brown colored, inactive form of hemoglobin, in which ferrohemes (with divalent Fe^{++}) are oxidized to ferrihemes (with trivalent Fe^{+++}). As with carboxyhemoglobin, some methemoglobin is normally present and mechanisms for reversing the inactivation are known to be active and (in normally endowed persons) effective in keeping methemoglobin levels low. The naturally present methemoglobin is enzymatically reduced to hemoglobin, but in some families this enzyme is deficient[6,7]. In addition, to familial or congenital methemoglobin-emia, ingestion of sodium nitrite[8] or other toxic materials, or of water with elevated levels of nitrate[9] can produce methemoglobinemia, an illness which is characterized by a dusky cyanosis and methemoglobin levels usually in excess of ten percent. This latter hazard is especially noted in infants and because of this hazard the United States Public Health Service has recommended a drinking water standard of 45 mg/l of nitrate, equivalent to about 10mg/l of nitrate

nitrogen[10].

Since nitric oxide and nitrogen dioxide in vitro will produce nitrite ion, and since this ion reacts with hemoglobin to produce methemoglobin, there is sufficient reason to determine if persons exposed to high levels of inhaled nitrogen oxides manifest elevated levels of methemoglobin. Data from occupational exposures of welders using coated rods[11,12] exposed to an estimated 13 parts per million of "nitric gas" showed elevations of methemoglobin up to 3.0%.

In our study of methemoglobin levels in infants living in an area with high nitrate levels in the drinking water[13] we observed a bimodal age dependent distribution of methemoglobin (Figure 1). Some of the variability was related to the presence of respiratory and gastrointestinal illness, and some was related to amount of nitrate nitrogen ingested in water of formula (Table I).

This paper reports on our findings among populations of school children and commuting adults in the Los Angeles Basin. Since the two populations were not primarily being studied for this purpose, we can only infer the magnitude and and sources of exposure. By the methods we use, however, a high proportion of the values exceed the conventional "normal" level of 0.5% MHb[14]. We shall therefore discuss the normal value and its possible sources of physiological variation.

II. Methods

Three population studies are reported, of which the first has already been published[14].

The first study was of infants born to residents of two communities, in the Central Valley of California, with a combined population of about 20,000. The water supply is from ground water with occasionally elevated nitrate levels. For all infants born in the area, an invitaition to be examined and for the parents to be interviewed was issued. Examinations were carried out in a clinic specially organized and staffed for the study. Over a one year period (March 1970 - March 1971) 487 examinations with capillary blood test for methemoglobin were performed. This involved 256 infants. Laboratory equipment and reagents were brought to the clinic and the chemist measured hemoglobin and methemoglobin with half an hour after the blood sample was drawn, since methemoglobin present in whole blood is not stable. Water and formula nitrate and nitrite were measured and the infants were examined.

The second study was part of an examination of grade and high school children in six areas of Southern California at which three were thought to have more photochemical air pollution than the others. The study sites included Culver City, Riverside, and Azusa (more polluted) and Lancaster, Long Beach, and Oceanside (less polluted). Both male and female primary school children were studied. Since athletic performance was to be studied, only male high school student athletes were included. Of the six communities, Oceanside, Culver City, and Long Beach have water supplies with low nitrate levels, but Lancaster sometimes has slightly higher levels (to 7 mg/l); Azusa and Riverside supplies may contain as much as 23 and 30 mg/l. Neither water nor food samples were taken during the study. Venous blood samples were drawn and the methemoglobin was measured by the chemist within half an hour. The study period of Semptember-October 1972 was not one in which ambient air pollution showed much contrast between these communities.

In the third study, adult employees working in downtown Los Angeles, were subjects in a study primarily oriented to estimation of carbon monoxide uptake during commuting. In order to validate the expired air samples which were used for the study, venous blood samples were drawn and analyzed for carboxyhemoglobin; methemoglobin was measured within half an hour. The study was done a week at a time during September and December 1972 and January, February, and March 1973. These are designated weeks 1-2 and weeks 3-6.

Both hemoglobin and methemoglobin were measured and the results expressed as percent methemoglobin. The method used[14] was based on colorimetric estimation of cyanomethemoglobin published by Hainline, with modifications suggested by Winton and Tardiff, and Hegesh et al. Following hemolysis in Tritonborate, centrifugation was continued in order to assure a clear supernatant solution.

Results:

Figure 1 and Table I show the results of our first study. Among infants in the age range of 31 to 60 days, 33 percent had methemoglobin values above 3 percent and 8 percent exceeded four percent methemoglobin.

Table II shows for the study of school children the means and standard deviations as well as the number exceeding 2.5% methemoglobin. The means for the population-days sets vary from 1.37 (Long Beach 10/2) to 2.09 (Riverside 10/11). There appears to be no systematic sex and age difference. Long Beach and Culver City are relatively low. Among the Azusa samples there are not significant differences between the mean results for the two study days. In Riverside, the high values were for the high school study day, and in Oceanside and Lancaster the high values were found on the grammer school study days. Air pollution measurements on the day of sampling are unlikely to account for these variations.

Figure 2 is a cumulative frequency chart showing that the distributions of tests for males and females are virtually identical. Three percent had methemoglobin greater than three and for 1 percent of the school children, the levels were above 4% methemoglobin.

The third set of results are shown in Table III along with comparable means, standard deviations and sample sizes for sex and smoking by groups of weeks and by whether the sample was taken after arrival at work or before leaving in the evening. Figure 3 shows the cumulative frequency plot. (The lines drawn in the figures do not represent computed regression, but are drawn "freehand")

No systematic differences occur between males and females or from morning to afternoon.

In Table IV the data for both sexes are therefore combined and the weeks are shown separately. There are no differences in methemoglobin between smokers and non-smokers, although there are consistent differences between them for carboxyhemoglobin. There is a suggestion of higher methemoglobin values during week 2 September 18-20 (still in photochemical pollution period) compared to the other weeks, especially among non-smokers. In Table V we examine the tendency for a person with a methemoglobin above 3.0% to have a repeat test with results above the median. No effect is found. There is a tendency for males and females to deviate together on a given day. However the sample size on a given day was not sufficient for testing for the presence or absence of a day effect.

Discussion:

In all three populations, by the methods we use and based on prompt determination of methemoglobin, the median values exceed 2 percent methemoglobin and range up to 5 percent. This is several times the previously accepted normal value. If confirmed, it places variability in methemoglobin in the range which may, analogously with carboxyhemoglobin, be active in aggravation of circulatory impairment due to interference with oxygen transport. Since nitrites, with their potential for causing methemoglobin, are used to treat angina pectoris, the occurrence of methemoglobin in some such patients, either from iatrogenic or environmental causes could be of clinical importance.

The finding, in all populations, of a bimodal distribution, such that from five to ten percent appear to have high values (say above 2.5%) requires further scrutiny; since methemoglobin may be thought of as a normal but dynamically reversible form of hemoglobin, elevated values may be related to increased production or diminished recycling of the substance. The former may be thought of as subject to environmental influence and the latter as enzymatically controlled. So at a random time in a random subject one may have elevated methemoglobin due to ingested nitrite, inhaled oxides of nitrogen, and/or relatively inactive methemoglobin reductase. Without further data on the time course of methemoglobin reduction, and the factors which influence population distribution of methemoglobin reductase, no further inferences seem appropriate. The distribution may be influenced by the direct or interactive role of measurement variance.

