HUMAN RESPONSES TO CONTROLLED EXPOSURES OF METHYLENE CHLORIDE VAPOR

By

Richard D. Stewart, M. D., M. P. H., Hubert V. Forster, Ph. D.,
Carl L. Hake, Ph. D., Andre J. Lebrun, M. D.,
Jack E. Peterson, Ph. D., and Staff

Report No. NIOSH-MCOW-ENVM-MC-73-7

December, 1973

From the Department of Environmental Medicine, The Medical College of Wisconsin, Milwaukee, Wisconsin.

This investigation was supported by Contract No. HSM-99-72-84 from the National Institute of Occupational Safety and Health.

50272-101

REPORT DOCUMENTATION PAGE	1. REPORT NO. HSM-99-72-82	2. NA	3. Recipient's Accession No. PB83 109710
4. Title and Subtitle: Human Responses to Controlled Exposures of Methylene Chloride Vapor			5. Report Date: December 1973
			6. NA
7. Author(s): Stewart, R. D., H. V. Forster, C. L. Hake, A. J. Leburn, et al			8. Performing Organization Rept. No. NA
9. Performing Organization Name and Address: Department of Enviornmental Medicine, The Medical College of Wisconsin, Milwaukee, Wisconsin			10. Project/Task/Work Unit No. NA
			11. Contract(C) or Grant(G) No. (C) HSM-99-72-82 (G) 4
12. Sponsoring Organization Name and Address: NIOSH, Cincinnati, Ohio			13. Type of Report & Period Covered: Contract
			14. NA

15. Supplementary Notes

NA

16. Abstract (Limit: 200 words)

The behavioral and health effects of exposure to methylene-chloride (75092) (CH_2Cl_2) vapor were studied. Eleven male subjects were exposed to 500 parts per million (ppm) of CH_2Cl_2 for 7.5 hours per day for 2 days or to concentrations of up to 250ppm for 5 days. Subjects were given a comprehensive medical examination prior to and following exposure. Blood counts and urinalyses were conducted. Alveolar breath samples were taken at various intervals. No deleterious health or behavioral effects were seen at any exposure dose. Carboxyhemoglobin (CoHb) concentrations were consistently increased and averaged over 5 percent at CH_2Cl_2 exposures of 250ppm for 7.5 hours. The authors conclude that exposure to methylene-chloride increases CoHb concentrations. They note that this measurement is not a good indicator of CH_2Cl_2 exposure because of the unknown presence of carbon-monoxide in industrial environments.

17. Document Analysis a. Descriptors

Chlorinated-hydrocarbons, Humans, Hematology, Blood-disorders, Toxicology, Behavior

b. Identifiers/Open-Ended Terms

c. COSATI Field/Group

18. Availability Statement: Available to Public	19. Security Class (This Report): NA	21. No. of Pages: 85
	20. Security Class (This Page)	22. Price

The discovery by Stewart, et al [1], that the exposure of humans to the vapor of methylene chloride (dichloromethane, CH_2Cl_2) is associated with an increase in carboxyhemoglobin saturation has initiated a review of the threshold limit value (TLV) [2] for this substance. It can be assumed from the work in Stewart's laboratory [3] that exposure to the 1971 TLV of 500 ppm (1,740 mg/M^3) for an eight-hour work day would likely result in a blood carboxyhemoglobin concentration higher than that caused by the 1972 TLV of 50 ppm (55 mg/M^3) for carbon monoxide. It has been shown that the continuous exposure of humans to this level of carbon monoxide results in a carboxyhemoglobin concentration of approximately 8% saturation [4]. Even this concentration of blood carboxyhemoglobin is now considered by some to be deleterious to the behavioral performance of healthy humans. Therefore, it was of considerable interest to study the behavioral and health effects of methylene chloride on humans repeatedly exposed to vapor levels that included the 1971 TLV, and lower levels which may be proposed as TLV's in the future. This report encompasses these behavioral and health effects, with their relation to carboxyhemoglobin concentrations, while a companion report [5] details the correlation of the post-exposure breath levels of methylene chloride with magnitudes of exposure. An additional report [6] will discuss cardio-pulmonary measurements carried out during the exposures to methylene chloride.

EXPERIMENTAL

Subjects:

A total of eleven healthy, male, volunteer human subjects were exposed to the vapor of methylene chloride (CH_2Cl_2) in a controlled environment chamber. Their ages ranged from 20 to 39 years, height from 170 to 190 cm and weight from 57 to 93.5 kg. Prior to any exposure to CH_2Cl_2, the objectives of the study and the nature of the procedures to be used were fully explained. Informed consent was obtained. The subjects were divided into three groups depending upon their availability for $7\frac{1}{2}$, 3 or 1 hr of exposure per day. Four volunteered for the $7\frac{1}{2}$-hr, 3 for the 3-hr, and 3 for the 1-hr exposures. Just prior to the exposure to CH_2Cl_2 on the fifth day of the first week of consecutive daily exposures, one subject from the 1-hr group experienced a brief fainting spell due to his inadequate eating and sleeping habits. He was not exposed that day, and was replaced with another subject the following week.

One subject from each of the three groups smoked cigarettes, all other subjects were non-smokers. The smokers from the $7\frac{1}{2}$-hr and 1-hr groups smoked $\frac{1}{2}$ to 1 pack of cigarettes per day, while the smoker from the 3-hr group smoked approximately 2 packs of cigarettes per day. Another subject from the 3-hr group worked in a garage 2 or 3 evenings per week, while a non-smoker from the 1-hr group worked the third shift in an industrial plant. All other subjects were temporarily unemployed or were students. None were exposed to CH_2Cl_2 during the study period outside of the times that they were in the controlled environment chamber.

Exposure Chamber:

Exposures to the vapor of CH_2Cl_2 were conducted in a controlled environment chamber (room) 20 ft x 20 ft x 8 ft in size, which contained a 5 ft x 4 ft x 8 ft toilet facility and a 5 ft x 4 ft x $6\frac{1}{2}$ ft commercial audiometric booth. Both the toilet facility and the audiometric booth were ventilated with air from the chamber. Air flow through the chamber was approximately 1500 cu ft/min, and approximately 25% of this flow was exhausted. A slight negative pressure was maintained in the chamber at all times. Ambient temperature was 72 - 74° F, while relative humidity ranged between 45 - 55%. The CH_2Cl_2 was introduced by sweeping the concentrated vapor from a warm flask with a stream of air into the chamber's circulating air. A reciprocal dual-piston pump maintained a steady flow of liquid CH_2Cl_2 into the flask.

Analysis of Exposure Chamber Atmosphere:

The CH_2Cl_2 (Fisher Scientific Co., Certified ACS, D-37) used in these studies was shown by infrared analysis to be $> 99\%$ pure. The concentration of CH_2Cl_2 in the chamber atmosphere was continuously recorded by an infrared spectrometer (MIRAN I, Wilks Scientific Corporation) equipped with a 20-m path-length gas cell which was continuously flushed with air drawn from the chamber through a $\frac{1}{4}$-inch diameter polyethylene tubing. The absorbance at 13.3μ through a path-length of 2.25 m was measured. The infrared signal to the recorder was monitored each second by an on-line PDP-12 (Digital Equipment Corp.) computer, which displayed the mean vapor

concentration, as compared to standards, for each 30-second time interval of exposure and calculated the daily time-weighted-average exposures. Calibration standards of CH_2Cl_2 in purified air were prepared in saran bags and analyzed before and hourly during each daily exposure. A second infrared spectrometer was connected to an independent polyethylene tubing loop which sampled the environmental chamber atmosphere in a manner similar to the first. However, the signal to the recorder was not monitored by computer. This second monitor was used as a back-up and a check on the infrared spectrometer connected to the computer.

The ambient concentration of carbon monoxide (CO) in the chamber was measured once or twice daily while exposures were in progress. An aliquot from a grab sample of air obtained from the chamber was analyzed by gas chromatography using the method of Porter and Volman [7].

Exposure Schedule:

The three groups of volunteer subjects were exposed to five concentrations of the vapor of CH_2Cl_2. The original schedule called for weekly exposure at only four concentrations; they were 0, 50, 250 and 500 ppm. However, when it was found that a $7\frac{1}{2}$-hr daily exposure to an atmospheric concentration of 250 ppm caused carboxyhemoglobin (COHb) saturation to occasionally exceed 10% in some non-smokers, a daily exposure to 100 ppm CH_2Cl_2 for five consecutive days was added to the study and the 500 ppm concentration exposure was limited to two days. The final plan called for Group I (4 subjects for $7\frac{1}{2}$ hrs/day), Group II (3 subjects for 3 hrs/day), and Group III

(3 subjects for 1 hr/day) to be exposed to 0 ppm CH_2Cl_2 on days 4 and 5 of week 1, to 50 ppm CH_2Cl_2 on days 1 through 5 of week 2, to 250 ppm CH_2Cl_2 on days 1 through 5 of weeks 3 and 4, to 100 ppm CH_2Cl_2 on days 1 through 5 of week 5, to 0 ppm CH_2Cl_2 on days 1 and 2 and 500 ppm on days 3 and 4 of week 6. All concentrations were to be as stable as possible with the exception of week 4 when the CH_2Cl_2 concentration was to fluctuate from 187 to 312 ppm, but also result in a time-weighted-average exposure for each subject of 250 ppm. Figure 1 illustrates the exposure schedule as it was carried out, and Figure 2 illustrates the planned daily fluctuations in vapor concentration during week 4.

Subjects in groups I and III entered the chamber at 9:00 A.M., while the subjects in group II entered at 10:00 A.M., at the same time that the group III subjects exited. Group II subjects exited at 1:00 P.M., while group I subjects exited at 4:30 P.M. The latter group was served their lunches in the chamber. There were never more than 7 subjects in the chamber at one time.

Clinical Testing:

Each subject was given a comprehensive medical examination prior to the study and after the last exposure day of the study. These examinations included a complete history and physical examination with the following laboratory studies: complete blood count, SMA-12 survey panel of clinical chemistries and a 12-lead electrocardiogram (EKG). A complete blood count

and the SMA-12 survey panel of clinical chemistries was repeated at least once per week during the weekly exposures. Triglycerides were added to the clinical chemistries during the second week of exposure. Prior to each day's exposure, the subjects were given a brief medical examination which included blood pressure, temperature, subjective signs or symptoms, and urinalysis (Combistix®). During the time that they were in the environmental chamber, each subject's EKG (lead II) was continuously monitored by telemetry, and recorded at hourly intervals. The subjects were under continual surveillance by medical personnel while they were in the study.

Twenty-four-hr urine collections were begun daily as each subject entered the chamber. Urobilinogen determinations were carried out on fresh 24-hr urine specimens using the method of Watson, et al, as modified by Henry, et al, in Henry[8]. A Coleman Model 6/20A Junior® II A spectrophotometer was used to determine linear absorbance at 562 mμ. The PSP dye standard was adjusted in concentration to give an optical density of 0.384 at 562 mμ on a Beckman DB spectrophotometer, using water as a blank. Results were reported in Ehrlich units per 24 hours.

Breath Sample Collection and Analysis:

Alveolar breath samples were obtained daily from each subject prior to entry into the environmental chamber, immediately prior to exiting the chamber, and at the following times after exiting the chamber (post-exposure): 1, 15, 30 min; 1, 2, 3 hr. The last two samples were collected in duplicate.

A 16-, 21-, and 23-hr post-exposure sample (usually identical to the pre-exposure sample) for group I, II, and III, respectively, was also collected. Breath samples, with the exception noted below, were collected in 35-ml glass tubes fitted at each end with screw caps containing saran liners. One of the caps had a pre-drilled hole through which an aliquot of the alveolar breath samples could be withdrawn. The subjects flushed the tube twice, then after holding the third breath for 30 seconds, exhaled and capped the tube while finishing the exhalation. The alveolar breath sample obtained just prior to exit from the environmental chamber was collected by having the subject breathe through a polyethylene tube which extended through a porthole in the chamber door to a saran bag. Sampling of the saran bag was accomplished by puncturing with the syringe needle.

Fifteen min after the end of the exposure on the fifth day of weeks 2, 3, 4, and 5, a second alveolar breath sample was collected from each subject, this one in a 4-liter saran bag. This breath sample was slow-scanned in a Beckman® IR-10 infrared spectrometer with a 10-m gas cell.

A Varian Aerograph Model 2700 gas chromatograph equipped with a hydrogen flame ionization detector was used to determine CH_2Cl_2 in the breath samples. The gas chromatograph (GC) was fitted with two stainless steel columns; 3 ft x 1/8 in and 1 ft x 1/8 in, each packed with Poropak Q, 50-80 mesh (Waters Associates, Inc.). The columns were preconditioned at 200°C overnight prior to use. The operating conditions of the GC were as follows: carrier gas (helium) flow rates, 10 and 20/ml min; column temperature, 75°C;

injection port, 150°C; and detector, 200°C. Standards at several concentrations to bracket the unknown levels were prepared with purified air as diluent. One-ml aliquots were injected and the concentration of CH_2Cl_2 in unknowns was obtained by direct comparison of peak heights to the standards.

On days 1 and 3 of weeks 3, 4, 5 and 6, one of the duplicate alveolar breath samples obtained at 2 and 3 hrs post-exposure was assayed for CO by the gas chromatographic procedure of Porter and Volman[7]. In addition, the CO in an additional duplicate breath sample was assayed at 15 min post-exposure for one subject in lieu of determining his blood carboxyhemoglobin level.

Blood Sampling and Carboxyhemoglobin Analysis:

Approximately 2 ml of blood was withdrawn from an antecubital vein of each subject each morning prior to chamber entry, and the sample was analyzed for baseline carboxyhemoglobin (COHb) saturation. Smokers were not allowed to smoke between the withdrawal of this sample and the last blood sample of the day. Additional blood samples for COHb assay were obtained immediately pre-exit from the chamber, and 1 hr post-exposure on days 1, 3, and 5 of each week. Fewer samples were obtained from one 3-hr subject who had difficulty with the procedure. His breath sample was analyzed for CO in lieu of the assay of blood COHb at some of these times.

Blood COHb percent saturation was determined directly in a CO-Oximeter (Instrumentation Laboratories, Inc.), an automated instrument

which also determined the hemoglobin concentration. There were two instruments used, and they were cross-checked for accuracy with blood samples obtained from non-exposed laboratory personnel.

Neurological Studies:

Within 5 min of entry into the environmental chamber, and within 10 min prior to exit, each subject performed a modified Romberg equilibrium test which was video taped for later inspection if necessary. The test consisted of standing upon each leg singly with arms at the side for a minimum of 3 sec, and walking heel-to-toe in a straight line for approximately 5 ft. This was first done with the eyes open and then repeated with eyes shut.

During the first five weeks, electroencephalograms and visual evoked response(VER) measurements were made each Monday, Wednesday and Friday on group I subjects. During the sixth week, measurements were made one day at each concentration. On each measurement day, four recordings were made, one during the first hr of exposure and three between the fifth and seventh hrs of exposure. A modified 10 - 20 international electrode arrangement was used for EEG recordings. One electrode was placed 2 to 2.5 cm above the inion, and the VER was recorded from this electrode with the left ear as the reference. The details of our recording arrangements have been presented in a previous publication [9].

The EEG and VER were both analyzed by visual examination. In addition, the amplitude of the 3rd, 4th, and 5th waves of the VER complex

were measured (mm ruler). Our objective was to ascertain whether changes occurred within a day, during a week at each CH_2Cl_2 level, and from one concentration to another.

Pulmonary Function Studies:

Four specific functions were studied during the CH_2Cl_2 exposures; (1) functional integrity of airways, (2) alveolar to capillary gas exchange, (3) pulmonary ventilation, and (4) hemoglobin affinity for oxygen. Each of these was assessed at least once on group I subjects during each week of the exposure (Thursday or Friday; 5th to 7th hr), and once before and once after CH_2Cl_2 exposure. These will be discussed in detail in a companion report[6].

Behavioral Testing:

A series of behavioral tests was performed by the seven subjects in groups I and II. One of the series was an alertness test lasting $1\frac{1}{2}$ hrs which was performed on days 1, 3, and 5 of each week. It began $2\frac{1}{4}$ hrs after the start of an exposure of group I, and $1\frac{1}{4}$ hrs after "zero time" of group II. No training was required for this test. On days 2 and 4 of each week, the remaining tests in the series were performed beginning at 3 and 2 hrs after "zero time" for groups I and II, respectively. The subjects were trained to a performance plateau before these tests were used during exposures.

For each of the behavioral tests, the subjects sat in comfortable chairs at individual carrels assigned to them for the duration of the study. The

subjects were not permitted to talk or have access to watches, food, soft drinks, radios, etc., during the tests. All instructional commands were made from outside of the chamber via an intercom system. The tests are described below in the order in which they were performed.

Alertness Test. This test was also called the "clock" test because the subjects watched a black clock face from which all numerals and hands were removed except the sweep second hand. The test, administered and graded by a PDP-12 computer, consisted of a primary and a secondary task. For the primary task, the subject pressed a hand-held micro-switch as rapidly as possible whenever he observed a "stop and start" of the sweep second hand. The clock stoppages were for 0.23 seconds or 0.13 seconds, each occurring randomly 50% of the time. During the 90-min test, only 30 of these signals were presented. For the secondary task, the subject pressed the hand-held switch whenever a tone was heard through the headphones he was wearing. These auditory signals were presented randomly for one second, 3 to 6 times per test. At the completion of the test, the computer printed the results in table form for each subject. The table listed signal duration, time of occurrence, reaction time or miss for each signal, percent correct for the day, and the average reaction times for clock stoppages and for tones.

Ten- and Thirty-Second Time Estimation Test. Each subject, upon verbal signal (ready, begin), depressed a hand-held, silent, push-button micro switch for an interval of time he estimated to be 10 seconds. This was

repeated two additional times, and then 3 thirty-second estimates were made. The micro-switches were connected to a polygraph whose pen-deflection could be read to the closest 10 msec. This test took approximately 3 min to perform.

Marquette Time Estimation Test. This test consisted of a series of nine tone stimuli followed by nine light stimuli of approximately 1, 3, or 5 seconds' duration presented in a random sequence but always with three stimuli of each time interval. At the termination of each stimulus, the subject depressed the push-button for that interval of time he estimated to be equal in length to the original auditory or light stimulus. A detailed description of the test and the instrumentation used to carry it out has been described by Stewart, et al[10]. This test took approximately 7 min to perform.

Coordination Test. This test was the Flanagan Aptitude Classification Tests, 7A, Coordination, published by Science Research Associates, Inc., 259 East Erie Street, Chicago, Illinois. This test asked the subject to rapidly follow a spiral pathway with a pencil. The subject was allowed 40 seconds to complete each of 6 spirals. The first 2 were considered practice and the last 4 were scored and totaled. The total score depended upon the longest distance attained in each spiral minus the number of times the sides of the spiral pathway were touched with the pencil. This test took approximately 5 min to perform.

Arithmetic Test. This test, which measured the subjects' ability to work with numbers, was divided into two parts. The first part, lasting 5 min,

consisted of simple addition and subtraction problems while the second part, lasting 3 min, consisted of multiplication and division. The maximum score attainable if all answers were correct was 125, however, no subjects completed the tests in the allotted time. In order to minimize memorization of answers, four permutations of problem order were used.

Inspection Test. This test was a measure of the subjects' ability to spot the number 3 in rows of random numbers on an $8\frac{1}{2}$ x 11" page. The subject was asked to scan each row, beginning at the top of the page, and slash out with a red pencil each "3" encountered. The subject was given 2 min to strike out as many as possible. No subjects ever finished the entire page. A subject's score was the total number of "3's" struck. Six differing pages with random numbers were utilized so that no subject received an identical number sheet on a given day.

RESULTS

The daily time-weighted-average concentrations of methylene chloride (CH_2Cl_2) vapor in the chamber atmosphere as determined by continuous infrared spectroscopy are listed in Table I. The computer tabulating the data also calculated the daily standard deviation from the mean ($\pm$ S. D.) for each group of subjects. It is evident from the results that all actual mean exposure concentrations to CH_2Cl_2 were within 6% of those planned, and usually they were within 1% of planned concentrations. It is also evident that absenteeism was

no great problem as only two subjects missed an exposure.

Analysis of the chamber atmosphere for ambient carbon monoxide (CO) revealed concentrations varying from 0.4 to 4.3 ppm. In the majority of cases, the daily sample contained <1 ppm CO.