In infants, nitrate-nitrite ingestion and presence or absence of minor illness are major determinants of the elevated levels observed. We do not know if this is the case for school children and adults. However the daily variation among groups of school children may provide a clue, but unfortunately, no food or water ingestion data were collected during this study.

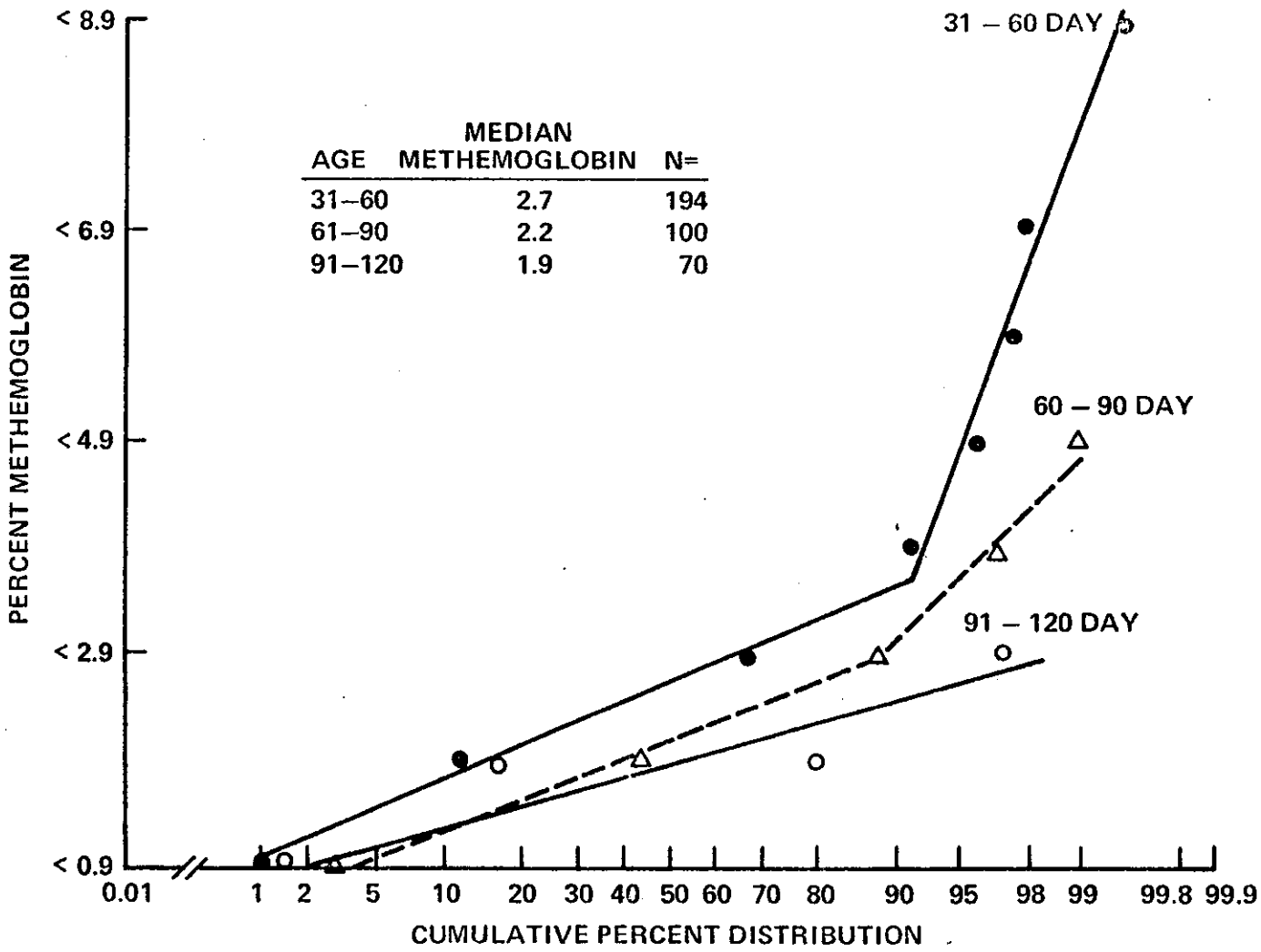
In infants the observed high levels in babies 31-60 days, compared to older and younger children are thought to be a reflection of a) the immaturity of methemoglobin reductase enzymes and b) the relatively high PH of the gastric contents which could permit bacterial reduction of nitrate to nitrite.

The respiratory exposure to inhaled oxides of nitrogen had seemed to be an important possible source of nitrite ion and hence of methemoglobin. Although the data are consistent with a small contribution of community air pollution, there certainly is no discernable effect of cigarette smoking, which involves greater nitrogen oxide exposures, by several orders of magnitude. Conceivably cigarette smokers could have, as a result of enzyme induction, efficient methemoglobin reductase systems, in which case non-smokers smoking a cigarette might manifest increased methemoglobin, whereas regular smokers would not.

With respect to environmental exposures, and methemoglobin, our data support only the effect of nitrate-nitrite ingestion in infants. However the day to day variation among school children is suggestive of an environmental factor or factors. Only further study of exposure-response relationships will permit an answer. In comparison with carboxyhemoglobin, the apparent levels of methemoglobin in populations studied are of concern, as indicating both clinical and public health problems. The validation of the procedure has been a concern: so far in stability tests, we find on the basis on preliminary data, that test values are at a maximum in half an hour after the sample is drawn.

A great deal of interest has been expressed concerning the formation of the class of potent nitrosamine carcinogens in the gastro-intestinal tract through reaction of nitrite with the precursor amines. While this has been observed in animal studies[15,16], the process if it occurs in man will be difficult to study. However, the measured variation in methemoglobin, as an indicator of the ingestion of nitrate (or nitrite), may be a meaningful index of risk.

CUMULATIVE PER CENT OF INFANTS BY AGE GROUPS FOR PER CENT METHEMOGLOBIN MARCH 1970 - MARCH 1971



AGE	MEDIAN METHEMOGLOBIN	N=
31–60	2.7	194
61–90	2.2	100
91–120	1.9	70

Figure 1

CUMULATIVE PROBABILITY DISTRIBUTION OF METHEMOGLOBIN LEVELS AMONG LOS ANGELES SCHOOL CHILDREN

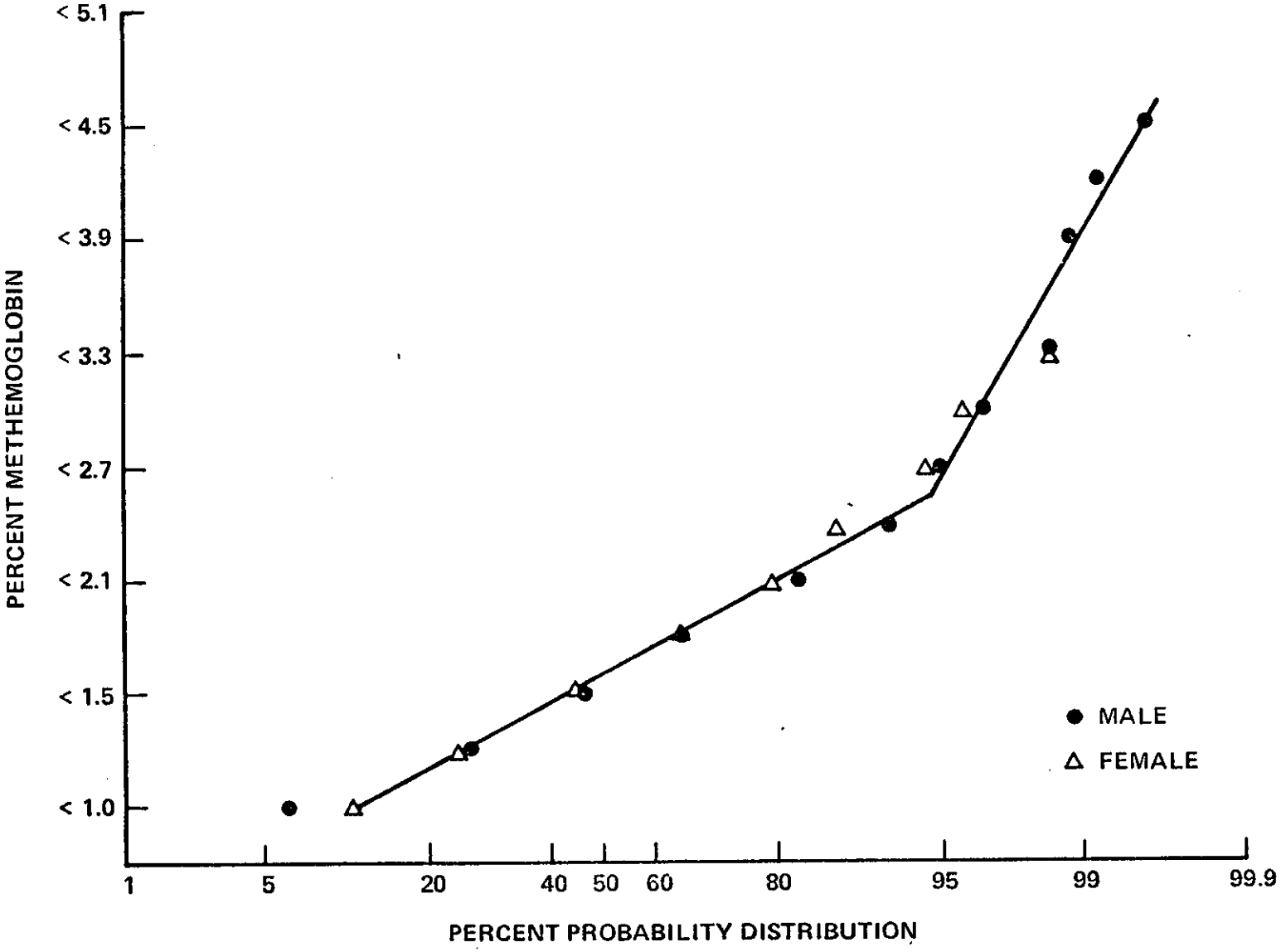


Figure 2

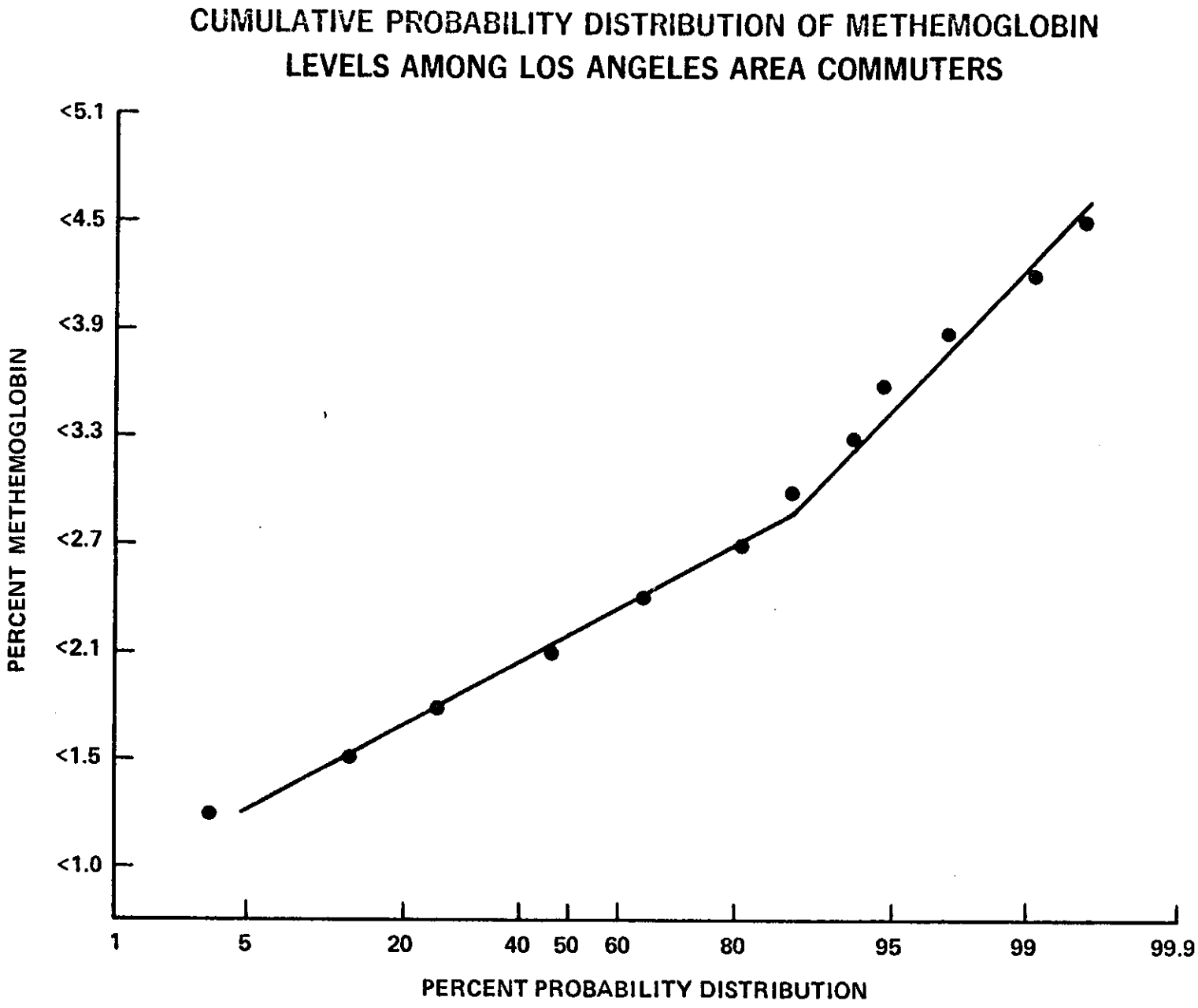

CUMULATIVE PROBABILITY DISTRIBUTION OF METHEMOGLOBIN LEVELS AMONG LOS ANGELES AREA COMMUTERS
PERCENT METHEMOGLOBIN
<5.1
<4.5
<3.9
<3.3
<2.7
<2.1
<1.5
<1.0
1
5
20
40
50
60
80
95
99
99.9
PERCENT PROBABILITY DISTRIBUTION