The health of all subjects, with the exception of minor colds and an occasional sore throat, remained excellent throughout the study. The only abnormal laboratory values reported which could be correlated in degree of abnormality with the magnitude of exposure to CH_2Cl_2 were those for blood carboxyhemoglobin (COHb) percent saturation. There were no other consistently abnormal results from any laboratory studies, no individual gross abnormalities were reported, nor were abnormals more prevalent after the high exposure days than after the four days of zero exposure. As an example, 24-hr urobilinogen values were $>$ 10 Ehrlich units in 4 of 29 specimens collected after daily 0 ppm exposures, 1 of 50 specimens after 50 ppm, 5 of 100 after 250 ppm, 3 of 49 after 100 ppm, and 0 of 19 after daily 500 ppm exposures to CH_2Cl_2.

The results of the analysis of breath samples for CH_2Cl_2 are being reported in a separate paper, along with the resultant breath decay curves. It should be noted here that breath decay curves can be used to estimate the magnitude of a CH_2Cl_2 exposure. There were no unusual peaks in the infrared scan of 15-minute post-exposure breath samples except those expected in the CH_2Cl_2 and CO regions of the spectrum. The values obtained for the CO in breath samples by GC were converted to COHb [11] and will be reported

in the next section.

The blood COHb percent saturation studies were limited in number by the need to maintain normal hemoglobin values throughout the study. Blood samples for COHb analysis were obtained pre-exposure on a daily basis, on Saturday morning, and at the end of the exposure and 1-hr post-exposure on Monday, Wednesday and Friday. At 2 and 3 hrs post-exposure on Monday and Wednesday of each week the COHb percent saturation was calculated for each subject from the analysis of breath samples for CO. However, this indirect method of determining COHb saturation is not as accurate as the direct determination on blood because the indirect method always assumes a normal hemoglobin. The results of all COHb determinations are listed in Tables II, III, and IV. Values obtained by the indirect method are noted. Table II lists the values for the $7\frac{1}{2}$-hr subjects. Because three of these subjects were nonsmokers and had no obvious exposure to more than ambient levels of CO, their COHb values were averaged. All subjects in groups II and III are listed individually in Tables III and IV, because of either smoking or possible work exposure to CO.

Equilibrium studies of subjects as determined subjectively by the modified Romberg test revealed no abnormalities either prior to, during, or after exposure to CH_2Cl_2 at various magnitudes. Other objective CNS studies on subjects from group I also revealed no abnormalities. During studies at 0 ppm the frequency and wave amplitude of each subject's EEG were within normal limits at each recording site. There were no consistent significant

changes during exposure at any of the CH_2Cl_2 concentrations. Exposure to CH_2Cl_2 did not cause any consistent significant changes in the amplitude, latency, and configuration of the VER. There were only two minor VER changes. The peak to peak amplitude of one subject's 4th and 5th wave of the VER complex tended to decrease slightly as CH_2Cl_2 concentration increased. In addition, relative to 0 ppm, the amplitude of another subject's 3rd, 4th, and 5th wave was slightly reduced throughout exposure to CH_2Cl_2.

Behavioral tests were performed by the group I ($7\frac{1}{2}$-hr) and group II (3-hr) subjects. The alertness test was performed on days 1, 4 and 5 each time there was an exposure on these days, with the exception of the 3rd day of the 6th week (500 ppm). For group I it began $2\frac{1}{4}$ hrs after the start of an exposure while for group II it began at $1\frac{1}{4}$ hrs. The data generated included the percent of correct responses and the reaction time for responding. The analyses of this data using the paired t-test to compare the performance for each day of exposure to CH_2Cl_2 versus the two zero exposure days (Monday and Friday) are found in Tables V and VI for groups I and II, respectively. It can be seen that for the group I ($7\frac{1}{2}$-hr) subjects, only on two occasions were the exposure day results significantly different than those on non-exposure days, and these were both faster reaction times at 250 and 100 ppm. In group II (3-hr), there were two significantly different results found, however, in this group the percent of correct responses at 50 and 250 ppm, both on Fridays, were lower than controls. Because this finding was not dose related, it was considered to be a Friday fatigue effect.

The additional behavioral studies were performed by group I and group II subjects on days 2 and 4 of each week beginning at 3 and 2 hrs, respectively, after start of exposure for each group. Nine training sessions were conducted for each of these additional behavioral tests before the first zero chamber concentration session (session 10) was conducted. Table VII correlates the session numbers and exposure concentrations. Graphs to depict the mean scores plus one standard deviation were developed for each group from the results of each individual session and for the combined results from sessions at 0, 50, 100, 250 and 500 ppm CH_2Cl_2. These four graphs for the 10- and 30-second time estimations are shown in Figures 3 through 6, the 24 graphs for the Marquette time estimation test, including the subsections of estimate/stimulus, the difference between estimate and stimulus, and the reaction times for both sound and light stimuli in Figures 7 through 30, the four graphs for the coordination tests in Figures 31 through 34, the four for the arithmetic tests in Figures 35 through 38, and the four for the inspection test in Figures 39 through 42. For a determination of the significance of the results obtained, paired t-tests were used to compare the control (from two 0 ppm exposures) values versus those obtained for each subject at each CH_2Cl_2 vapor concentration. Table VIII shows the results of these statistical comparisons for group I, and Table IX for group II. In group I ($7\frac{1}{2}$-hr), reaction time to the light stimulus improved significantly ($P = 0.05$) at 50, 100 and 250 ppm, but not at 500 ppm, when compared to control days. Also in group I, test scores increased significantly ($P = 0.01$) over control days for the inspection test at

500 ppm CH_2Cl_2. Likewise, the only significant difference in paired t-tests for group II (3-hr) was an improvement in performance ($P = 0.01$) of the coordination test at 250 ppm versus control.

DISCUSSION

Previous studies reporting on well-controlled exposures of human subjects to CH_2Cl_2 have not included repeat daily exposures. Therefore, in this sense, this study was unique. Fortunately, this study did corroborate previous single exposure studies in that no deleterious effects upon the health or performance of human males could be detected when they were repeatedly exposed to 250 ppm or less of CH_2Cl_2 for $7\frac{1}{2}$ hrs per day, five days per week, or to 500 ppm on two consecutive days.

Confirmation of the discovery by Stewart, et al[1], that exposure to CH_2Cl_2 vapors results in increased COHb blood levels has come from other laboratories[12,13], along with proof that in animals the CO source is the carbon of the CH_2Cl_2 molecule[13]. The work reported herein further confirms the CH_2Cl_2 to CO conversion, as all subjects showed increases in blood COHb saturation values when exposed to the higher concentrations of CH_2Cl_2.

It was of interest to study the difference in COHb levels between pre- and post-exposure blood samples obtained from these subjects. Table X lists these differences for the $7\frac{1}{2}$-hr exposures. The results for the light smoker

in this group were kept separate, though it is obvious that his COHb level increased very similarly to the average of the other three non-smoking subjects. Actually, his pre-exposure values varied little from theirs, indicating that he smoked only occasionally. At 50 or 100 ppm exposure for $7\frac{1}{2}$ hrs, all increases in COHb levels remained below 5%. However, at 250 ppm, average COHb levels increased 5.4 to 8.8%, and at 500 ppm the average increase was slightly over 10%. In group II, where 3 subjects were exposed to CH_2Cl_2 vapors for 3 hrs per day, each subject started from a different baseline and, therefore, the differences in COHb were not averaged in Table XI. It was of interest to follow the subject who was a heavy smoker and consequently had pre-exposure levels ranging from 6.1 to 9.3%. When he was exposed to 50 or 100 ppm CH_2Cl_2 for 3 hrs, his COHb levels actually decreased because he did not smoke during this period of time. The non-smoker and garageman both showed slight increases during these exposures. The latter two subjects showed significant increases of from 2.1 to 7.2% at 250 ppm, while the heavy smoker's level increased 0.2 to 2.5%. Again, it is obvious that the smoker's COHb level increased less because of discontinuing the exposure to the CO from the cigarettes. Table XII lists the values for each subject in group III, the 1-hr per day subjects. The exposure to 50 ppm CH_2Cl_2 had no significant effect upon COHb levels, while at 100 and 250 ppm, the COHb level generally remained within 1% of the pre-exposure value. At 500 ppm, the non-smoker and industrial worker both showed significant increases in COHb values.

It was also of interest to look at the 16-hr post-exposure (identical to pre-exposure on days 2, 3, 4 and 5) COHb levels to ascertain the clearance of the increased COHb due to the previous exposure. Figure 43 denotes the daily average post-exposure levels for the 4 group I subjects. The 64-hr post-exposure sample is identical to the pre-exposure sample of a Monday morning. It is obvious from the graph that COHb levels had not returned to baseline the morning following $7\frac{1}{2}$ hrs of exposure to 100, 250 or 500 ppm CH_2Cl_2. However, on Monday mornings (64 hrs after Friday's exposure), a baseline level was always achieved. Carryover was negligible after a daily exposure to 50 ppm CH_2Cl_2.

The group-average blood COHb levels of the seven subjects, excluding amokers, as they exited the chamber and for 3 hrs thereafter, are depicted in Figure 44. It is obvious that the levels varied directly with the concentration of the CH_2Cl_2 in the chamber and also with the length of exposure. Although the number of samplings was limited because of the need to conserve blood, it is evident that the metabolism of the CH_2Cl_2 to CO had already begun at 1 hr of exposure, and it continued after termination of exposure to maintain the COHb level for at least 3 hrs. A subject from the faculty who volunteered for serial blood sampling after single 3-hr exposures to three levels of CH_2Cl_2 gave curves plotting blood COHb versus time as shown in Figure 45. His COHb climbed steadily during exposure and peaked at 1-hr post-exposure. The half-life from the decay of the COHb after peaking appears to be approximately 7 to 10 hrs, or perhaps 1.5 to 2 times the half-life of COHb

after an exposure to CO. Obviously, the blood was being exposed to endogenous CO from stored CH_2Cl_2 long after the CH_2Cl_2 exposure was discontinued.

It is interesting to speculate as to where the CH_2Cl_2 metabolism was taking place. Though the liver is the most likely place, we ruled out metabolism in the red cells by incubating the blood of a CH_2Cl_2-exposed subject overnight at 37°. There was no increase in COHb even though CH_2Cl_2 was present in the blood. Although the site of metabolism is still unproven, it can be said with certainty, contrary to papers published as recently as 1972 [14], that absorbed CH_2Cl_2 vapor is not metabolically inert.

Results of the previous study [3] suggested that the peak to peak amplitude of the 3, 4, 5 complex of the VER was increased during 2 hrs of exposure at 986 and 700 ppm. In the present study, there was no indication of increased amplitude even at the highest CH_2Cl_2 concentration (500 ppm). In fact, exposure at these low concentrations resulted in a slight reduction in amplitude in two of the subjects. The results of these two studies are not incompatible. Anesthetic agents are also known to induce a similar pattern of change in cortical electrical activity [15]. In the initial stage of anesthesia, EEG amplitude is decreased due to a direct excitatory effect of the agent on cortical neurons. As anesthesia is deepened, EEG amplitude increases due to the depressant effect of the agent on the CNS arousal mechanism (reticular activating system).

Behavioral tests on subjects undergoing exposure to CH_2Cl_2, and thus under both the influence of CH_2Cl_2 and COHb, showed no deleterious effects

at the magnitudes of exposure studied. Although exact COHb levels at the time of testing are unknown, it can be assumed without doubt that the COHb levels were well above 2% saturation when the groups I and II subjects were tested in an atmosphere of 250 and 500 ppm CH_2Cl_2. This is a most important finding as some previous reports have indicated that CO alone at low levels had a deleterious effect upon time perception[16], although we were unable to confirm this finding in our laboratory[17].

CONCLUSIONS

There were no deleterious effects observed on the health or behavioral performance of male human subjects exposed in a controlled environment chamber to CH_2Cl_2 vapors at concentrations of 500 ppm for $7\frac{1}{2}$ hrs per day for 2 days, or at concentrations of up to 250 ppm 5 days per week. The only consistent change in laboratory findings during these studies was a consistent increase in blood COHb percent saturations. At a CH_2Cl_2 time-weighted-average concentration of 250 ppm, the 1973 TLV, the COHb of subjects exposed for $7\frac{1}{2}$ hrs per day consistently averaged over 5% saturation. Although the measurement of blood COHb saturation is a very accurate indicator of CO exposure, the general use of this measurement as an indicator of the magnitude of exposure to CH_2Cl_2 is not recommended because of the ubiquitous and often unknown presence of low levels of CO in the atmosphere of industrial plants.

ACKNOWLEDGEMENT

The authors wish to acknowledge Joyce Aasen and Susan Kamke for their aid in the preparation of this report.

BIBLIOGRAPHY

1. Stewart, R. D., Fisher, T. N., Hosko, M. J., Peterson, J. E., Baretta, E. D., and Dodd, H. C.: Carboxyhemoglobin Elevation After Exposure to Dichloromethane. Science. 176:295-296, 1972.

2. Threshold Limit Values for Substance in Workroom Air Adopted by ACGIH for 1972. Cincinnati, American Conference of Governmental Industrial Hygienists, 1972.

3. Stewart, R. D., Fisher, T. N., Hosko, M. J., Peterson, J. E., Baretta, E. D., and Dodd, H. C.: Experimental Human Exposure to Methylene Chloride. Arch. Environ. Health. 25:342-348, 1972.

4. Coburn, R. F., Forster, R. E., and Kane, P. B.: Considerations of the Physiology and Variables that Determine the Blood Carboxyhemoglobin Concentration in Man. Jr. Clin. Invest. 41:1899-1910, 1965.

5. Stewart, R. D., et al: Use of Breath Analysis to Monitor Methylene Chloride Exposures. (In Progress).

6. Forster, H. V., et al: Cardio-pulmonary Studies on Humans During Exposure to Methylene Chloride. (In Progress).

7. Porter, K., and Volman, P. H.: Flame Ionization Detection of Carbon Monoxide for Gas Chromatographic Analysis. Anal. Chem. 34:748-749, 1962.

8. Henry, R. J.: Clinical Chemistry Principles and Technics, Harper and Row, Publishers, New York. 612-613, 1964.

9. Hosko, M. J.: The Effect of Carbon Monoxide on the Visual Evoked Response in Man. Arch. Environ. Health. 21:174-180, 1970.

10. Stewart, R. D., Peterson, J. E., Baretta, E. D., Bachand, R. T., Hosko, M. J., and Herrmann, A. A.: Experimental Human Exposure to Carbon Monoxide. Arch. Environ. Health. 21:154-164, 1970.

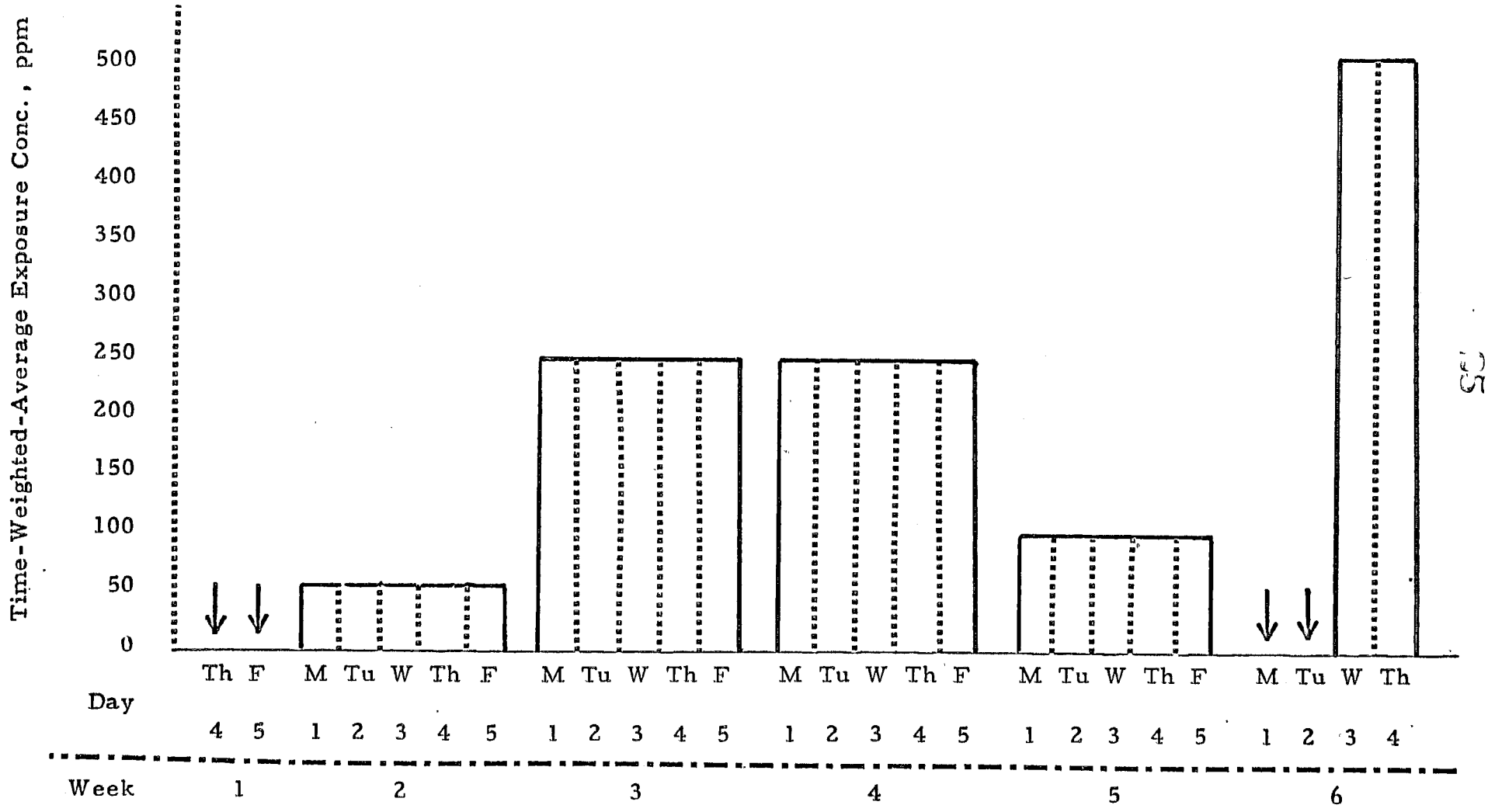
11. Peterson, J. E.: Post-exposure Relationship of Carbon Monoxide in Blood and Expired Air. Arch. Environ. Health. 21:172-173, 1970.