Figure 3

Table I

METHEMOGLOBIN LEVELS OBSERVED IN INFANTS BY AGE, PRESENCE OF DIARRHEA OR RESPIRATORY ILLNESS, AND TWENTY FOUR HOUR NITRATE-NITRITE NITROGEN IN WATER OR FORMULA

		Infants Without Illness				Infants with Diarrhea or Respiratory Illness			
Twenty Four Hour Nitrate-Nitrite Nitrogen in Water or Formula		<5 mg		5 mg +		<5 mg		5 mg +	
Methemoglobin Level		<4%	≥4%	<4%	≥4%	<4%	≥4%	<4%	≥4%
Age of Infant	Total No.								
<30 days	45	30	2	5	0	8	0	0	0
31 - 60 days	194	128	6	19	3	28	5	2	3
61 days +	240	156	1	21	2	60	0	6	0
Total	419	314	9	45	5	96	5	8	3
% of Babies with 4% or More Methemoglobin		2.78%		10%		4.9%		27.2%	

Source: Shearer et al (13)

Table II

METHEMOGLOBIN LEVELS IN SCHOOL CHILDREN IN SOUTHERN CALIFORNIA
PER CENT METHEMOGLOBIN (%MHb.)

ELEMENTARY

FEMALES

Date	9/21	10/2	10/5	10/10	10/13	10/16
Place	Lancaster	Long Beach	Culver City	Riverside	Azusa	Oceanside
N	28	24	21	23	22	17
Mean	2.04	1.37	1.63	1.41	1.66	1.81
Std. Dev.	0.616	0.502	0.429	0.650	0.716	0.754
Equal or greater than 2.5% MHb.	6	0	0	2	4	5

MALES

N	11	22	28	27	18	21
Mean	2.11	1.53	1.54	1.70	1.86	2.06
Std. Dev.	0.373	0.434	0.312	0.724	0.960	0.451
Equal or greater than 2.5% MHb.	2	0	0	3	2	3

HIGH SCHOOL

MALES

Date	9/22	10/3	10/16	10/11	10/12	10/17
N	20	30	20	17	23	26
Mean	1.44	1.38	1.43	2.09	1.82	1.55
Std. Dev.	0.555	0.387	0.535	0.773	0.846	0.609
Equal or greater than 2.5% MHb.	1	0	0	5	4	1

Table III

METHEMOGLOBIN AND CARBOXYHEMOGLOBIN CONCENTRATIONS IN PERCENT FOR ADULT LOS ANGELES COMMUTERS BY SEX, SMOKING, WEEK, AND TIME OF DAY

	MALE						FEMALE					
	Number	Mean	S.D.	Number	Mean	S.D.	Number	Mean	S.D.	Number	Mean	S.D.
	(Weeks 1-2)			(Weeks 3-6)			(Weeks 1-2)			(Weeks 3-6)		
METHEMOGLOBIN												
Non-smokers												
Morning	7	2.80	0.26	52	2.40	0.08	3	3.33	0.41	18	2.28	0.11
Afternoon	8	3.11	0.26	53	2.06	0.08	14	1.97	0.18	18	2.23	0.13
Smokers												
Morning	4	2.25	0.49	22	1.97	0.09	—	—	—	9	2.58	0.34
Afternoon	3	3.47	0.39	20	1.86	0.17	2	3.3	0.70	7	1.76	0.26
CARBOXYHEMO-GLOBIN												
Non-smokers												
Morning	28	1.91	0.08	39	2.22	0.09	9	1.46	0.13	13	2.47	0.21
Afternoon	20	1.70	0.09	57	1.86	0.04	—	—	—	20	1.65	0.07
Smokers												
Morning	18	4.53	0.56	15	6.16	0.64	3	3.40	1.57	7	5.84	1.01
Afternoon	9	5.67	0.97	21	6.76	0.97	6	6.90	1.03	7	7.33	1.64

Note: Methemoglobin values were not obtained during week 1; apart from this, differences in number of samples reported for methemoglobin and carboxyhemoglobin reflect only the number with satisfactory samples.

METHEMOGLOBIN AND CARBOXYHEMOGLOBIN CONCENTRATIONS IN PERCENT FOR ADULT COMMUTERS IN LOS ANGELES BY WEEK OF STUDY, BY SMOKING, AND BY TIME OF DAY (SEXES COMBINED)

	Week 1*			Week 2			Week 3			Week 4			Week 5			Week 6		
	Number	Mean	S.D.	Number	Mean	S.D.	Number	Mean	S.D.	Number	Mean	S.D.	Number	Mean	S.D.	Number	Mean	S.D.
% METHEMOGLOBIN																		
Non-smokers																		
Morning	–	–	–	8	2.94	0.26	17	2.57	0.13	16	2.28	0.12	14	2.53	0.13	23	2.19	0.12
Afternoon	–	–	–	11	3.17	0.21	12	1.97	0.14	19	2.09	1.15	23	2.06	0.11	17	2.28	0.15
Smokers																		
Morning	–	–	–	4	2.25	0.49	9	2.26	0.39	7	2.27	0.14	5	2.24	0.16	10	1.91	0.11
Afternoon	–	–	–	5	3.40	0.31	7	1.67	0.21	7	1.90	0.36	9	1.81	0.29	4	2.05	0.06
% CARBOXYHEMOGLOBIN																		
Non-smokers																		
Morning	24	1.69	0.08	18	2.25	0.11	20	2.32	0.10	16	2.44	0.22	15	2.13	0.09	–	–	–
Afternoon	9	1.73	0.09	20	1.58	0.10	12	1.77	0.09	22	1.77	0.06	26	1.83	0.07	17	1.86	0.10
Smokers																		
Morning	14	4.45	0.65	7	4.21	0.90	10	6.36	0.54	7	6.61	1.10	5	4.67	1.40	–	–	–
Afternoon	4	5.17	1.35	11	6.52	0.84	7	5.04	1.49	8	8.74	1.78	9	7.99	1.23	4	4.06	1.69
				September 18-20			December 12-15			January 8-12			January 29-February 2			February 26-March 2		

*Equipment not available for methemoglobin during week 1.

Table V

REPEATED VALUES OF METHEMOGLOBIN FOR THOSE WITH ONE VALUE EXCEEDING 3%

	Initial High Value	Repeated Tests with Same Subject				Proportion Exceeding Median Value of 2.2	
			NON-SMOKERS				
MALES							
Morning	3.9	3.0	3.0	4.0	2.2	3/4	
	3.8	3.1	2.3			2/2	
	3.8	2.1	1.9	1.3		0/3	
	3.4	2.8	2.3	2.3		3/3	
	3.4	3.1	2.6	1.4		2/3	
	3.1	2.3	2.0	2.0		1/3	
Afternoon	4.0	1.1	1.3			0/2	12/23
	3.2	3.1	1.3	1.8		1/3	
FEMALES							
Morning	3.9	2.5	2.0			1/2	
	3.2	2.3	1.7			1/2	4/7
Afternoon	4.0	2.6	2.7	2.1		2/3	
			SMOKERS				
MALES							
Morning	3.5	2.1	1.5	1.4		0/3	
	4.0	2.7	2.0	1.4		1/3	
Afternoon	3.8	1.8	2.2			0/2	
	3.7	1.9	2.1			0/2	
FEMALES							
Morning	5.2	2.4				1/1	2/11

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V

DETERMINATION OF NITRIC OXIDE IN BLOOD BY ISOTOPE DILUTION ANALYSIS*

by

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Introduction

Nitric oxide, a major air pollutant, is produced in internal combustion engines and during other high temperature combustion processes. Because the rate of oxidation of nitric oxide (NO) to nitrogen dioxide (NO_2) in the air is relatively slow, a considerable steady-state concentration of NO is built up. Nitric oxide is a critical ingredient in the mechanism of smog formation, but little is known about its biological effects at low concentrations.