12. Winneke, G., Personal communication.

13. Fassett, D., Personal communication.

FIGURE 1

METHYLENE CHLORIDE EXPOSURE SCHEDULE



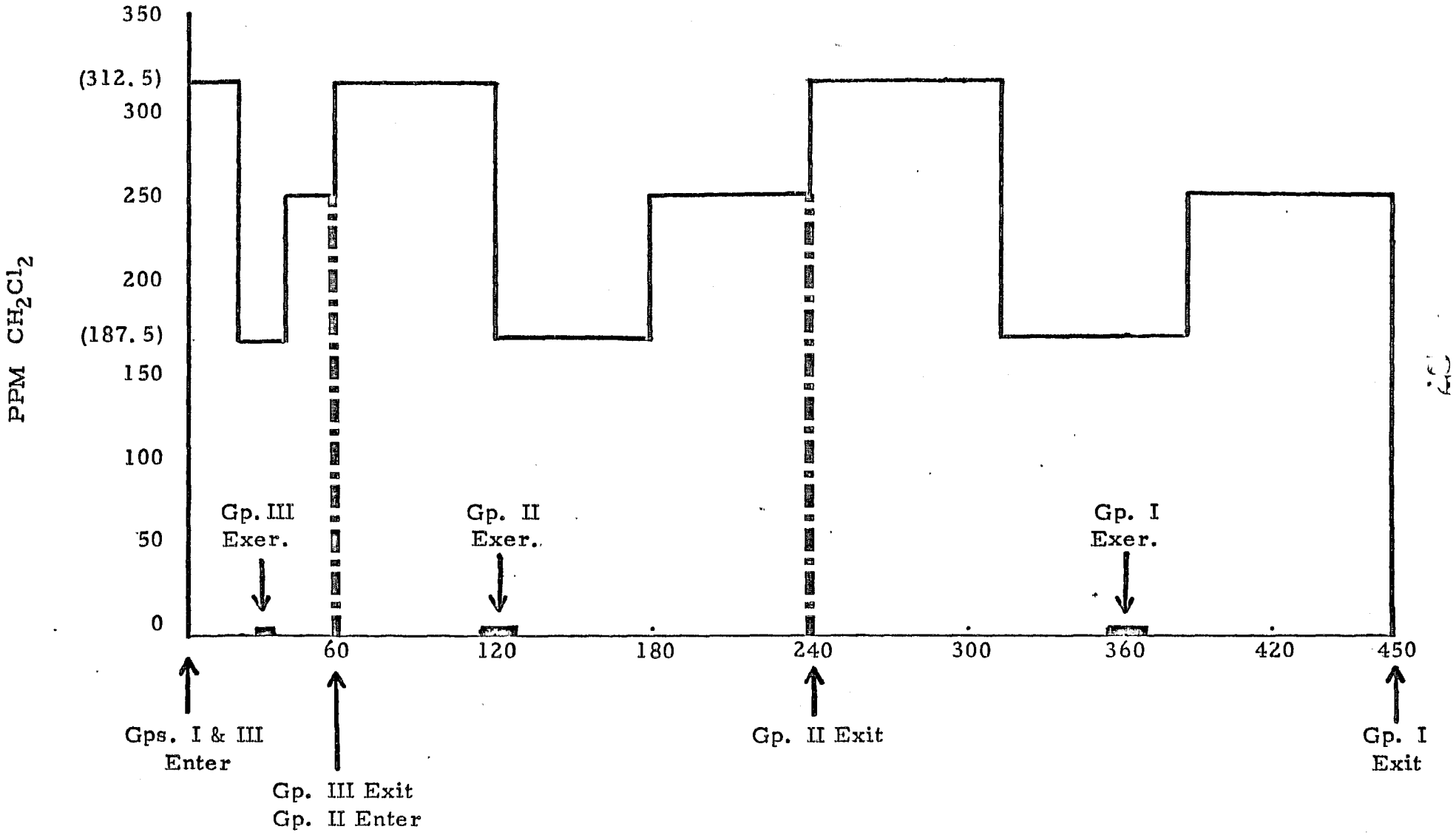
14. DiVincenzo, G. D., Yanno, F. J., and Astill, B. D.: Human and Canine Exposures to Methylene Chloride Vapor. Amer. Indust. Hyg. Assoc. J. 33:125-135, 1972.

15. Faulconer, A., and Bickford, R. G.: Electroencephalography in Anesthesiology. C. C. Thomas, Springfield, Illinois, 1960.

16. Beard, R. R., and Wertheim, G. A.: Behavioral Impairment Associated with Small Doses of Carbon Monoxide. Amer. Jr. Public Health. 57:2012-2022, 1967.

17. Stewart, R. D., Newton, P. E., Hosko, M. J., and Peterson, J. E.: Effect of Carbon Monoxide on Time Perception. Arch. Environ. Health. 27:155-160, 1973.

FIGURE 2


350
(312.5)
300
250
200
(187.5)
150
100
50
0
PPM CH_2Cl_2
Gp. III Exer.
Gp. II Exer.
Gp. I Exer.
60
120
180
240
300
360
420
450
Gps. I & III Enter
Gp. III Exit
Gp. II Enter
Gp. II Exit
Gp. I Exit
ELAPSED TIME - MINUTES

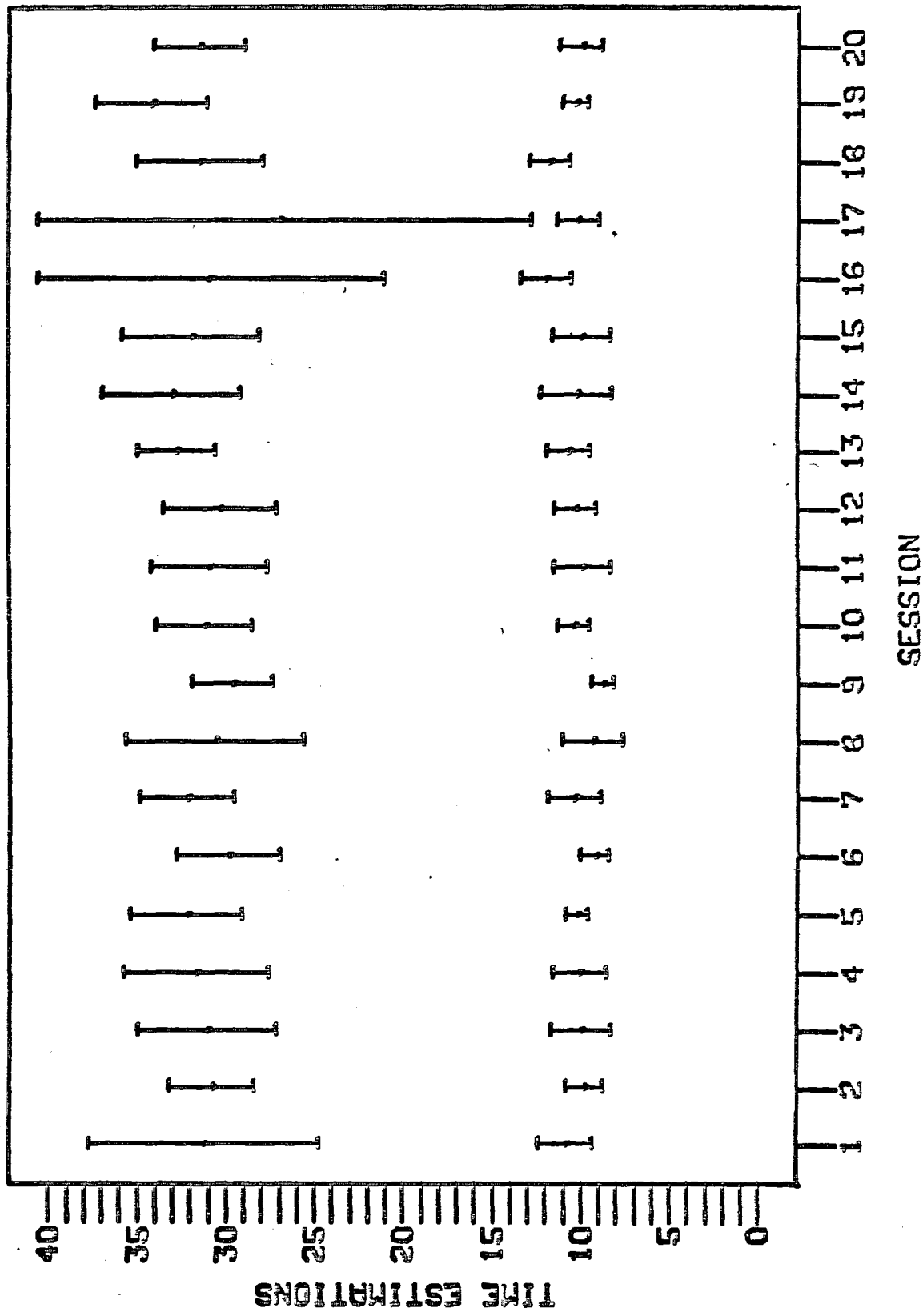


FIGURE 3

GROUP I

FIGURE 4

GROUP I

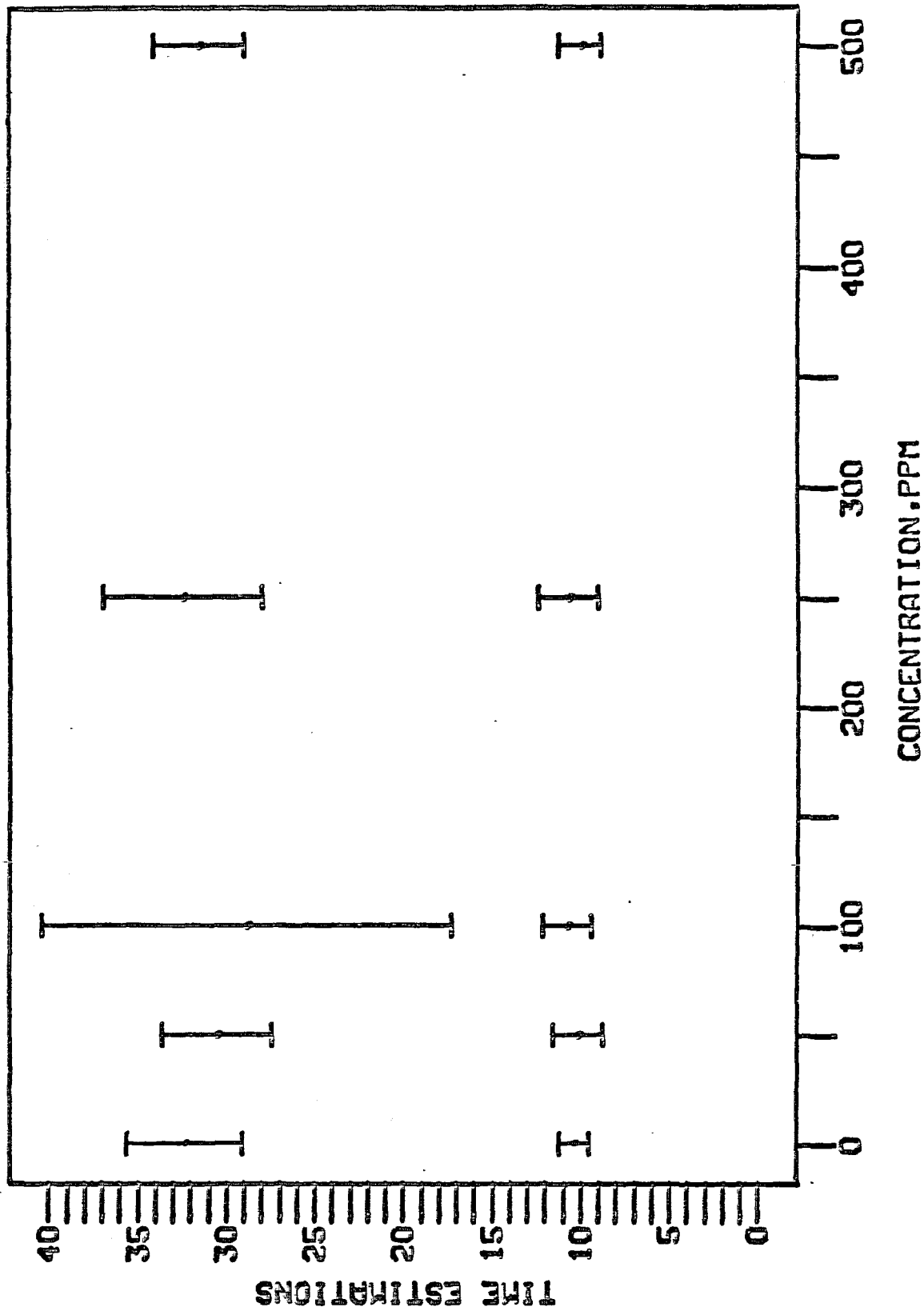


FIGURE 5

GROUP II

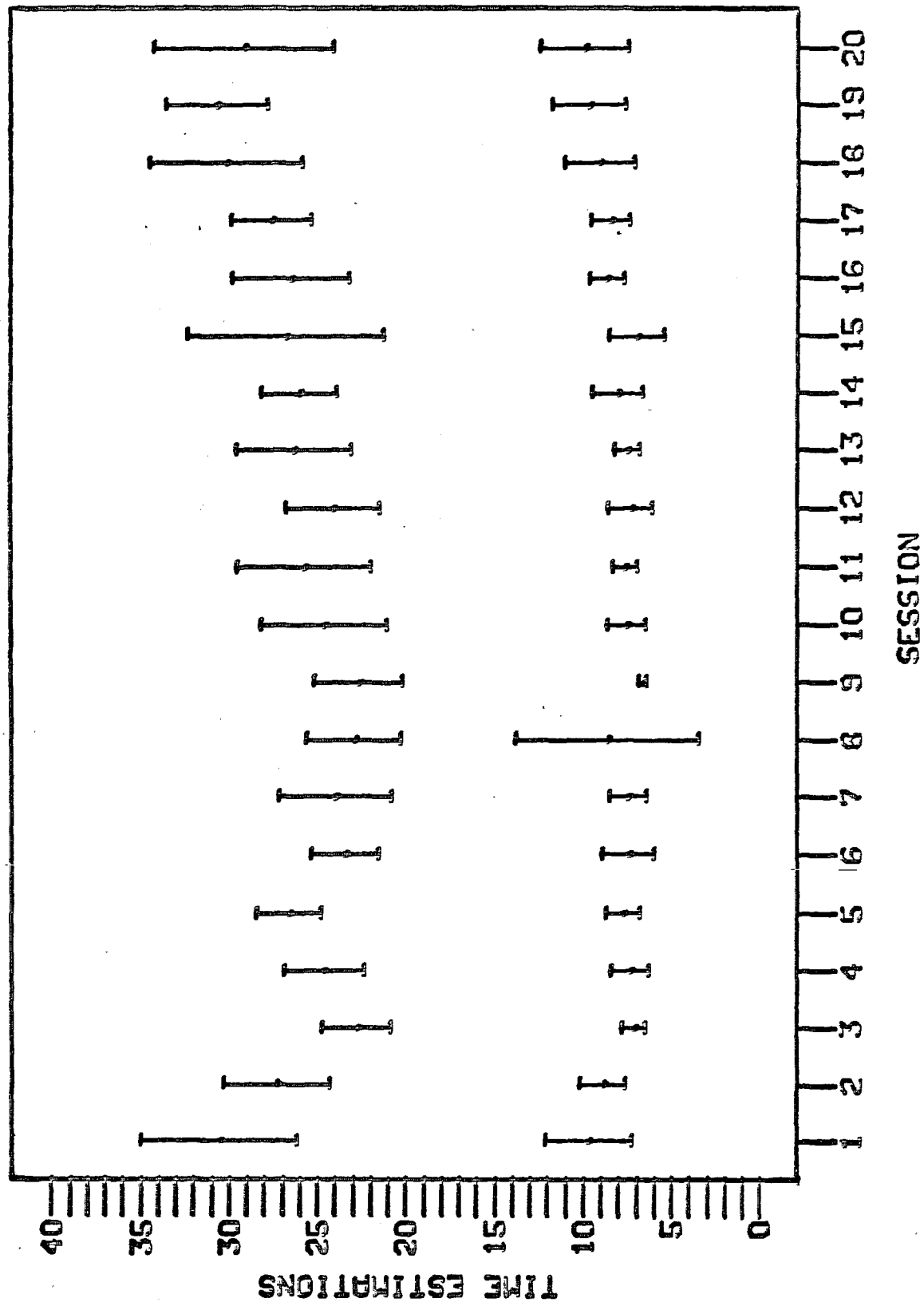


FIGURE 6

GROUP II

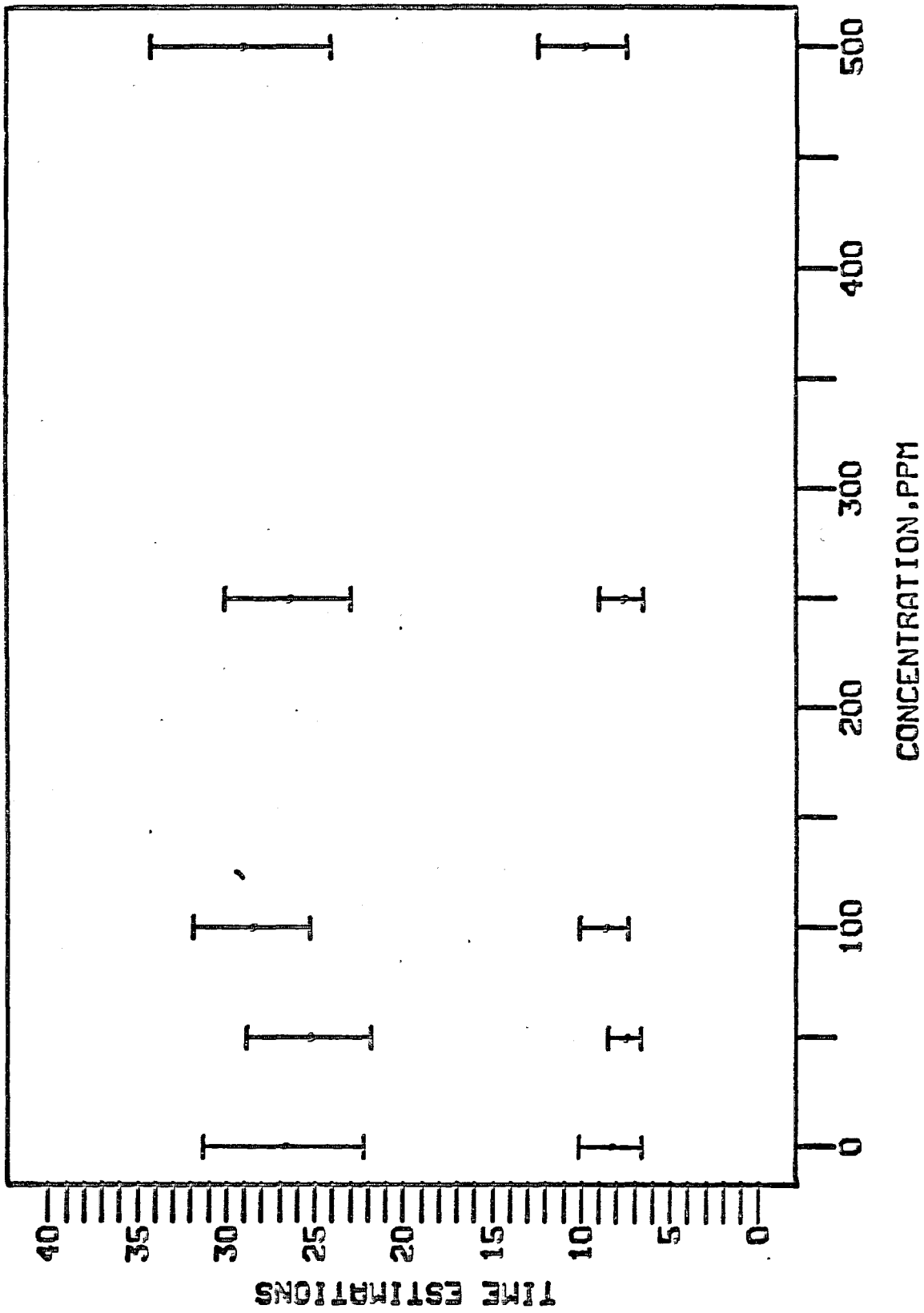


FIGURE 7

GROUP I

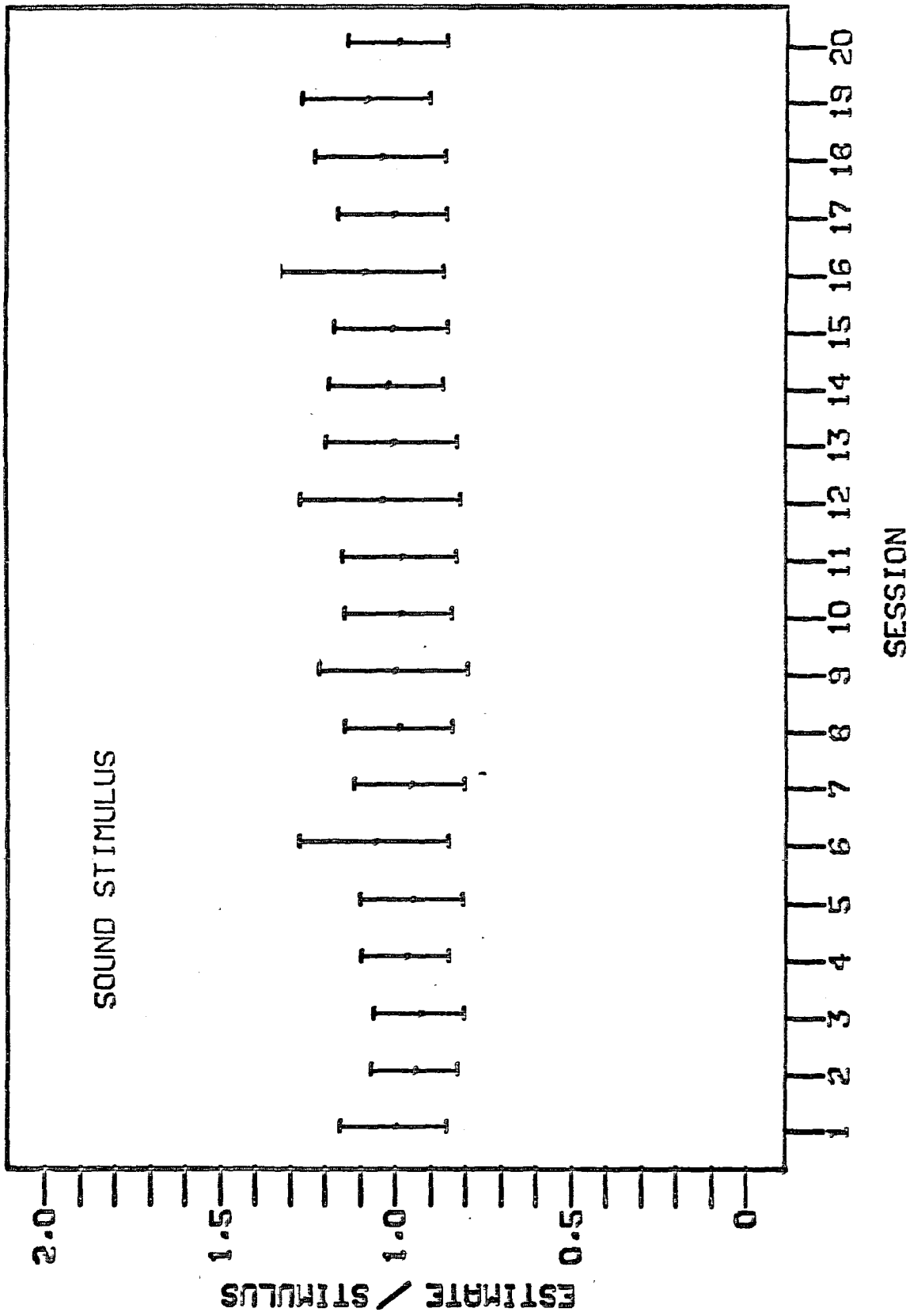
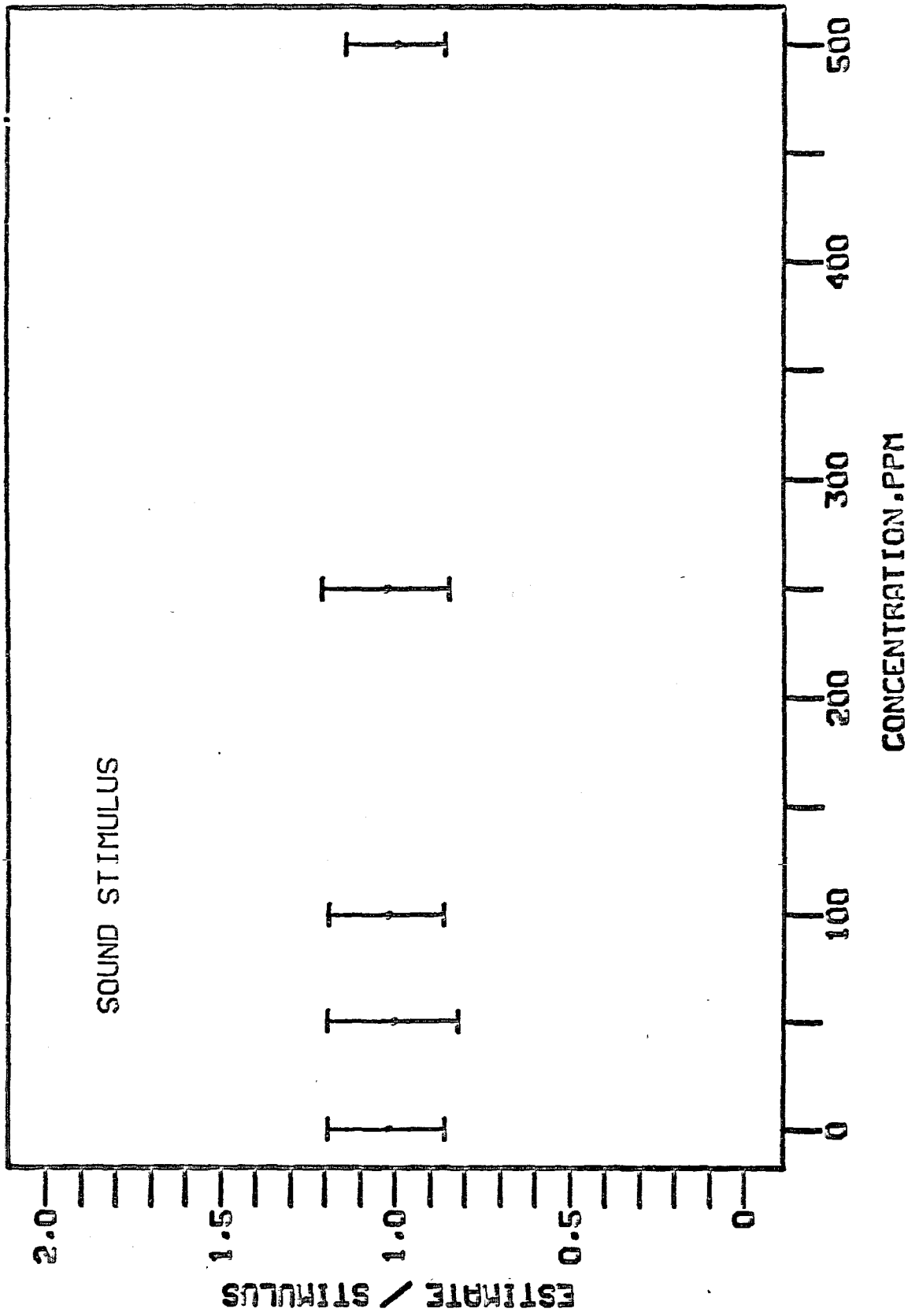