Gibson and Roughton demonstrated that NO binds to hemoglobin (Hb) many times stronger than does carbon monoxide (CO).[1] The mode of binding of NO to Hb has been recently investigated using electron spin resonance (esr) techniques.[2] The ready access of Hb in the lungs to inhaled CO suggested that NO might react similarly with Hb. Nitric oxide has not been, however, recognized as a significantly toxic material and though a lethal effect on mice has been reported,[3] it was not included among the substances having a threshold limit value by the American Conference of Governmental Industrial Hygienists (1967).[4]

There is a discrepancy between the expected binding of NO to Hb *in vivo* and the failure to detect the characteristic HbNO optical absorption band, despite the many years of observations on blood by spectrophotometry. Negative results were also obtained in a study using the more sensitive technique of esr to detect the free radical (either NO or NO_2) in blood of rats exposed to NO.[5] A recent study using practically the same technique indicates, however, the presence of Hb-NO in the blood of mice exposed to NO.[6] Preliminary experiments using the same isotope dilution analysis on which the present study is based suggest that human subjects carry minute amounts of NO in their blood.[7]

Owing to its stability the concentration of the Hb-NO complex in blood is expected to increase in time, reaching a steady-state concentration determined by the rates of NO intake and of its metabolization. Considering the reported stability constant of the Hb-NO complex, the Hb bound NO must be metabolized, most probably oxidized, rather effectively; otherwise NO would have been recognized a long time ago as a highly toxic substance.

The hypothesis that NO reaches a steady-state concentration in blood suggests that the level of HbNO in blood should increase in areas with high air pollution

or in persons who smoke excessively. The concentration of HbNO in blood, even if very low, could thus serve as a simple basis for epidemiological studies of populations exposed to NO-laden atmospheres.

It was of importance, therefore, to develop a sensitive quantitative analytical method for determination of NO in blood. We are interested in quantities of NO of the order of 10 nanomoles or less per ml blood ($NO/O_2 < 10^{-3}$). These are concentrations too low to be reliably determined in situ by esr. Although NO can be released from blood by denaturation of Hb, this cannot be used as a base for an analytical method because NO would be readily oxidized to NO_2 by the oxygen simultaneously released. Removal of the oxygen prior to denaturation by evacuation, by repeated flushing with an inert gas or by substitution with CO is not a practical solution either, because it is impossible to guarantee quantitative removal of O_2 to one part per ten thousand which is required if NO is to be determined with a 10% precision.

A unique solution to the analytical problem outlined above is isotope dilution analysis. This quantitative method allows the handling of the minute amounts of NO without necessitating a quantitative recovery.

Whole blood, hemolized or nonhemolized erythrocytes, when exposed to a known amount of $N^{15}O$ will exchange any bound $N^{14}O$ with the excess $N^{15}O$, resulting in an isotopic dilution of the latter. Part of the $N^{15}O$ added is consumed in the process of substituting O_2 and CO on the hemoglobin, as well as by being oxidized to NO_2 by the O_2 released. If excess $N^{15}O$ is added, the isotopic composition of the residual NO will still represent the isotope equilibrium value. Thus, if the amount of $N^{15}O$ added is known as well as the isotope ratio $N^{14}O/N^{15}O$ in the residual NO, the amount of $N^{14}O$ present in the blood sample can be readily calculated. Since commercially available $N^{15}O$ contains a small percentage of $N^{14}O$, this amount must be taken into account when calculating the $N^{14}O$ present in blood.

The same equilibrium value of $N^{14}O/N^{15}O$ is obtained when the hemoglobin is denatured in the presence of $N^{15}O$. The strong binding of NO to hemoglobin is

overcome by denaturation of the protein. The denaturation has the advantage of making the dilution analysis independent of the kinetics of isotopic equilibration between bound and unbound NO, which may lead to a possible error due to competition between the isotope exchange and the NO oxidation reaction. As the isotopic exchange with the NO released by denaturation is instantaneous, the much slower oxidation reaction which removes part of the NO is inconsequential. It is still advisable to remove the oxygen from the blood before denaturation by evaucation or by substitution with CO. This diminishes the losses of NO and allows, therefore, the use of smaller quantities of $N^{15}O$, thus increasing the sensitivity of the isotope dilution analysis. Furthermore, if most of the NO is being oxidized to NO_2, the isotopic composition of the residual NO may be modified by isotopic fractionation. This effect is not trivial when we are considering a mixture which contains a minute quantity of the more reactive $^{14}N^{16}O$.

The precision of the isotope dilution method depends on the reliability of the determination of the $\overline{30}/\overline{31}$ mass abundance ratio. In other words, it is imperative that the measured ratio represents the true $N^{14}O/N^{15}O$ abundance ratio. This imposes stringent requirements on the ionization mode of the mass spectrometer. When the classical electron impact ionization is used, the species produced from NO, in addition to NO^+, include N^+, O^+, N_2^+, N_2O^+, O_2^+, and NO_2^+. Under these conditions the formation of $^{15,15}N_2^+$, which would be the predominant form of N_2^+, would overshadow the minor peak of $^{14}N^{16}O^+$. Chemical ionization is also unsuitable for our purpose because if we used a carbon containing ionizing gas, we could not avoid the formation of minute amounts of H_2CO^+ and $C^{18}O^+$ in addition to some $^{15,15}N_2^+$. If we used an inorganic ionizing gas, e.g., argon, the ionization pattern would be similar to that obtained by electron impact.

The only mode of ionization suitable to our problem is field ionization. Here we produce NO^+ exclusively under conditions which do not produce any N_2^+ even from trace impurities of N_2. The multipoint field ionization source recently developed at the Stanford Research Institute[8,9] was found most suitable for our purpose.

Experimental

Vacuum Apparatus and Sample Handling

All nitric oxide manipulations were carried out in vacuo using an all glass vacuum manifold and a mercury diffusion pump backed up with a medium sized ($\sim$ 5 cfm) Welch mechanical pump. To eliminate oxygen from the system and thus avoid the unwanted reaction $2NO + O_2 = 2NO_2$, we flushed the vacuum system with either nitrogen or helium at one atmosphere pressure. Flushing with nitrogen and helium was also used to remove oxygen from blood before $N^{15}O$ exposure.