FIGURE 8

GROUP I



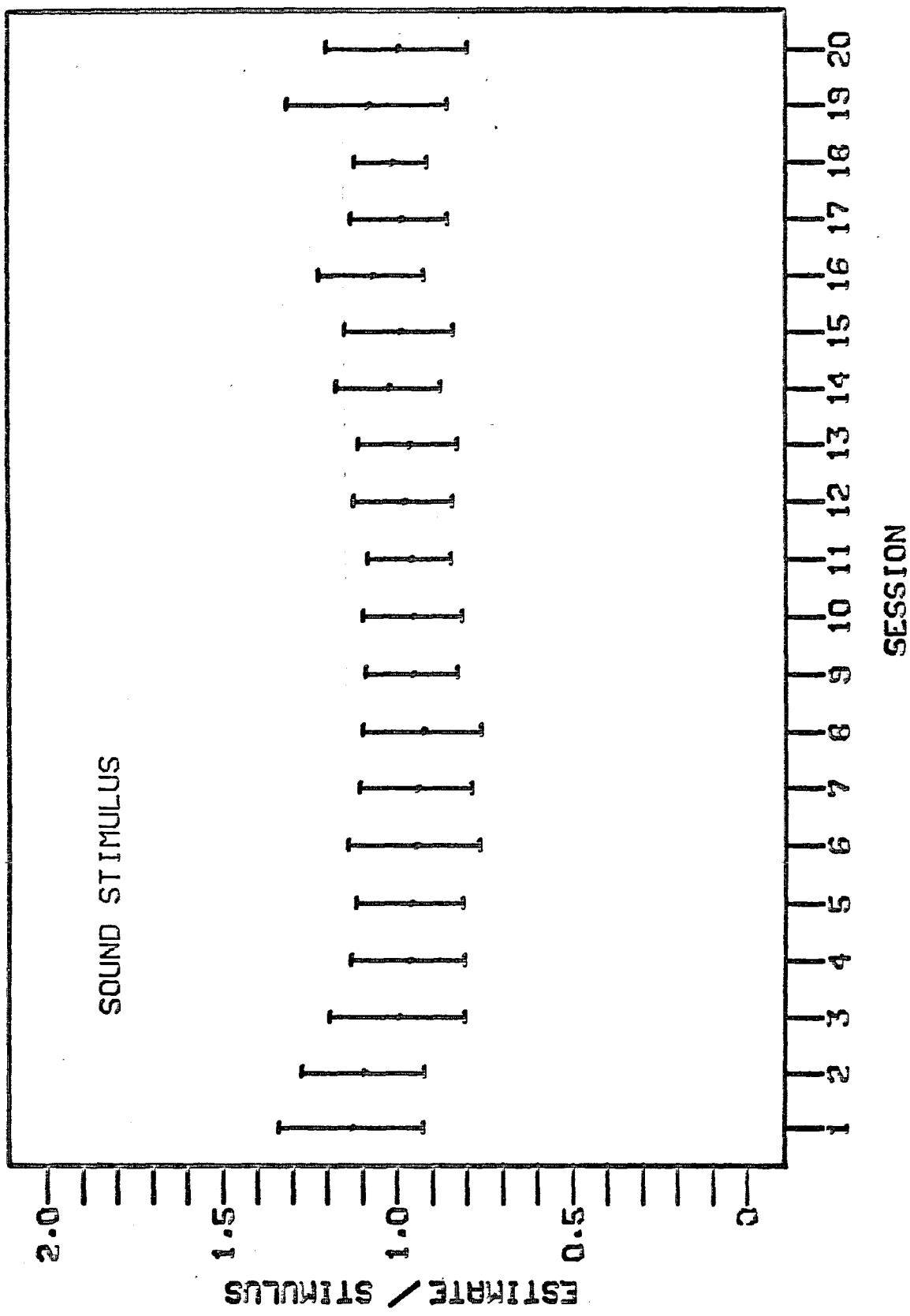


FIGURE 9

GROUP II

FIGURE 10

GROUP II

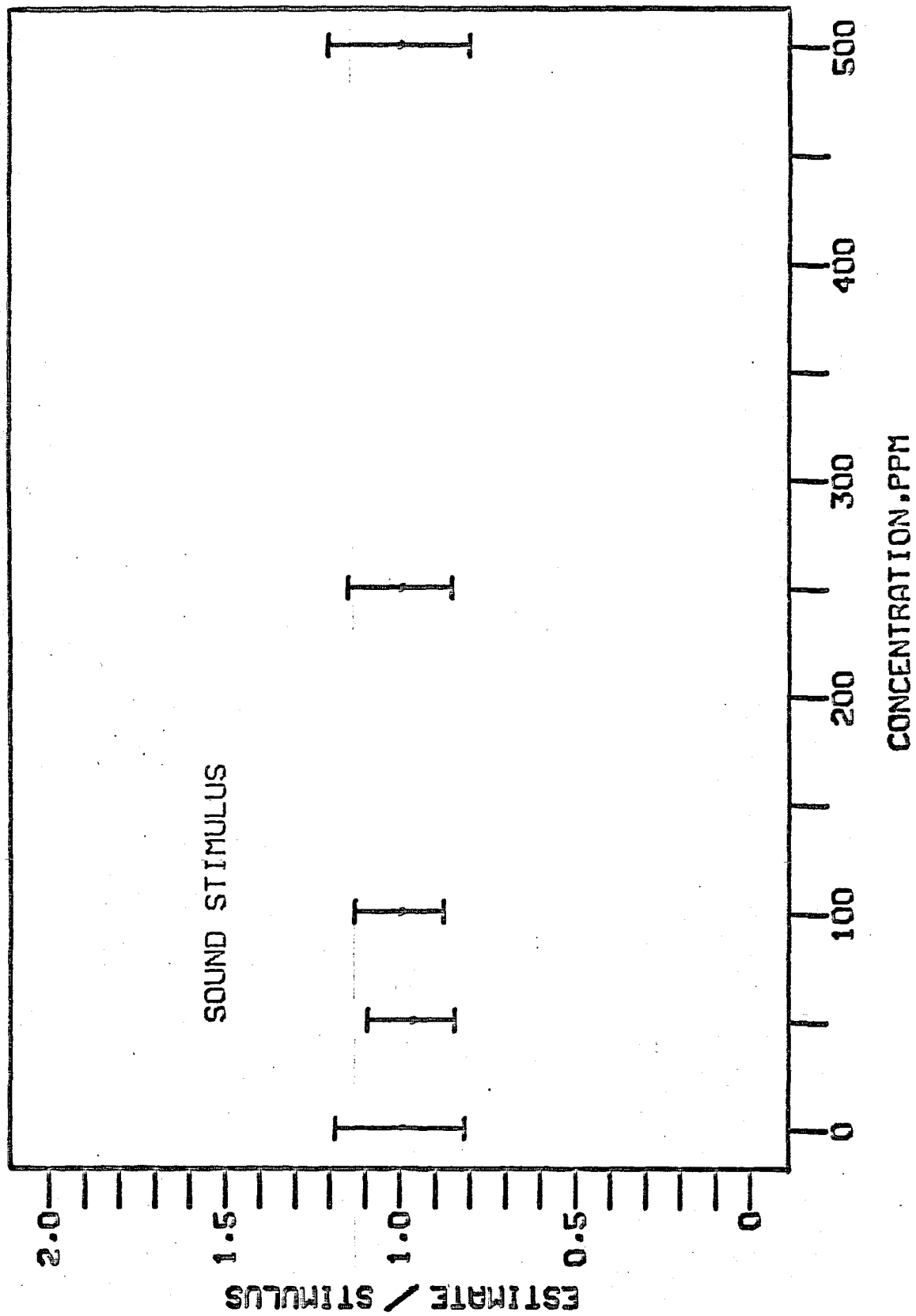


FIGURE 11

GROUP I

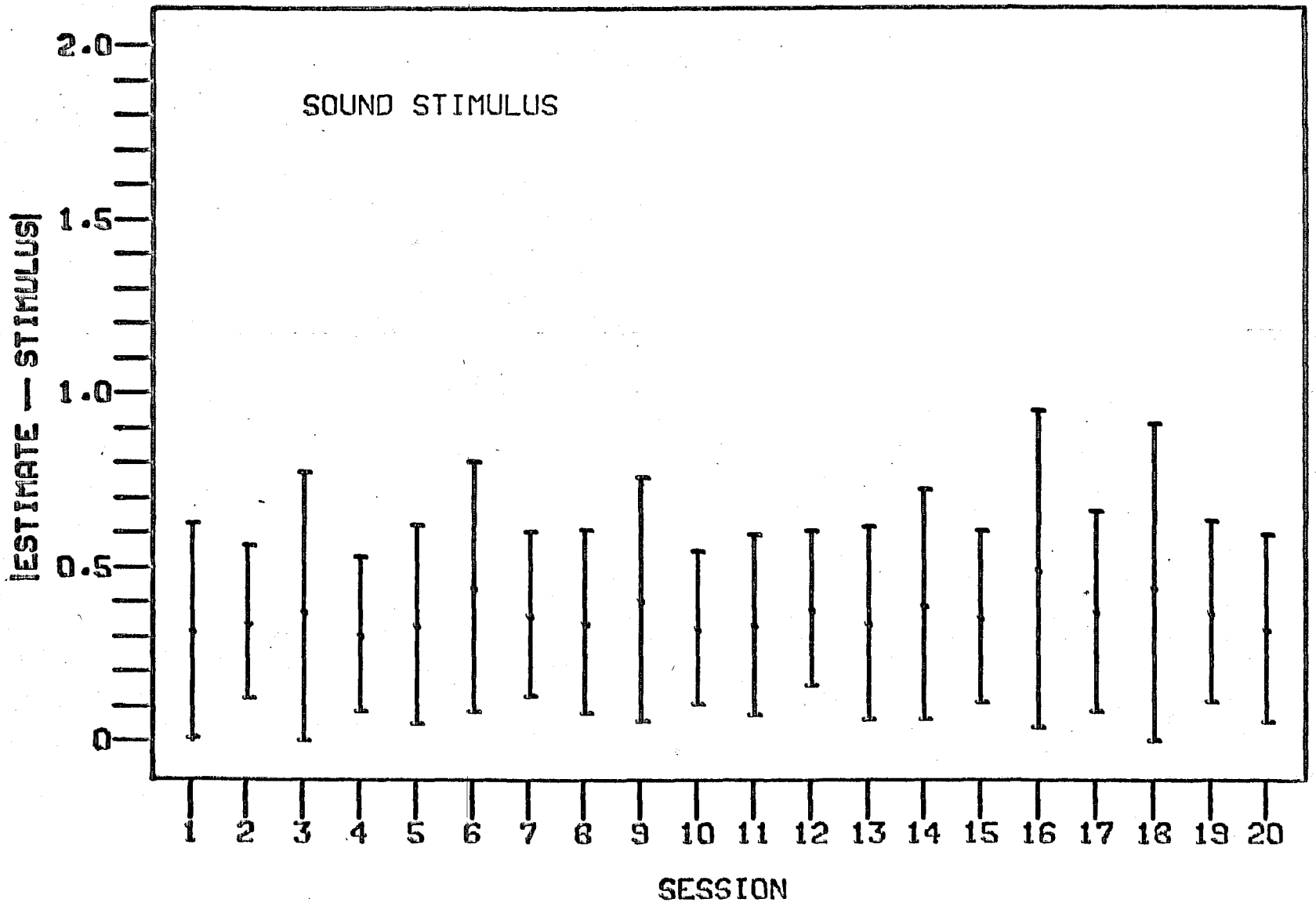


FIGURE 12

GROUP I

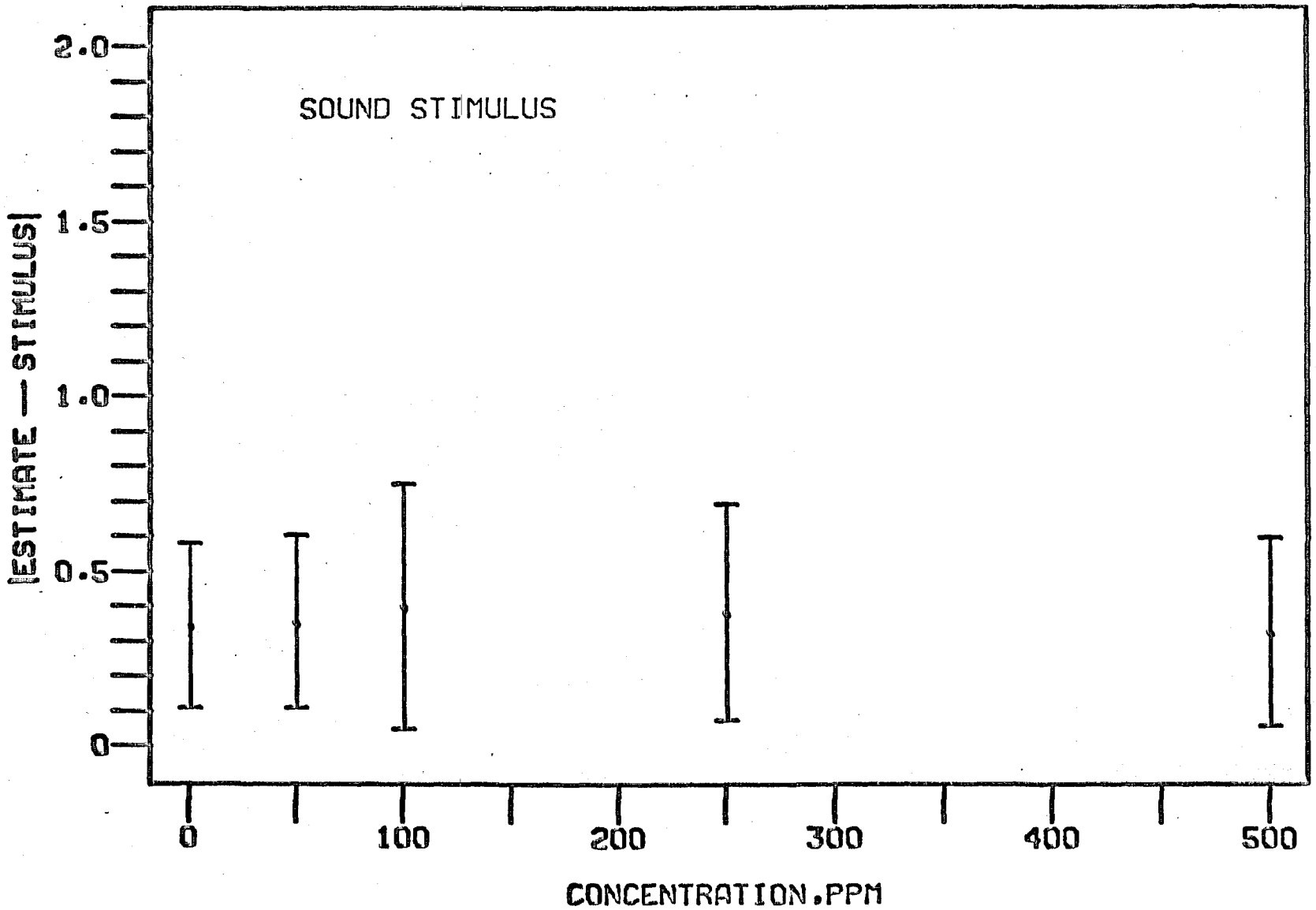


FIGURE 13

GROUP II

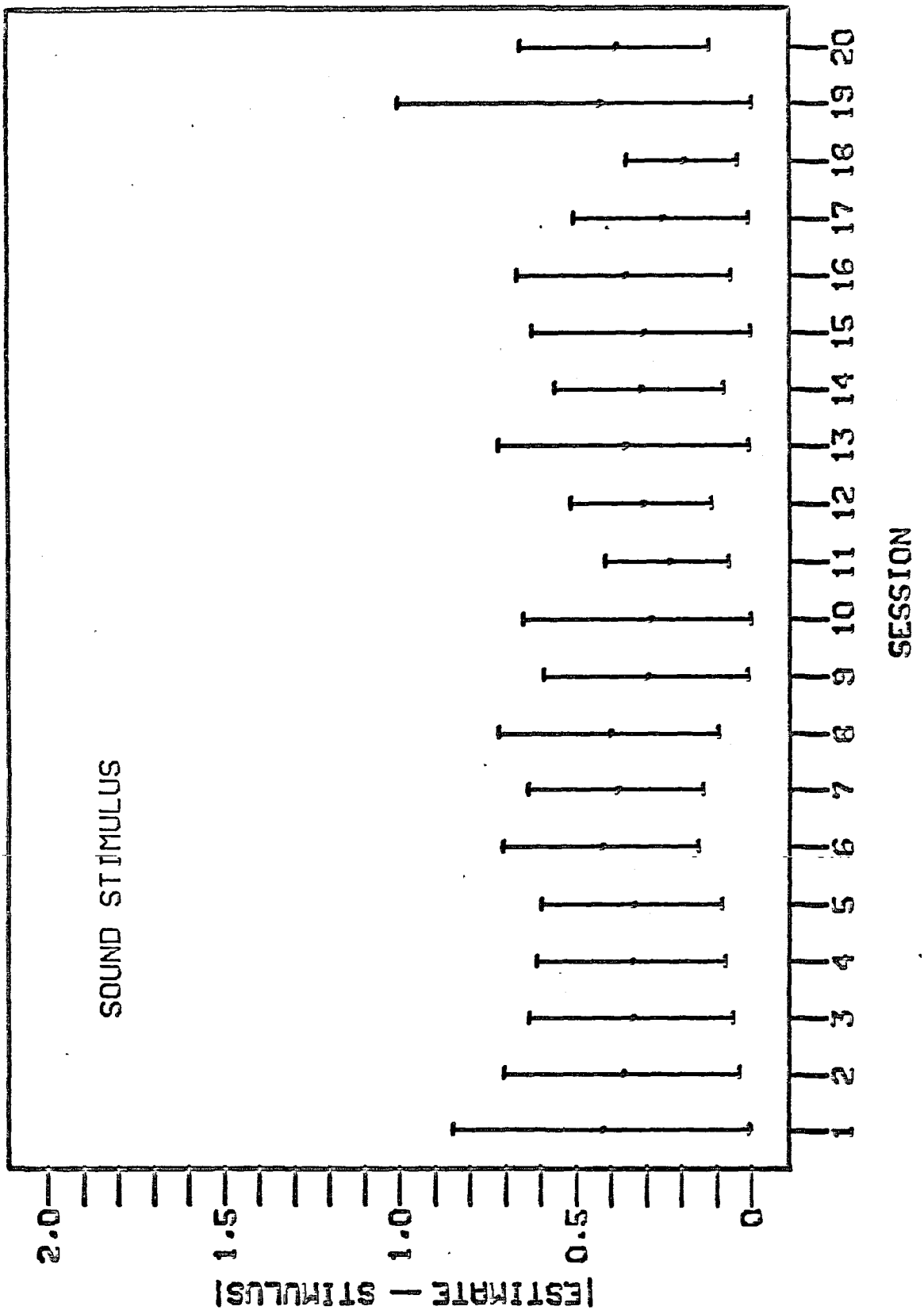


FIGURE 14

GROUP II

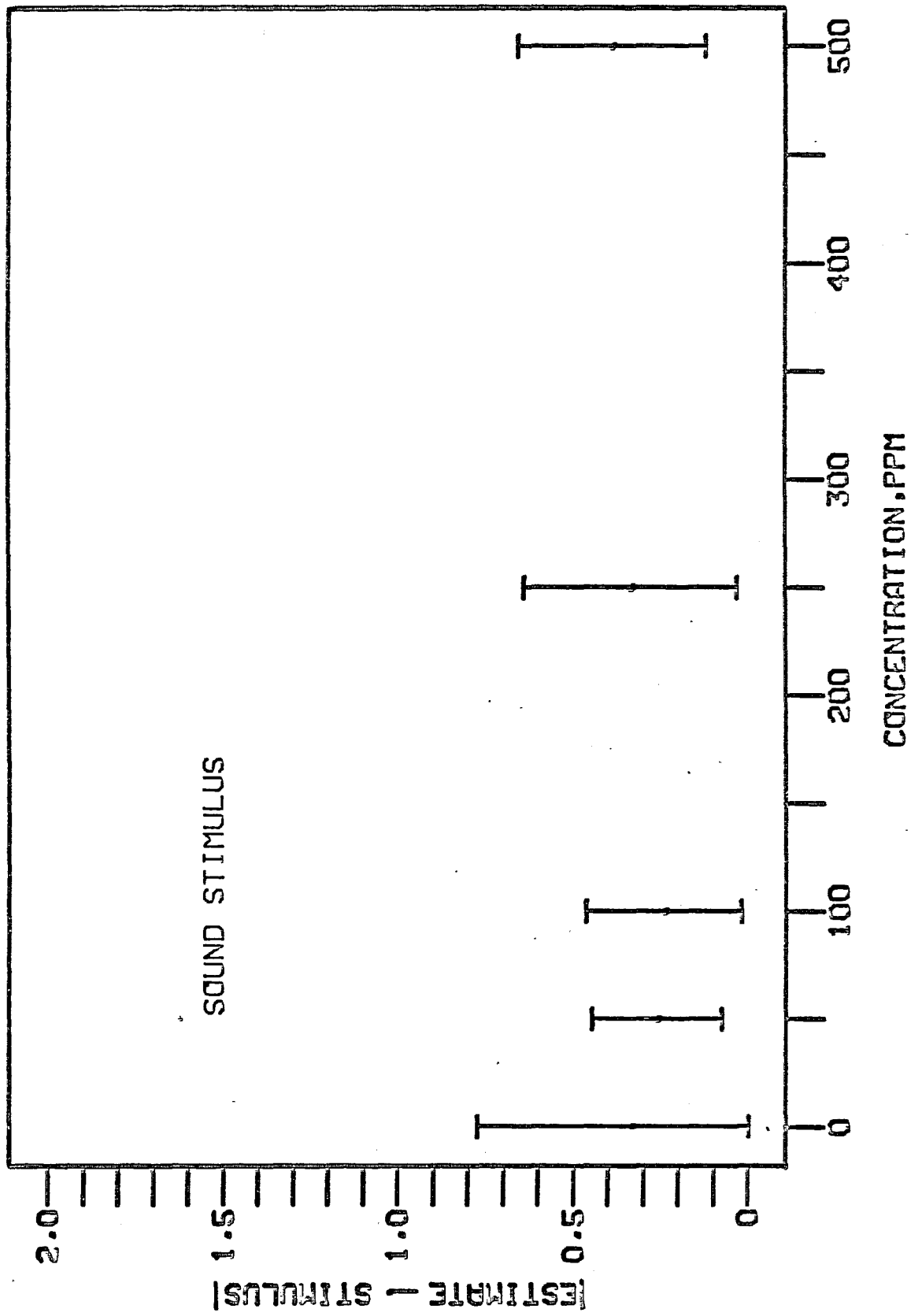


FIGURE 15

GROUP I

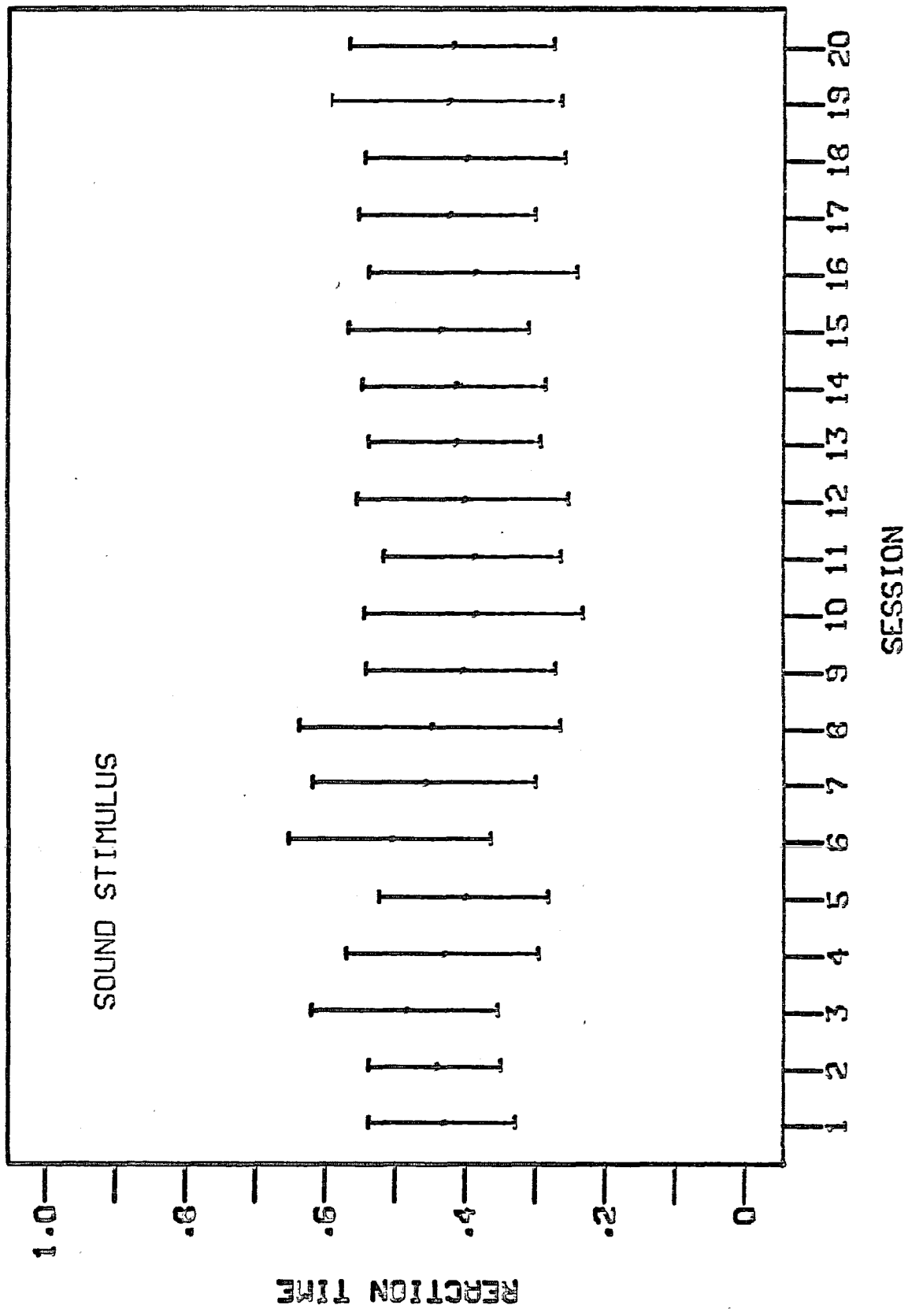


FIGURE 16

GROUP I

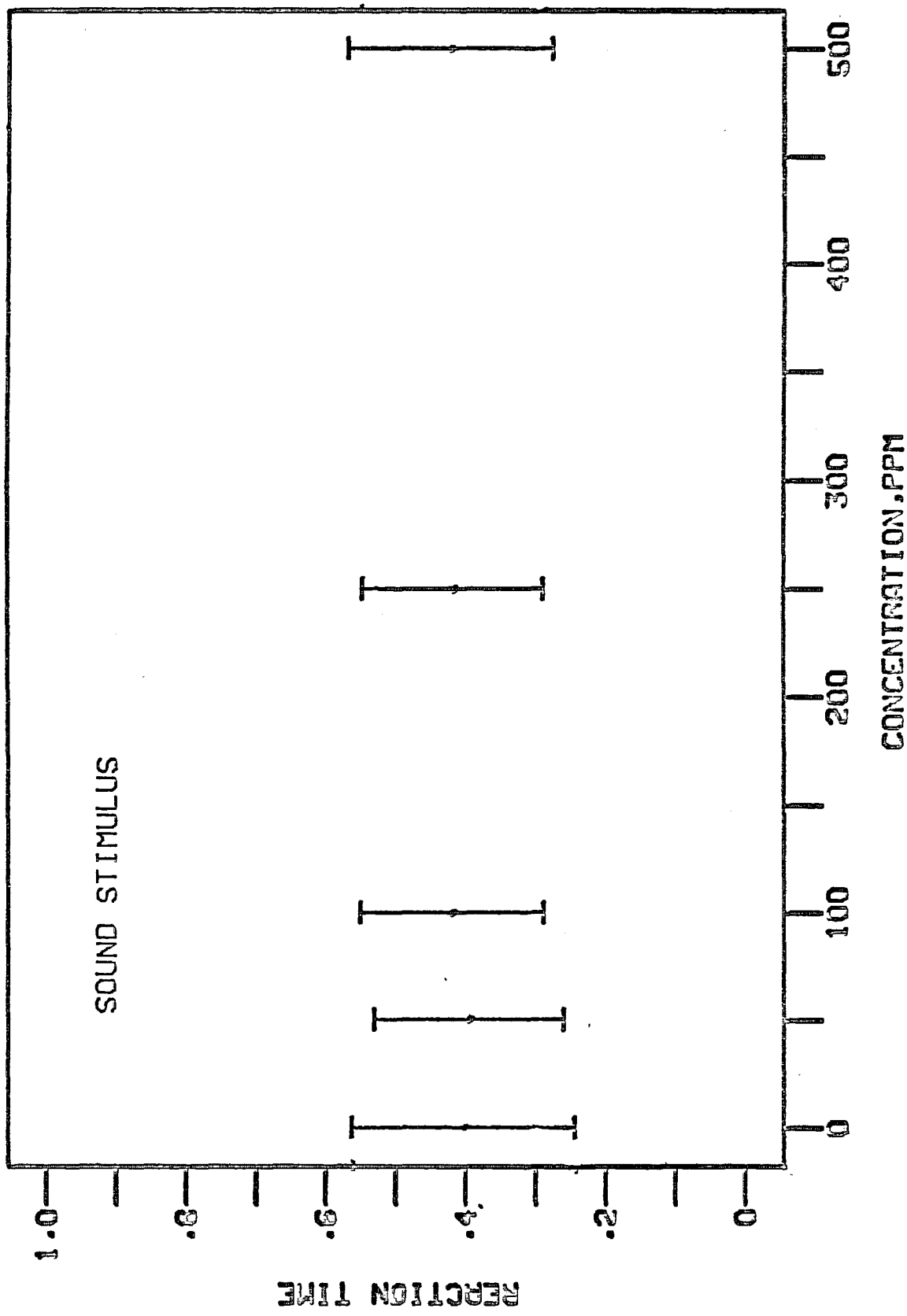


FIGURE 17

GROUP II

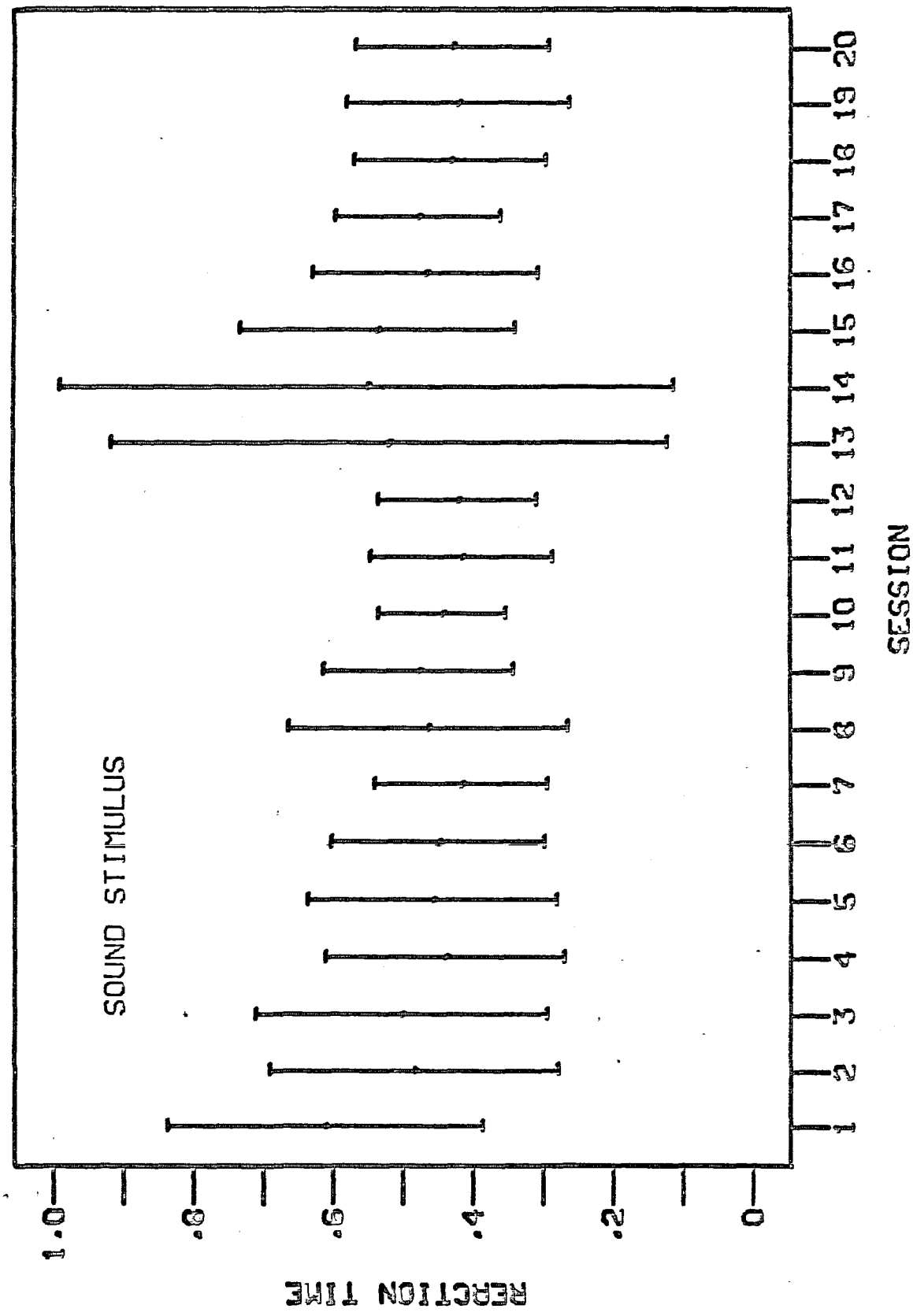


FIGURE 18

GROUP II

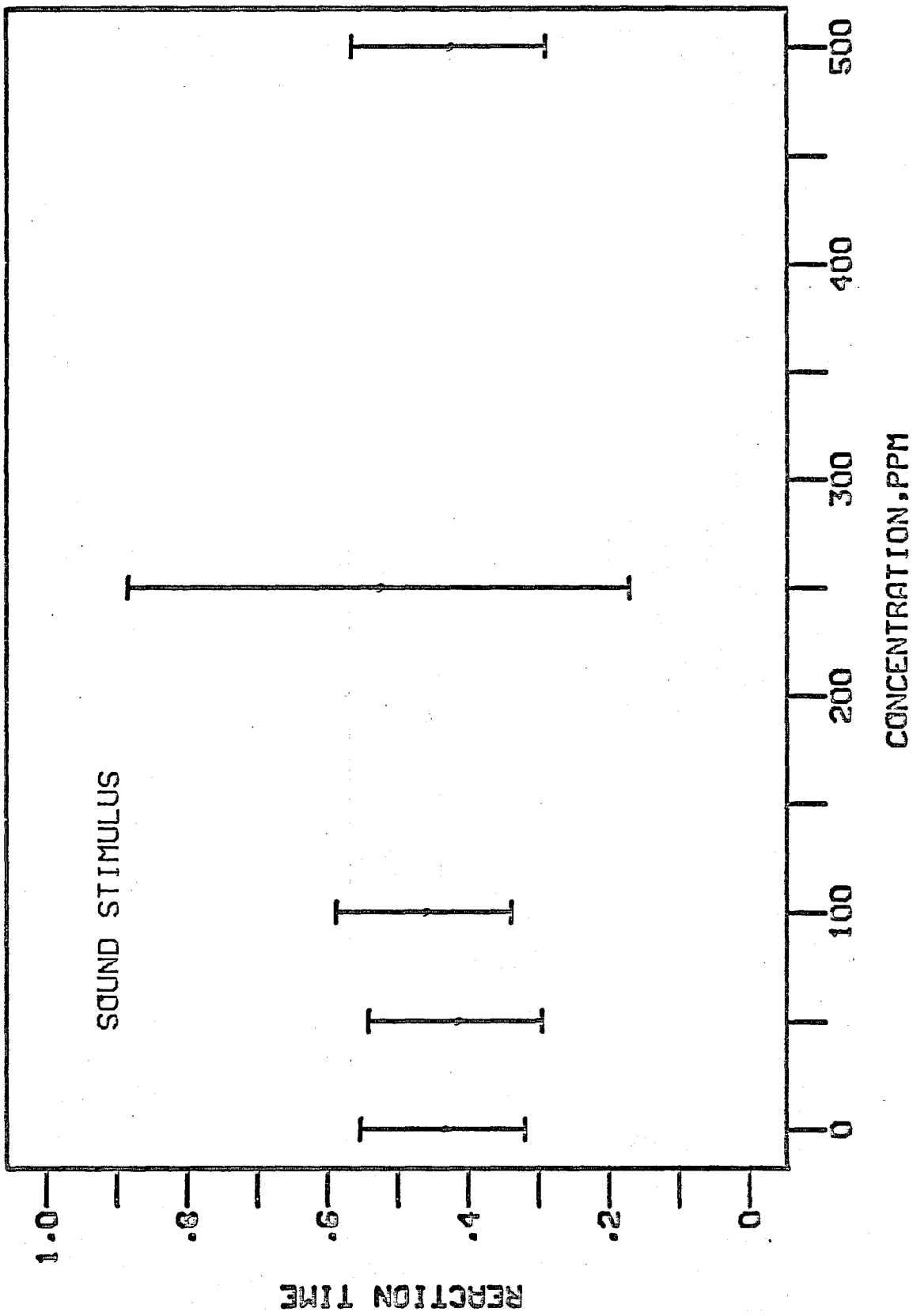


FIGURE 19

GROUP I

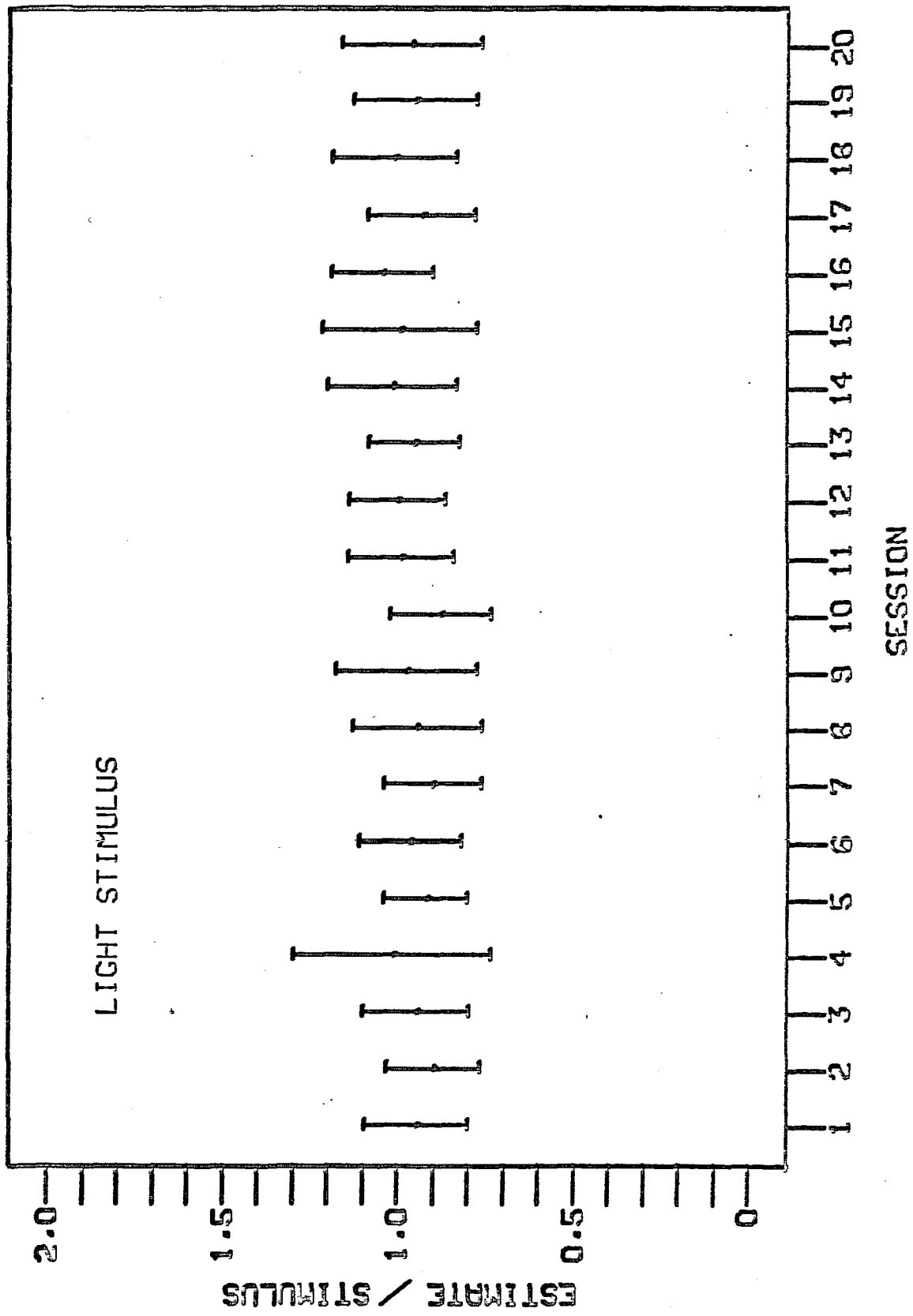


FIGURE 20

GROUP I

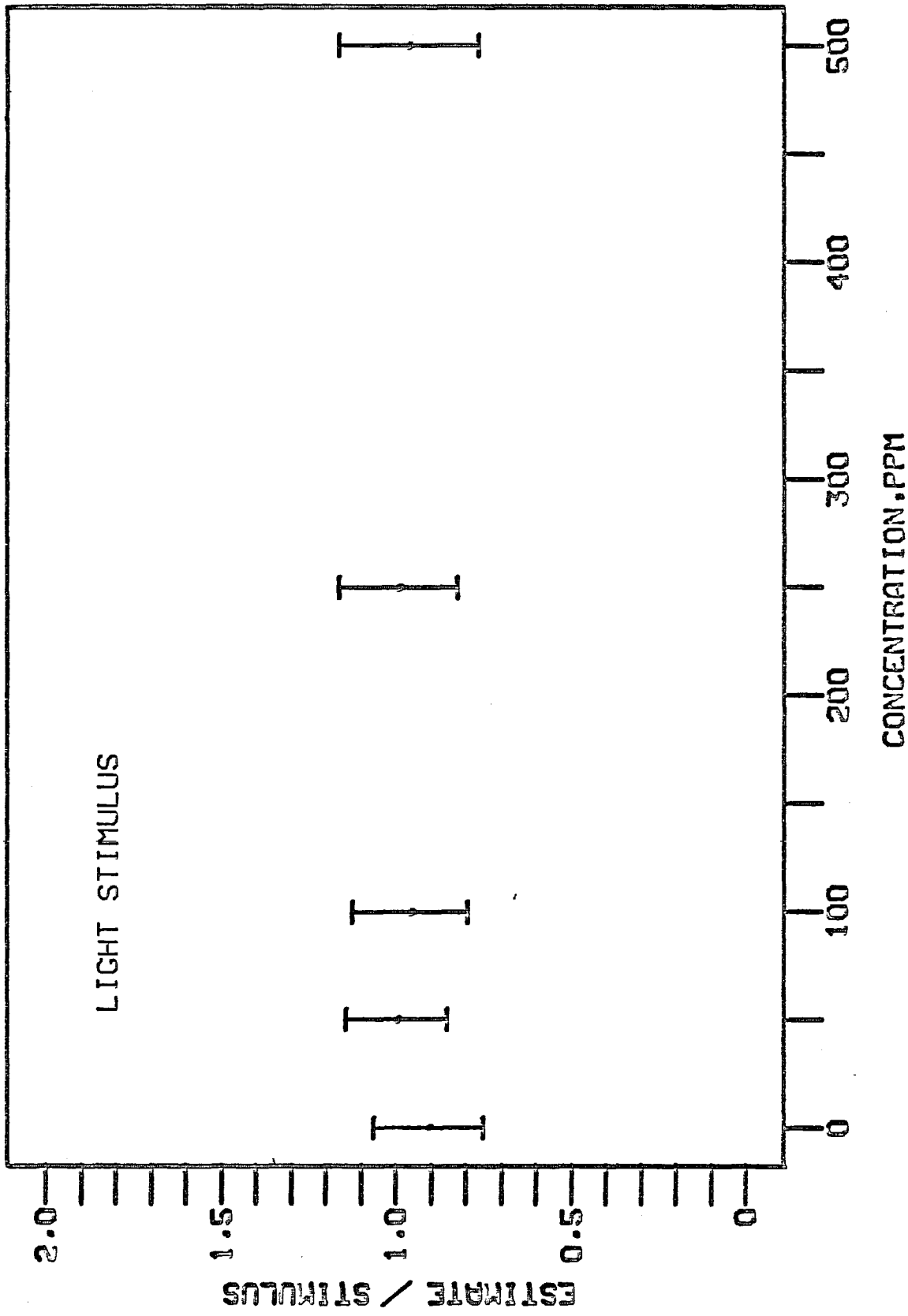


FIGURE 21

GROUP II