The general procedure for exposing blood to $N^{15}O$ was as follows: One cubic centimeter of whole blood (or any other form such as red blood cells or hemoglobin solution) is withdrawn using a standard glass syringe and placed into a stopcocked glass vessel with a rubber septum stoppered side arm. This vessel is connected to the vacuum manifold with a ground glass joint (see Figure 1). The blood is then cooled to 0°C and deoxygenated with He or N_2 by bringing the blood to one atmosphere pressure of N_2 and then evacuating. This is then repeated for a total of six exposures to N_2. During this type of degassing, the blood has a tendency to bubble during evacuation. This can be kept to a minimum by closing the stopcock whenever boiling starts. After the fourth exposure to N_2, the sample is removed from the manifold and rotated by hand to coat the inner surface of the glass vessel, so as to give maximum surface exposure of the blood to N_2. It has been shown that no NO is lost from the sample during this procedure. The flushing procedure has been preferred over freeze-thawing because the latter procedure may result in partial denaturation of Hb and in consequence, loss of NO. After the final evacuation from N_2, the sample is frozen in liquid nitrogen. The sample loses some water during degassing, but this is held to a minimum by avoiding prolonged pumping.

The $N^{15}O$ is expanded from its flask into a calibrated volume (B, Figure 1), which is connected via a stopcock to a second calibrated volume (A, Figure 1), which includes the measuring capsule of a Wallace and Tiernan pressure gauge. The pressure of the $N^{15}O$ gas can be read to within 0.2 torr. Knowing the pressure and volume permits calculation of the number of micromoles of $N^{15}O$ added to the blood sample.

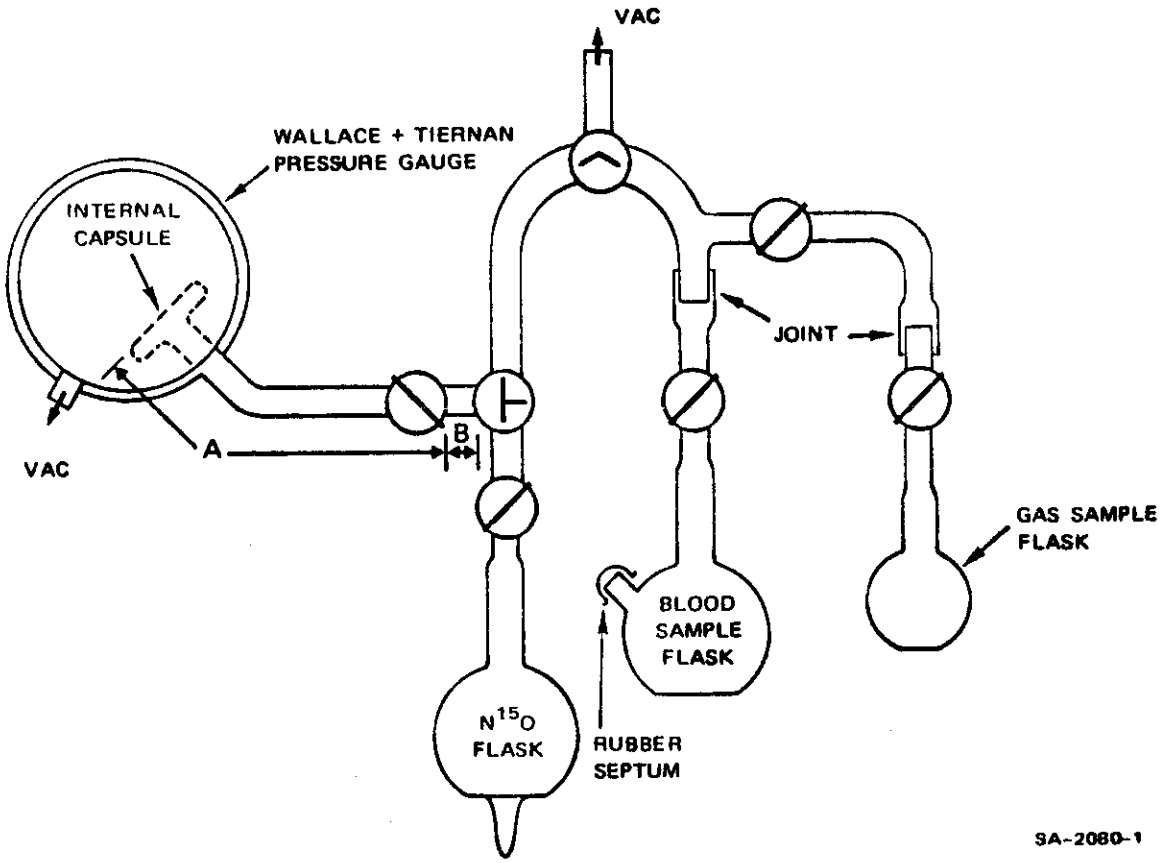

FIGURE 1 VACUUM APPARATUS FOR SAMPLE PREPARATION

After reading the pressure of $N^{15}O$, the $N^{15}O$ is condensed (using liquid nitrogen) into the flask containing the frozen degassed blood and the stopcock is closed. One ml of 10% sodium dodecyl sulfate (SDS) is then added using a syringe and needle via the rubber septum. The SDS is introduced under vacuum and while the blood is still at liquid nitrogen temperature. The blood sample flask is then removed from the manifold and thawed. Since hemoglobin does not bind NO when denatured, all bound $N^{14}O$ is released, forming a mixture of $N^{14}O$ and $N^{15}O$. Attempts to use trichlorocetic acid (TCA) as denaturing agent were unsuccessful, resulting in the total loss of NO. It seems that TCA induces oxidation or binding of NO by one of the cellular constituents.

After equilibration the flask is replaced on the vacuum line, and the blood is frozen in dry ice-acetone, which freezes out water vapor but not nitric oxide. The isotopic mixture of nitric oxide is then condensed with liquid N_2 into a previously evacuated small stopcocked receiving flask that can be removed from the system. This flask is designed to fit onto the vacuum inlet of the mass spectrometer and is now ready for isotopic ratio determination. This flask contains a droplet of mercury plus a small amount of Tobias' acid to remove NO_2 and formaldehyde.

There were two substances, which interferred with our analytical procedure, NO_2 and formaldehyde. NO_2, formed by oxidation of NO during the procedure or subsequently by trace amounts of oxygen which leaked into the gas sampling vessel, was found to attack chemically the microcones of our multipoint source and thus to diminish the ionization efficiency. We overcame this artifact readily by adding a droplet of elemental mercury to the gas sampling vessel. Mercury reduces NO_2 to NO forming HgO thus preserving the nitric oxide while keeping it from accidental oxidation.

It was also found that human blood undergoing the treatment described above releases small quantities of formaldehyde, of the order of 50 nanomoles per ml blood. Formaldehyde, H_2CO, having mass 30 thus interferred with the determination of NO in spite of its lower volatility. This artifact was eliminated by adding to the gas sampling flask a small amount of 2-rapthylamine 1-sulfonic acid (Tobias' acid) which removed the formaldehyde quantitatively forming a Schiff base.

We have also looked into the possible interference of ethane, C_2H_6, also mol. wt. 30. Gas chromatographic analysis of the gas sample set the upper limit of ethane present at 0.1 nanomole/ml human blood, which is equivalent to $< 0.5\%$ of the NO concentration found in the same blood. This observation does not exclude the presence of ethane in human blood, only that it is absent when the blood is denatured after repeated flushings with pure nitrogen. In any case, ethane should not be considered as an interfering substance in the NO dilution analysis.