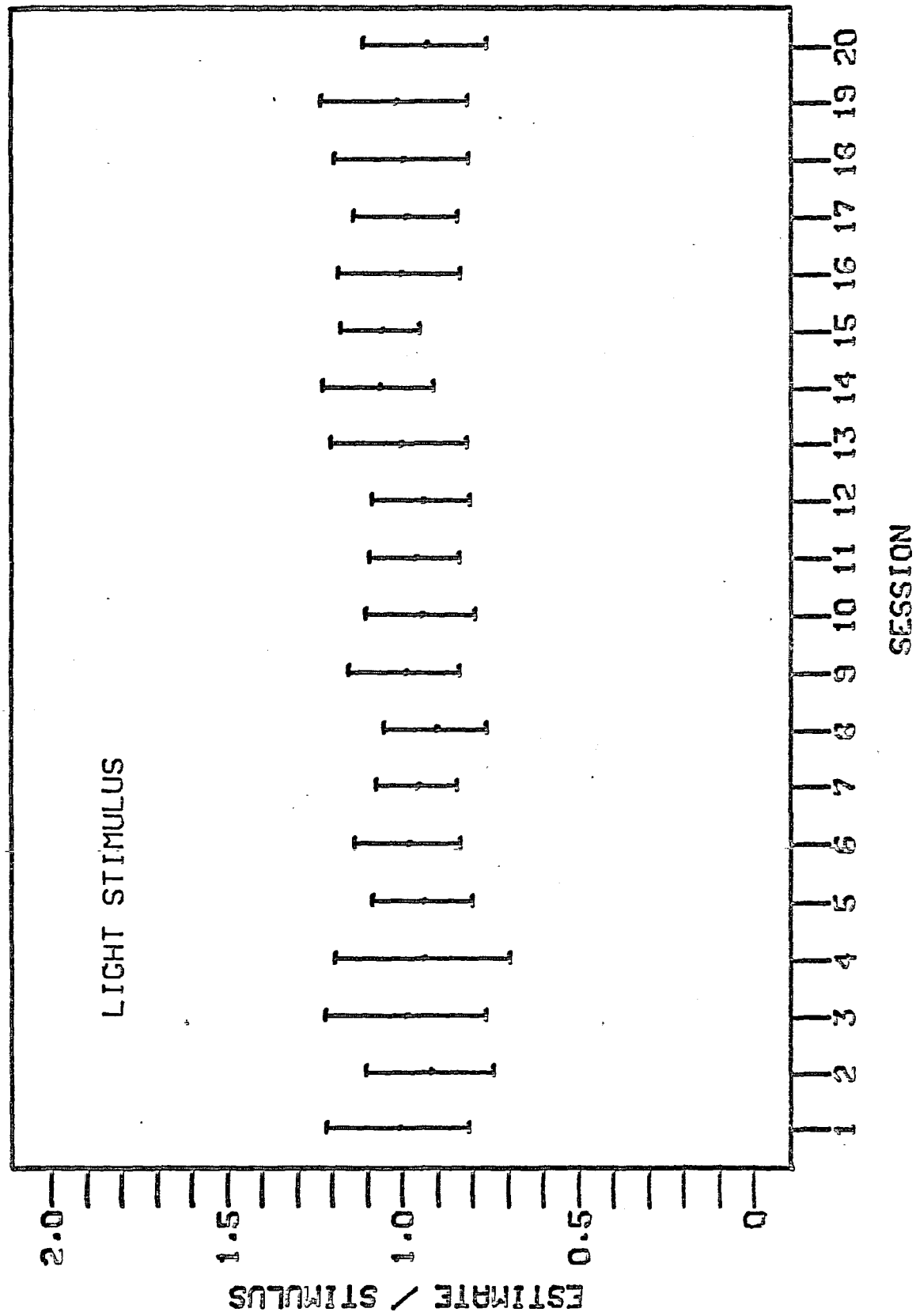


FIGURE 22

GROUP II

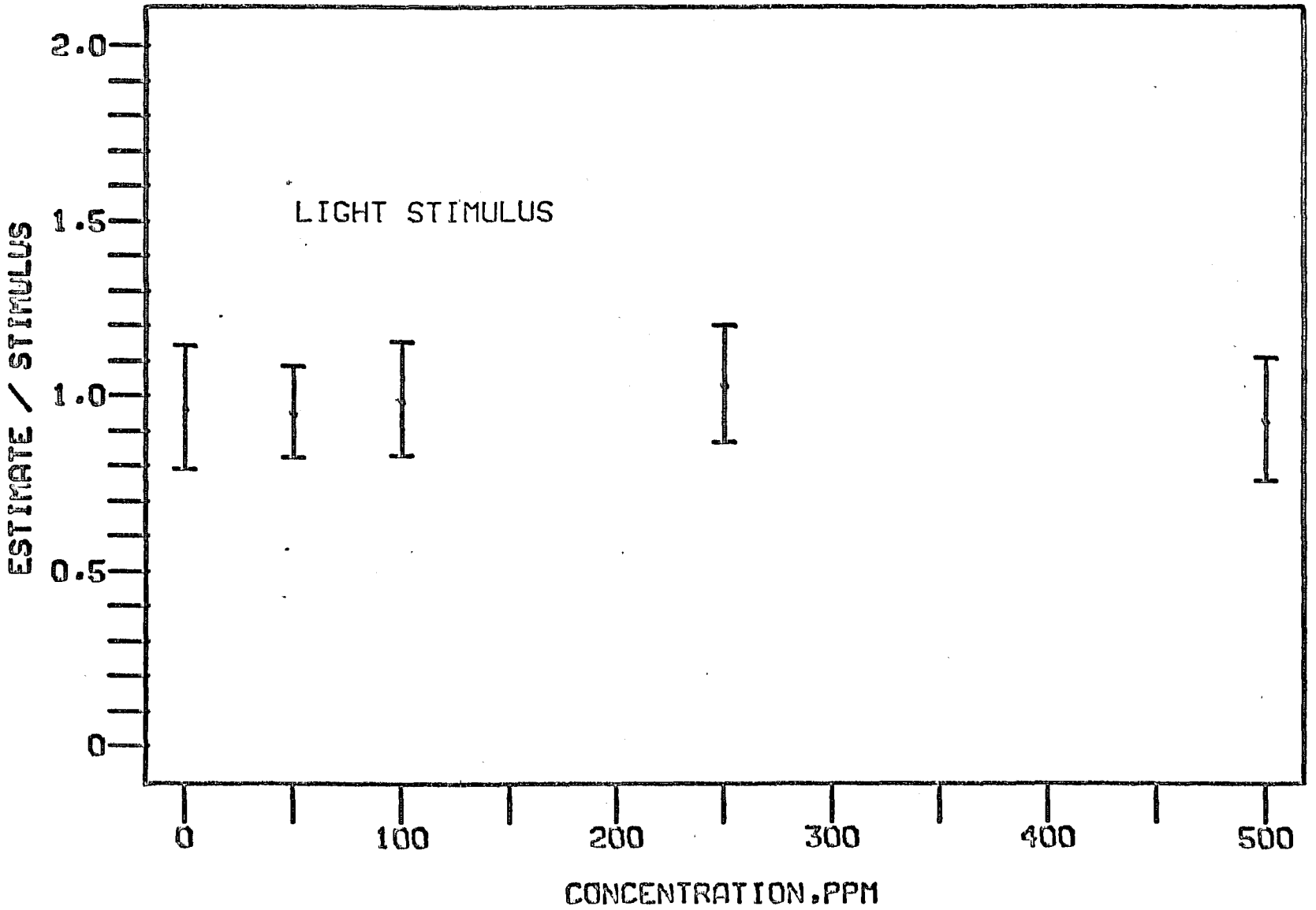


FIGURE 23

GROUP I

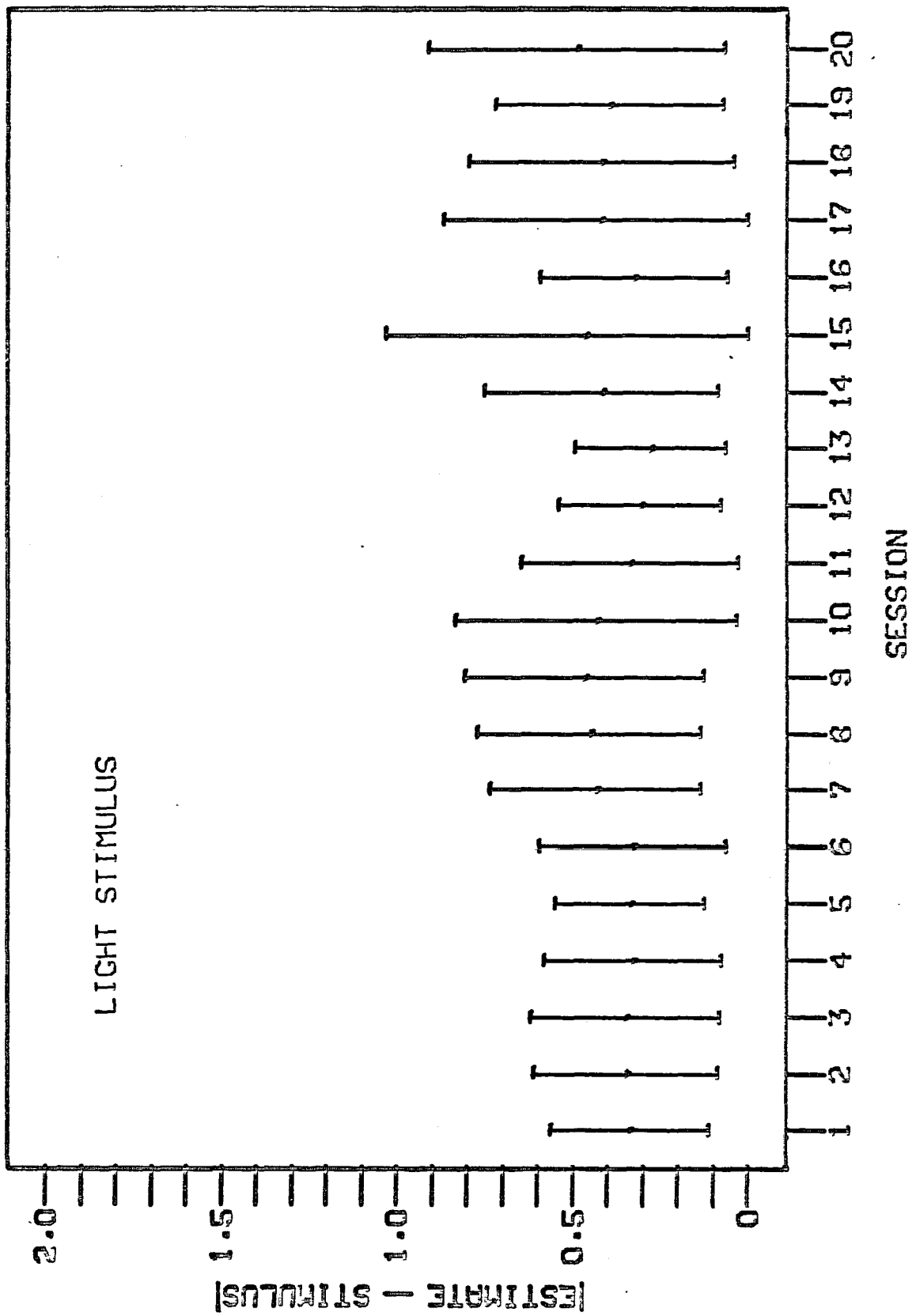


FIGURE 24

GROUP I

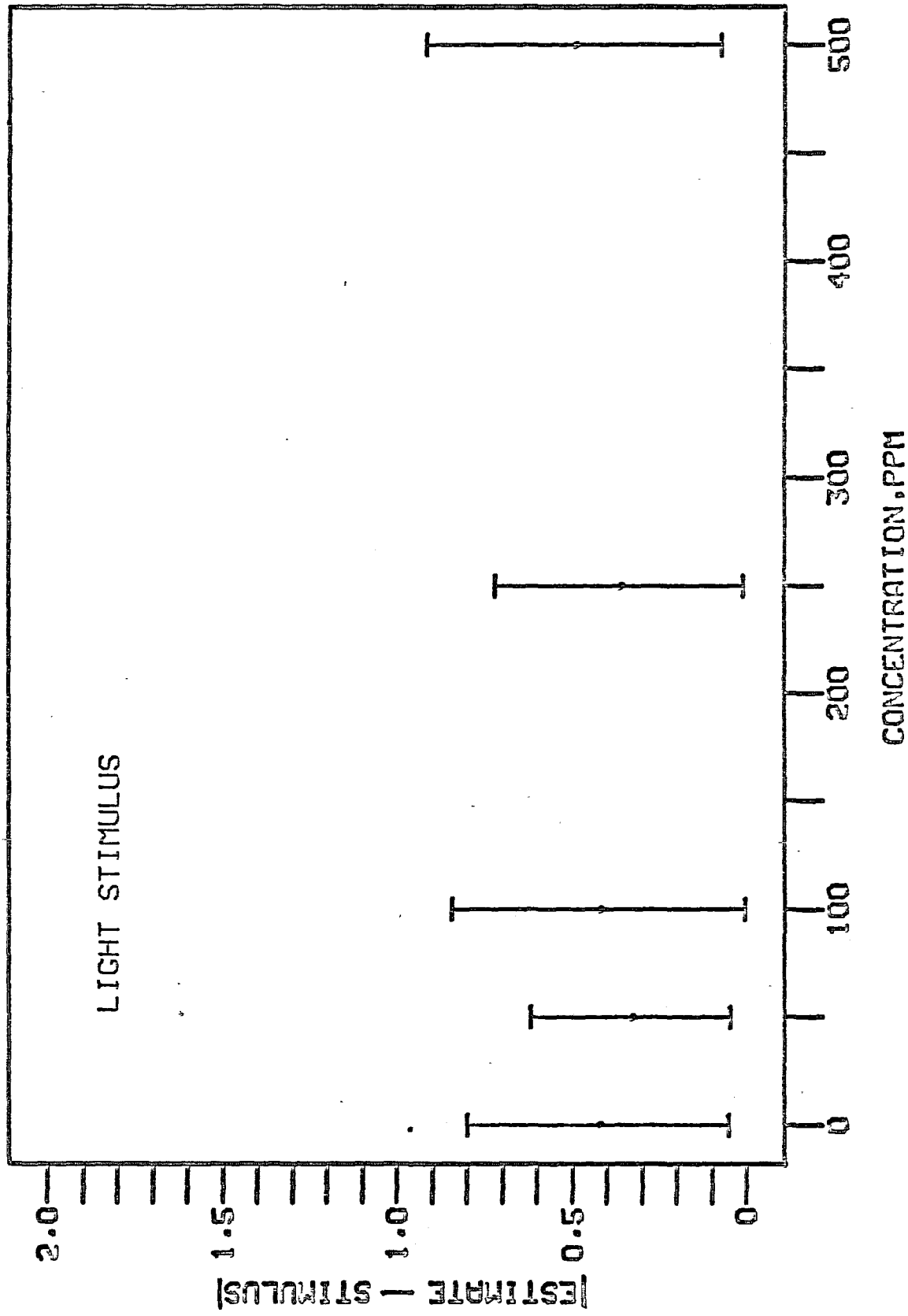


FIGURE 25

GROUP II

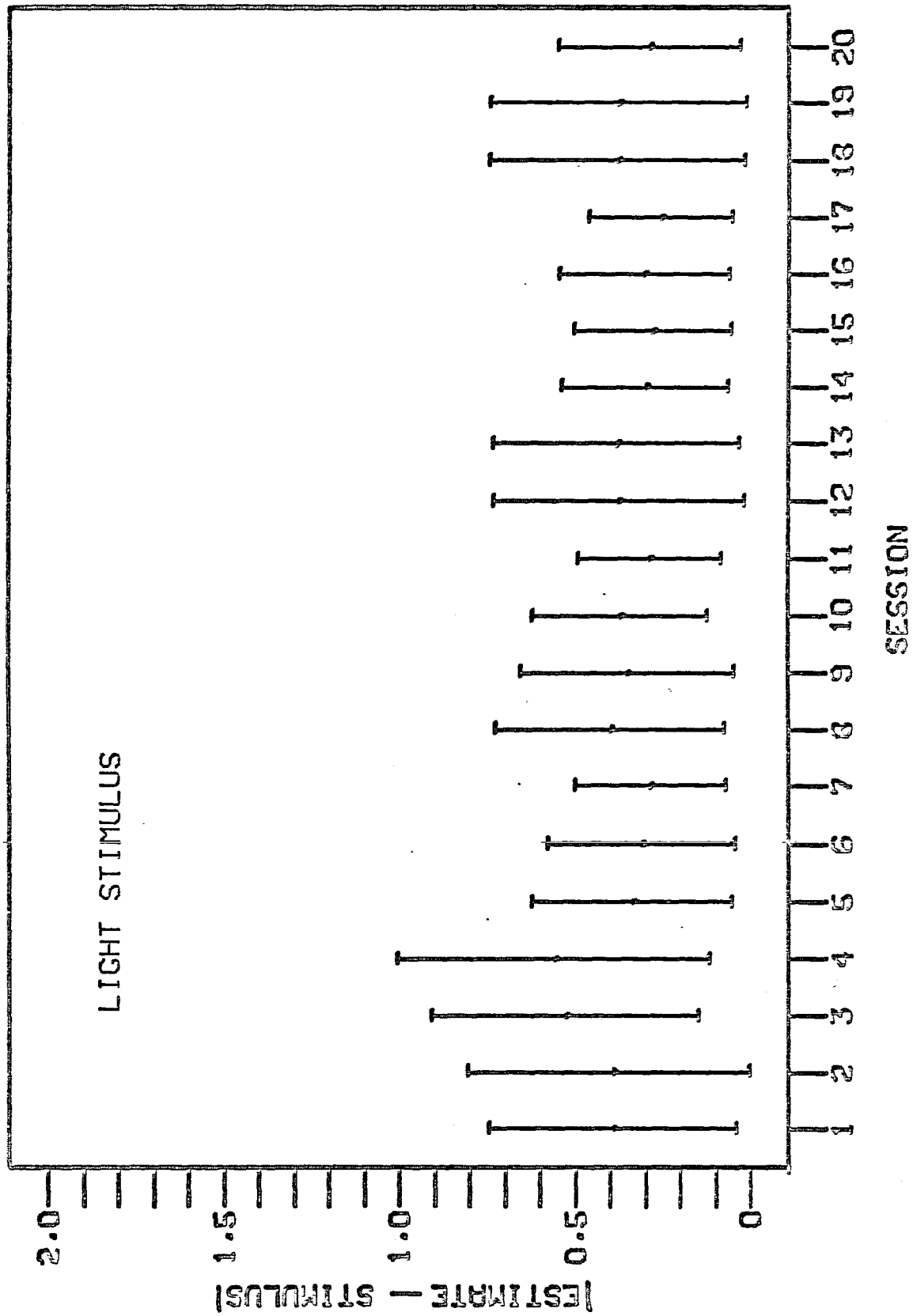


FIGURE 26

GROUP II

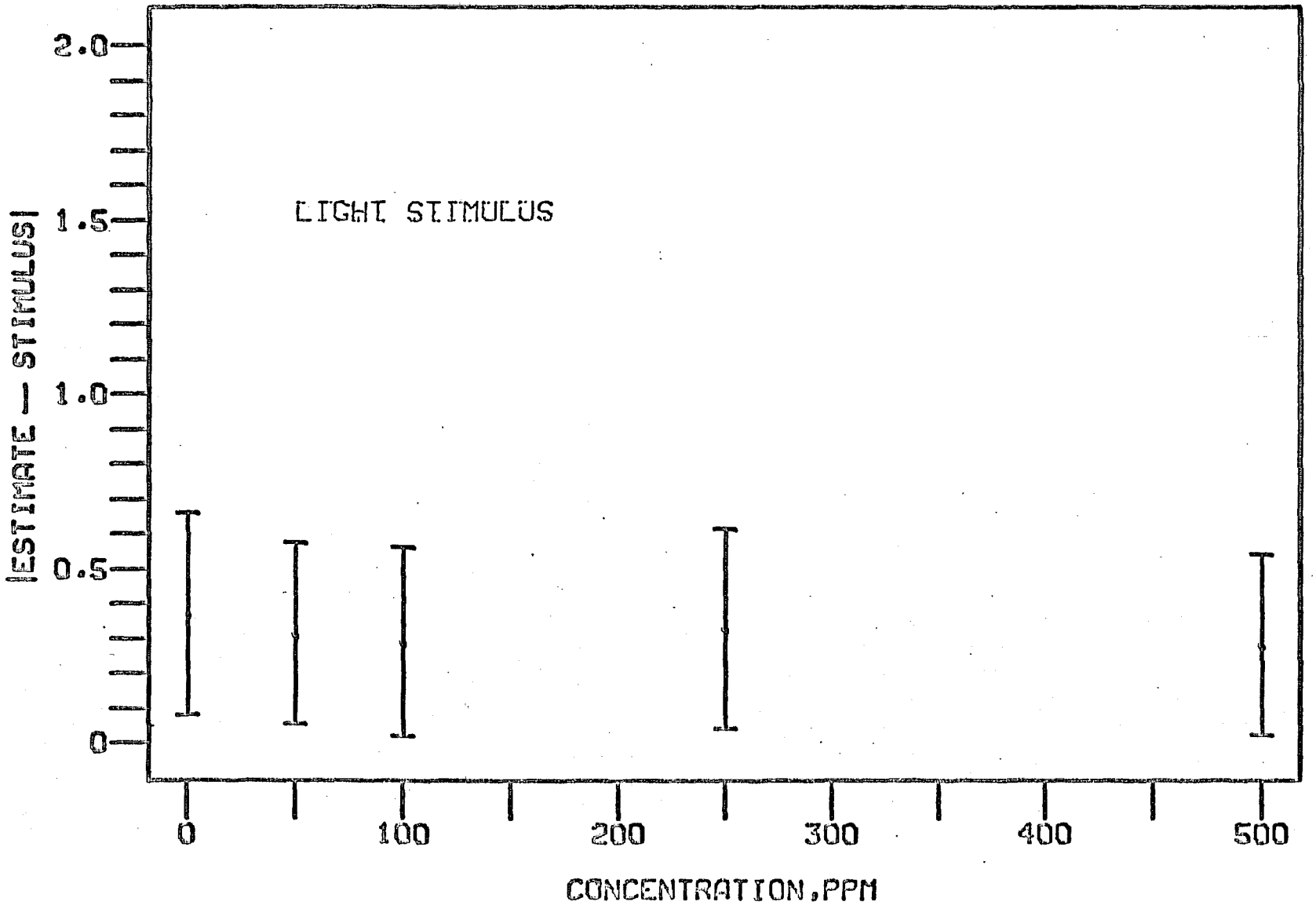


FIGURE 27

GROUP I

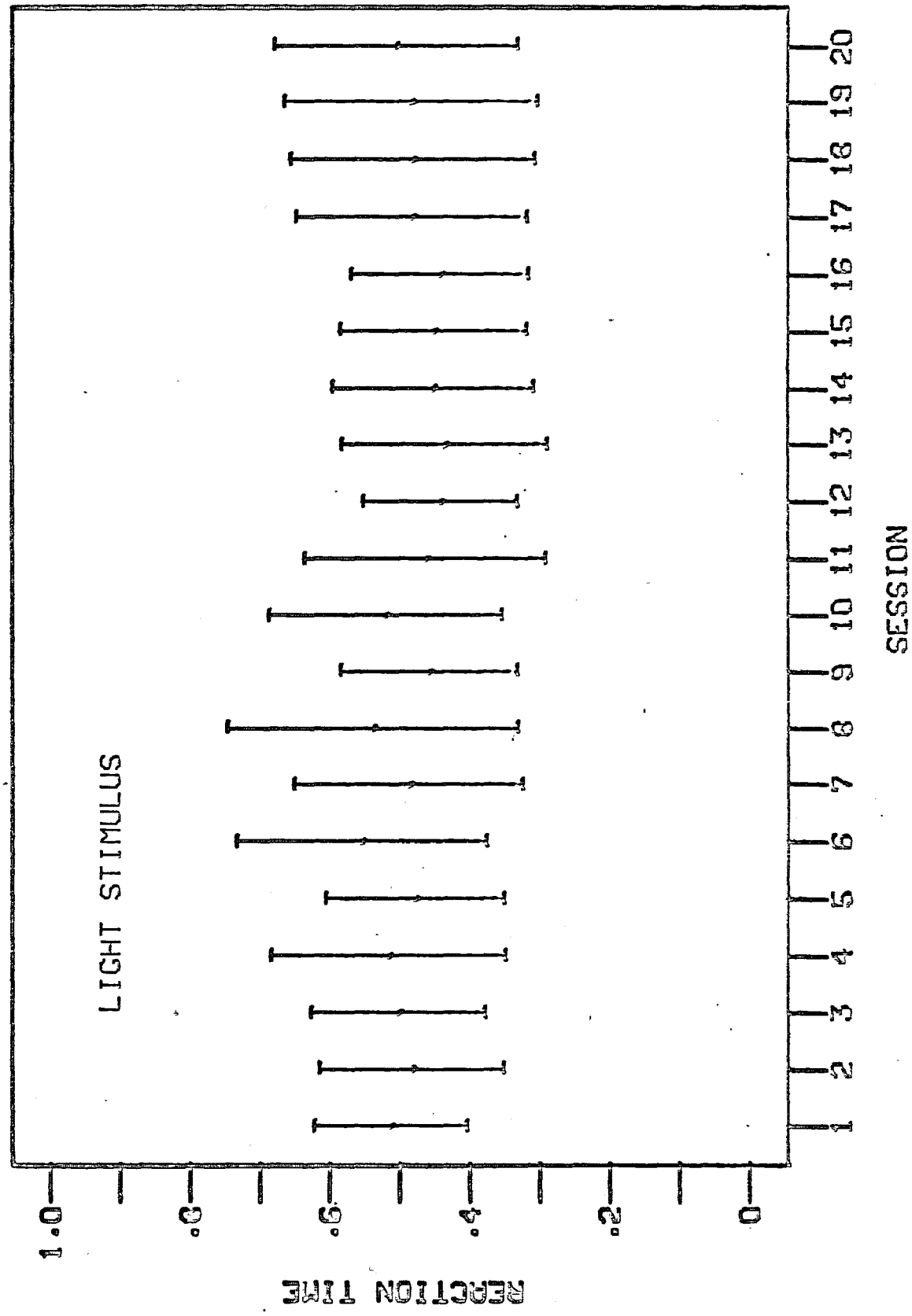