Mass Spectrometric Isotopic Ratio Determination

The mass spectrometer used in this study was a labmade 45° magnetic sector instrument fitted with a SRI multipoint porous field ionization ion source[9] and two nearly identical detectors. One detector is fixed and the other is movable in two dimensions to center the ion beam in the detectors (see Figure 2). The detectors are continuous dynode electron multipliers operated in pulse mode. The pulses are amplified, shaped, and fed through a discriminator set to respond to all true ion events but to reject random pulses down to a rate of ~ 0.5/sec, which constitutes the natural background dark current of the detectors. The counts are registered in an electronic ratio counter, which displays the mass 30 count while counting a preset mass 31 count. A second counter is used to display mass 31 count to determine the rate of count and to aid in aligning the two ion beams initially.

The NO sample is introduced into the ratio measuring mass spectrometer through a stainless steel leak valve. The gas passes directly through the ionizing field of the source into a pumped volume at a rate sufficient to raise the total pressure in the vicinity of the source by 3×10^{-8} torr over base pressure, which is $\sim 3 \times 10^{-8}$ torr. At this flow rate, $N^{15}O^+$ ions reach the detector at a rate of 3,000 to 10,000 per second. A ratio determination is made by counting the number of $N^{14}O^+$ ions detected while 10^5 $N^{15}O^+$ ions are counted. This measurement was repeated ten times and the ratios are averaged. The variance of the ratios was about 2%.

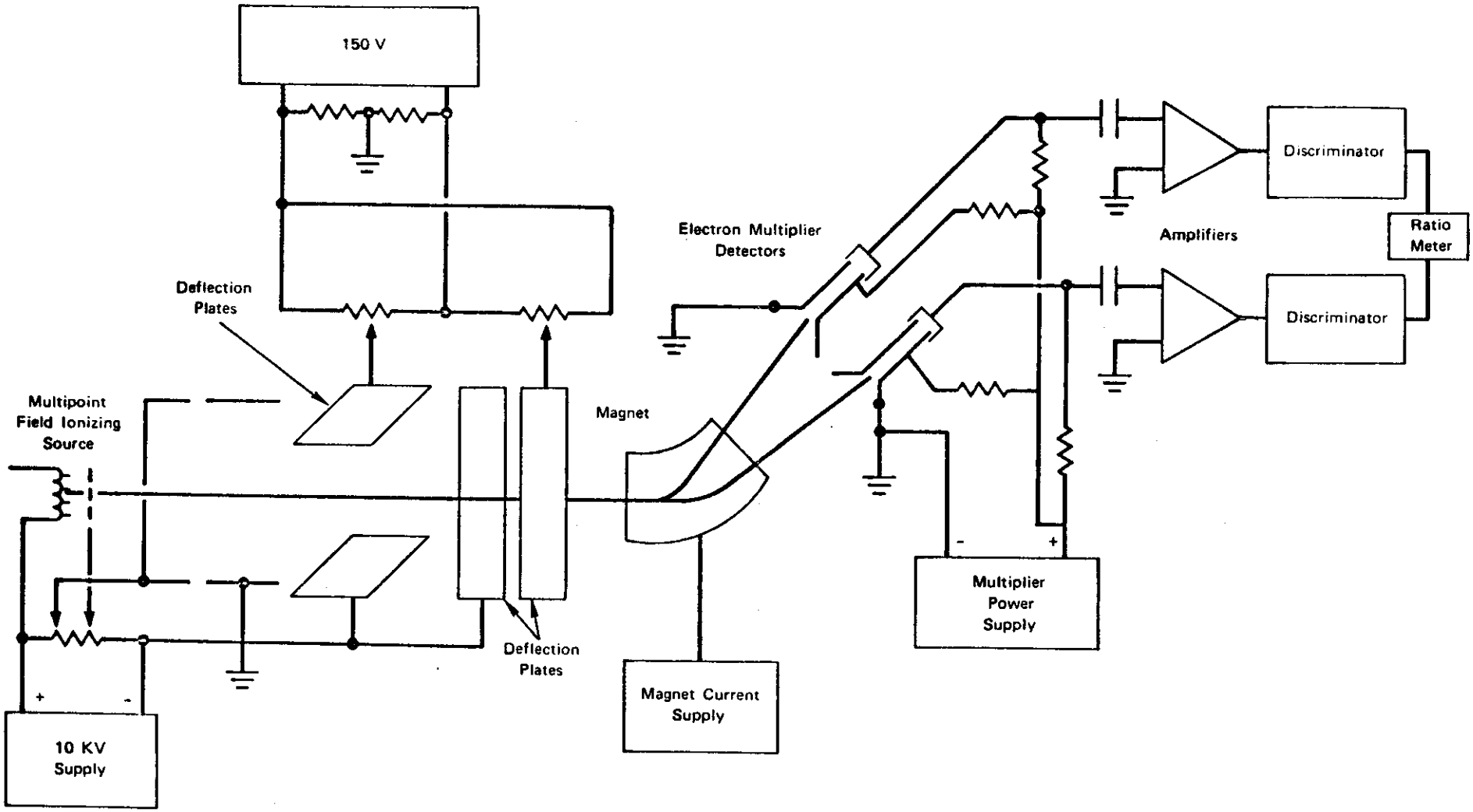

FIGURE 2 SCHEMATIC OF THE MAGNETIC SECTOR MASS RATIO SPECTROMETER

The flow of NO is then interrupted, and the background ion count at mass 30 is measured for 100 seconds. Knowing the time required to reach the 10^5 counts at mass 31, we substract the equivalent background counted of mass 30 from the rough ratio average. This correction seldom exceeded 5% of the counts at mass 30. A conversion factor is applied to the resultant ratios to equalize the detectors in terms of counting efficiency. This factor is arrived at by measuring a sample with a known ratio of $N^{14}O/N^{15}O$. The variance (S.D./average) when the same sample was measured several times on the same day was about ± 3%. This variance increased to ± 5% when the same sample was measured on different days over a period of several months.

The reproducibility of recovery of $N^{14}O$ from a number of samples of blood of the same exposure was fairly satisfactory; a variance of 10% was observed with a recovery of about 95%. This variance is not too surprising if we remember that the volumetric measurement of 1 ml of a heterogeneous fluid like blood may involve a 5% error. Further the boiling of the whole blood, when the nitrogen is being removed, may result in partial hemolysis and denaturation of hemoglobin with a consequential loss of NO. The relatively large variance in recovery of NO is tolerable when we are concerned with human blood samples, which seem to carry 10 to 20 nanomoles per ml. At these levels of NO, the precision of the mass spectrometric determination corresponded to ± 1.5 nanomole/ml. In other words, the variance in recovery was comparable to the variance in the mass spectrometric determination and was not, therefore, the limiting factor. The sensitivity and the precision of the mass spectrometric determination can, however, be increased by diminishing the amount of $N^{15}O$ added. Under the latter conditions, the reproducibility of $N^{14}O$ recovery would become the limiting factor of precision.

Scope and Limitations of the Isotope Dilution Method for Determination of NO

Isotope dilution analysis using field ionization mass spectrometry is competitive with esr techniques. As our method now stands, it will readily detect 10 nanomoles of NO in a milliliter of whole blood, i.e., 0.1% saturation of Hb. This sensitivity can be tripled without any special effort by decreasing the amount of $N^{15}O$ added by a factor of 3 or by a corresponding increase of the blood sample to 3 ml. The increase of sensitivity to 1 nanomole per ml could also be achieved after investment of some additional development.

The precision of the method as it stands today is ± 10%. The main limiting factor is the yield of NO recovery. The mass spectrometric determination is precise to within ± 2% and could be improved if necessary. The pressure measurement of the added $N^{15}O$ and the efficiency of gas handling may add 1 to 2% uncertainty to the determination. If substantial amounts of interfering substances, such as ethane or formaldehyde, occur in the biological samples, they will add another source of error that could, however, be completely eliminated by a gas chromatographic separation before the mass spectrometric determination.

It seems reasonable to suggest, however, that a 10% precision is sufficient for epidemiological or physiological investigation. Thus no upgrading of precision is required at this time.

Results and Discussion

The blood of six human subjects was analyzed for its NO content by the analytical procedure described above. The results are presented in Table 1. All samples of NO recovered after exposure to human blood show a significant increase in the $\overline{30}/\overline{31}$ abundance ratio. The calculated NO content of the blood is about 20 nanomoles/ml whole blood, corresponding to about 0.25% saturation of Hb with NO. No correlation between the smoking habits of the subjects and the NO content of their blood was found. In fact, it is rather surprising that the NO content of different human subjects is so similar--a variance of ± 13% may be calculated for our short series. On the other hand, the blood of rats showed no nitric oxide at all. If rats' blood contains a steady-state concentration of NO, it must be less than 1 nanomole/ml blood, i.e., below the sensitivity of detection by our method. The higher steady-state concentrations of NO found in humans may be due to a slower metabolization compared with rats. If this is the case, we would expect a difference in NO content between smokers and nonsmokers. The absence of such a significant difference may suggest that smokers exhibit enhanced NO metabolization to compensate for their higher rates of NO uptake. Another explanation for the relatively high but rather constant NO content of human blood could be the formation of endogeneous NO as a by-product or intermediate of nitrogen oxidation in vivo; this would be analogous to the well established physiological formation of CO. If this is the case, there

Table 1

DETERMINATION OF NO IN HUMAN WHOLE BLOOD

Subject No.	$\bar{30}/\bar{31}$ Abundance Ratio x 10^2	$N^{14}O$ Recovered[a] (nanomoles)	Percent HbNO[b]
$N^{15}O$ blank[c]	0.199 ± 0.005	--	--
1	0.240 ± 0.006	22.2	0.25 ± 0.04
2	0.242 ± 0.003	23.6	0.27 ± 0.02
3	0.238 ± 0.010	21.1	0.24 ± 0.05
4[d]	0.232 ± 0.001	18.1	0.21 ± 0.006
5[d]	0.244 ± 0.016	24.4	0.28 ± 0.09
6	0.212 ± 0.001	17.9	0.20 ± 0.003

[a] Average value. 54.9 μmoles $N^{15}O$ (containing 109.5 nanomoles $N^{14}O$) were added to 1 ml of whole blood.

[b] Calculated assuming that each milliliter of normal whole blood contains 2.2 μmole Hb each with 4 binding sites for NO. % HbNO = nmoles $N^{14}O$ recovered/8800.

[c] Commercial $N^{15}O$ containing 1994 ppm $N^{14}O$.

[d] Smoking subject.

ought to be only a small, and probably nondetectable, variation in NO content on increased uptake of exogeneous NO by humans.

The findings in humans have to be correlated with observations on animals. The low concentrations of NO found in blood of mice exposed to NO[6] cannot be the result of limited uptake of NO by the lungs as suggested,[5] because NO, being as small a molecule as O_2 or CO, must be able to diffuse through the air-blood barrier at the alveolus and into the erythrocytes. If no NO whatsoever were found in blood one could speculate that it is completely oxidized on contact with or during diffusion through the air-blood barrier. As it seems, the low level steady-state concentrations of NO in mice and man indicate that NO undergoes rapid oxidation inside the erythrocytes *in vivo*. This assumption is corroborated by preliminary experiments in an ongoing study on rats carried out by our isotope dilution technique. These experiments indicate that in the rat, erythrocyte-bound NO has a halflife of a few minutes.[10] On the other hand, samples were exposed to $N^{14}O$ and then to air before equilibration with $N^{15}O$ to determine whether the bound $N^{14}O$ would be oxidized *in vitro*. It appears that air oxidizes some of the bound $N^{14}O$ but at a slow rate, with a halflife of hours. It seems that the rapid disappearance of NO *in vivo* is a more complex process which may involve certain ATP dependent oxidases. The *in vitro* oxidation, which is oxygen dependent, may be a charge transfer process involving oxygen and NO bound to the same Hb molecule. Alternatively it may involve the same reactions as the *in vivo* metabolization of NO which are slowed down under the non-physiological conditions.

The fast metabolization of NO observed in the rat supports the suggestion that the steady-state concentration of NO observed in normal humans is the result of endogenous formation of NO, at least in humans. It is too early to speculate on the metabolic pathway which leads to the formation of NO *in vivo*.

Let us present some speculations on the basis of our preliminary findings. We do not know at this point what fraction of inhaled NO is oxidized at the air-blood barrier. Such an oxidation, if it occurs, is not extensive or it does not produce NO_2. Otherwise NO would be exhibiting toxicological effects identical to those of NO_2.[11] We have also found[10] that gaseous NO injected intravenously into rats is by far less acutely toxic than NO_2. This NO was metabolized, however,

with a halflife of a few minutes. These results suggest that NO_2 is not a major product of the metabolization of NO but that the latter is oxidized by a single electron transfer process. If this is the case, we may be confronted with two potentially detrimental effects. First, a certain oxidase reacts in a non-characteristic peroxidative mode, which may result in the occurence of additional unwanted single electron transfer processes. Second, the most likely product of oxidation of NO is the NO^+ ion which in the presence of secondary amines or peptides will rapidly produce nitrosoamines or nitrosoamides:

$$R_1R_2NH + NO^+ \rightarrow R_1R_2NNO + H^+$$

$$R_1CONHR_2 + NO^+ \rightarrow R_1CON(NO)R_2 + H^+$$

These products may be toxic and possibly carcinogenic.

Nitrogen dioxide, on the other hand, which acts as an oxidant, produces NO_2^- ions as the major product. The toxicological effects of the two nitrogen oxides are thus completely different. Although NO has no or very mild acute toxicological effects, it may have worse long term detrimental consequences. This would indicate that air pollution control of NO is as important as that of NO_2. On the other hand, if our hypothesis on the endogenous formation of NO in humans is corroborated, the inhalation of additional NO even from moderately polluted air may be inconsequential.

Further research is required to determine the mode of action of NO at the cellular level. This information is absolutely necessary before any conclusions are reached on the potential toxicity of atmospheric nitric oxide. It is evident that our preliminary results obtained by isotope dilution analysis open up a large number of questions most of which will have to be answered by aids of isotopic nitrogen.

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