FIGURE 28

GROUP I

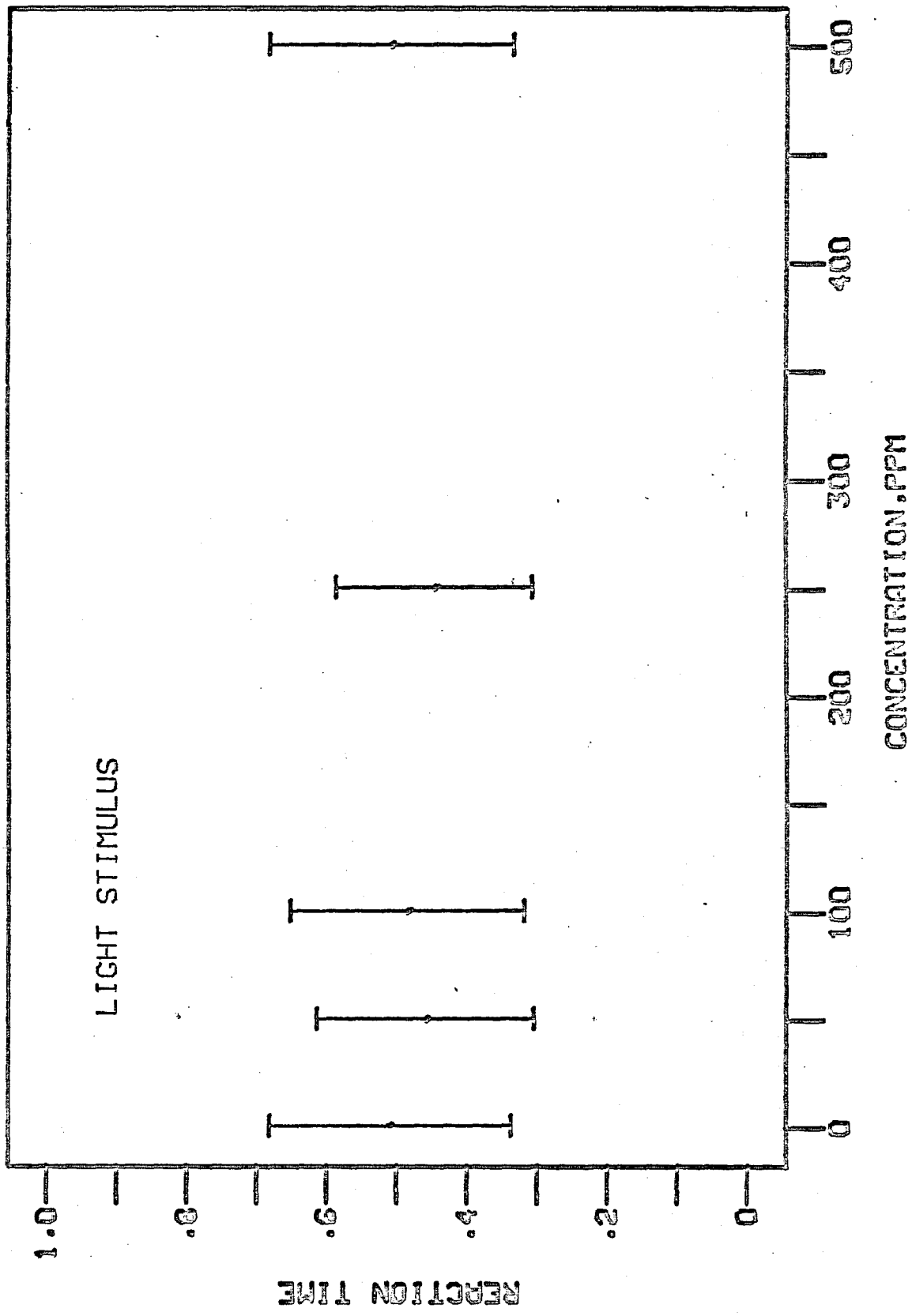


FIGURE 29

GROUP II

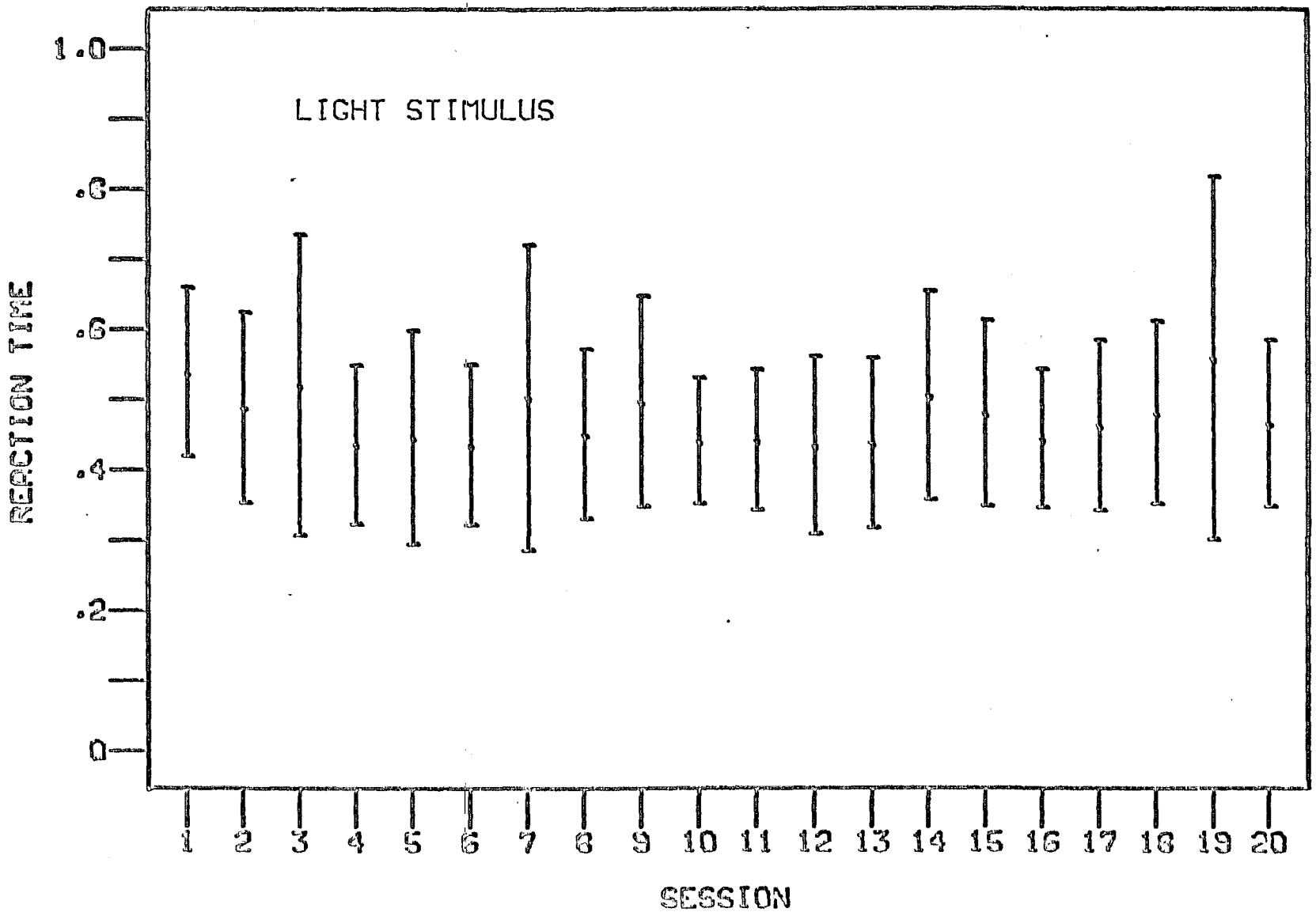


FIGURE 30

GROUP II

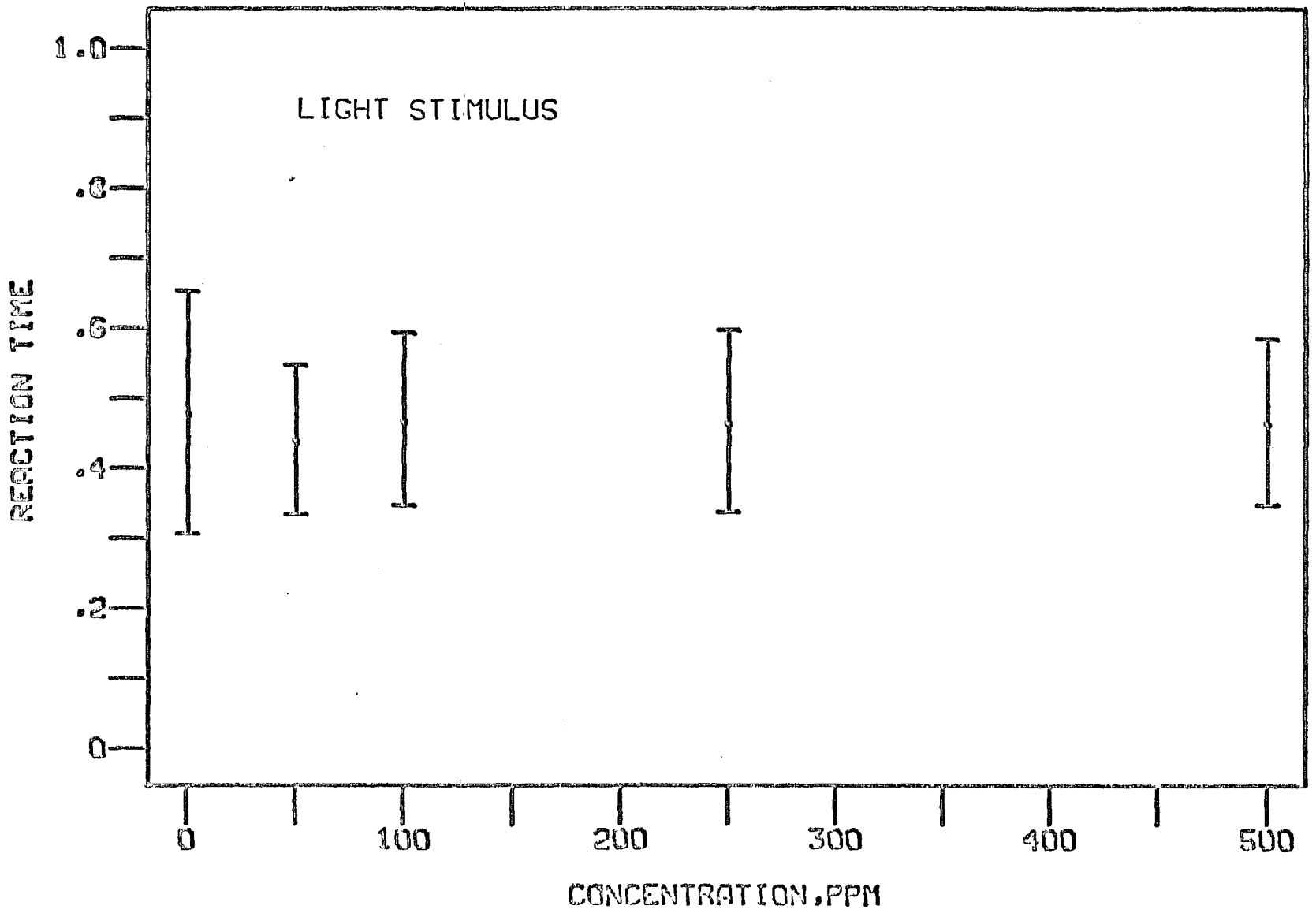


FIGURE 31

GROUP I

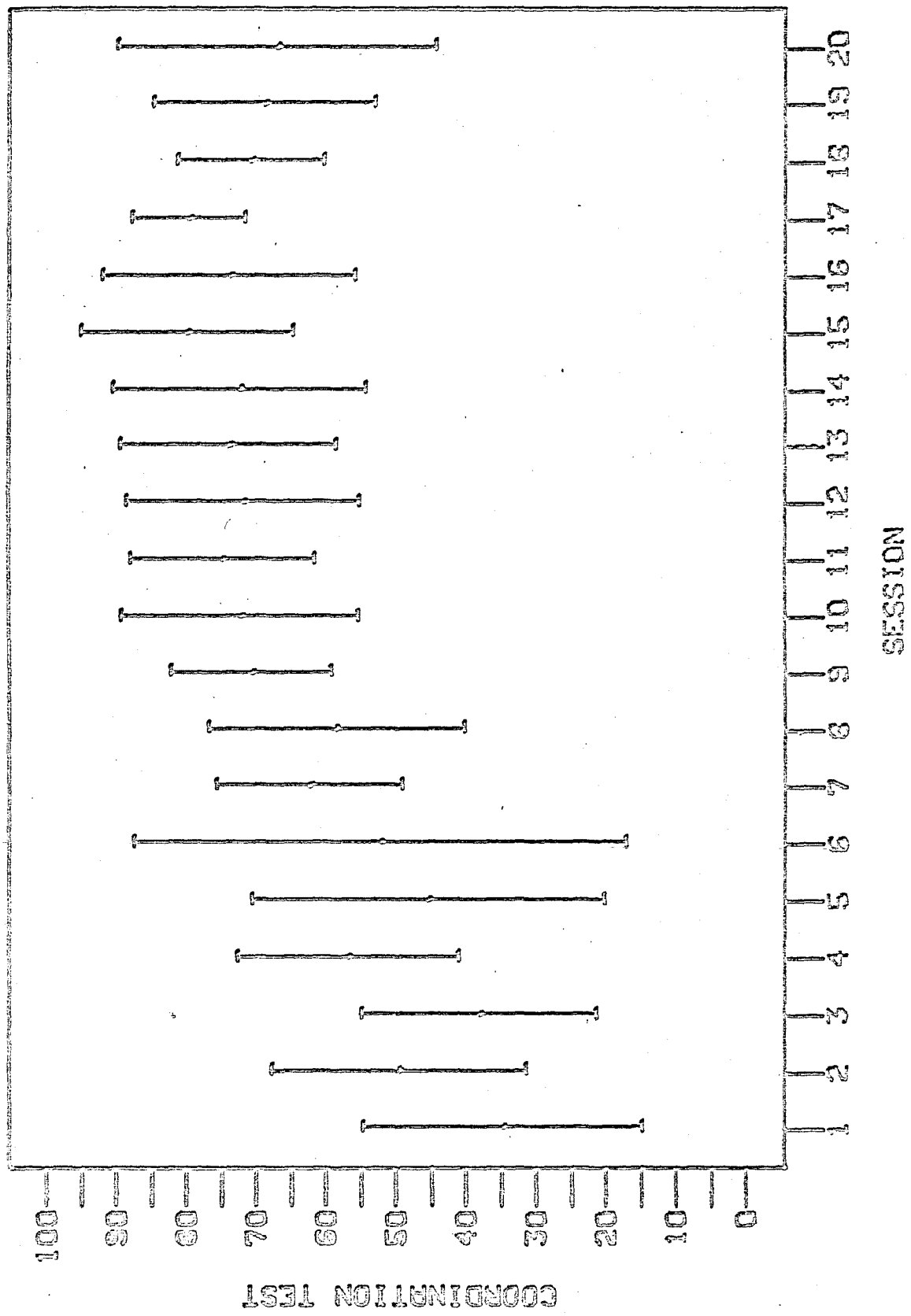
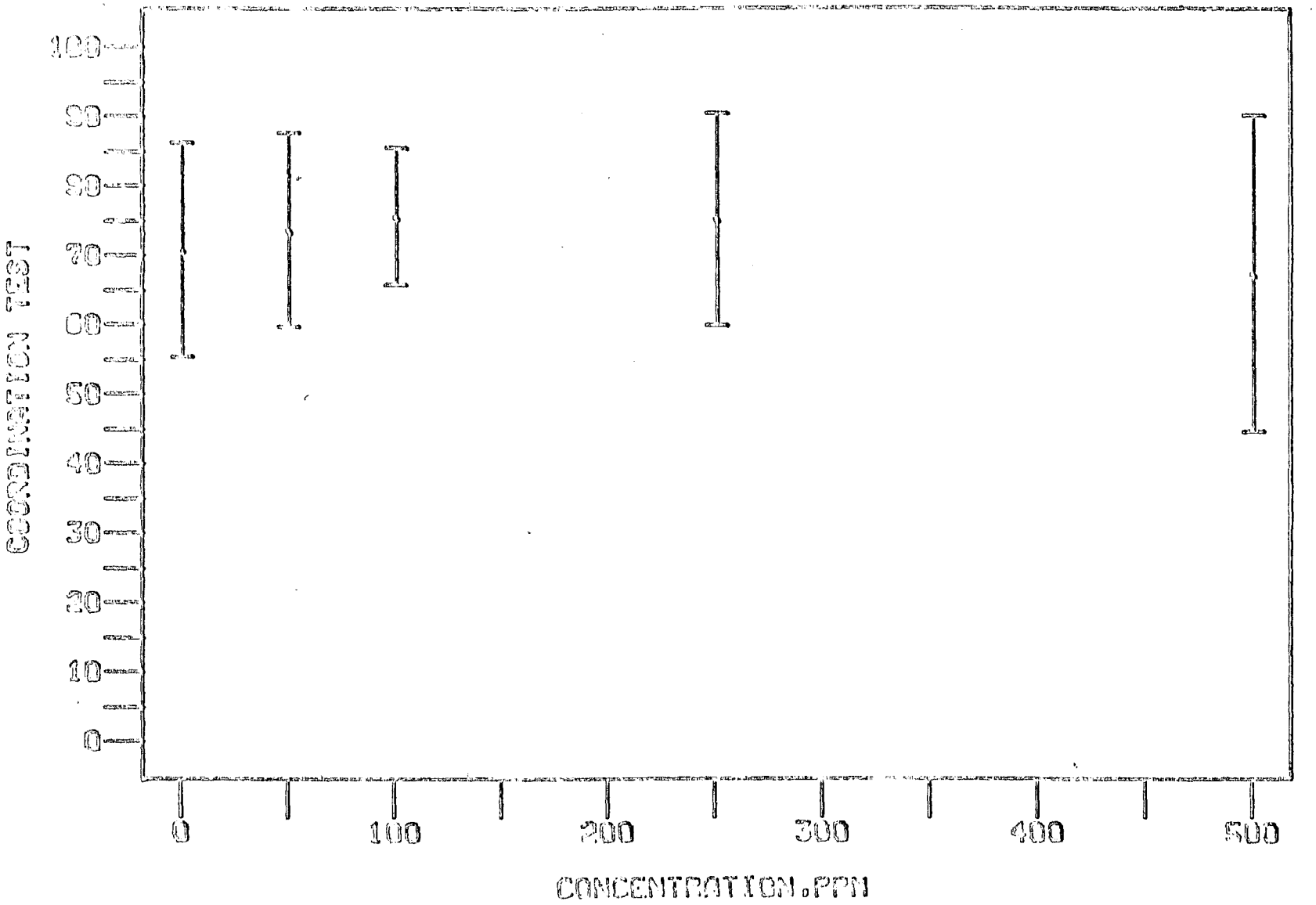


FIGURE 32

GROUP I



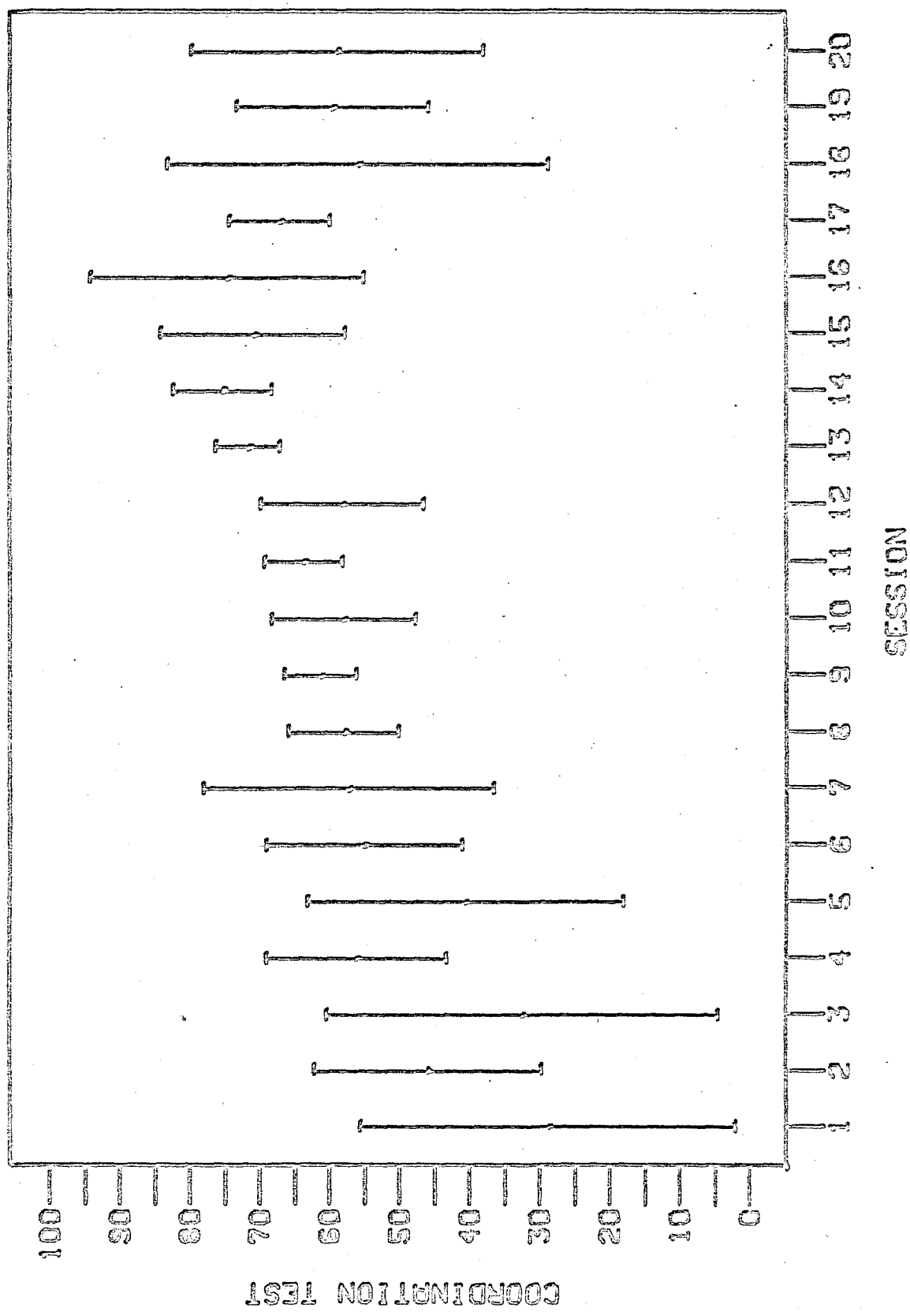


FIGURE 33

GROUP II

FIGURE 34

GROUP II

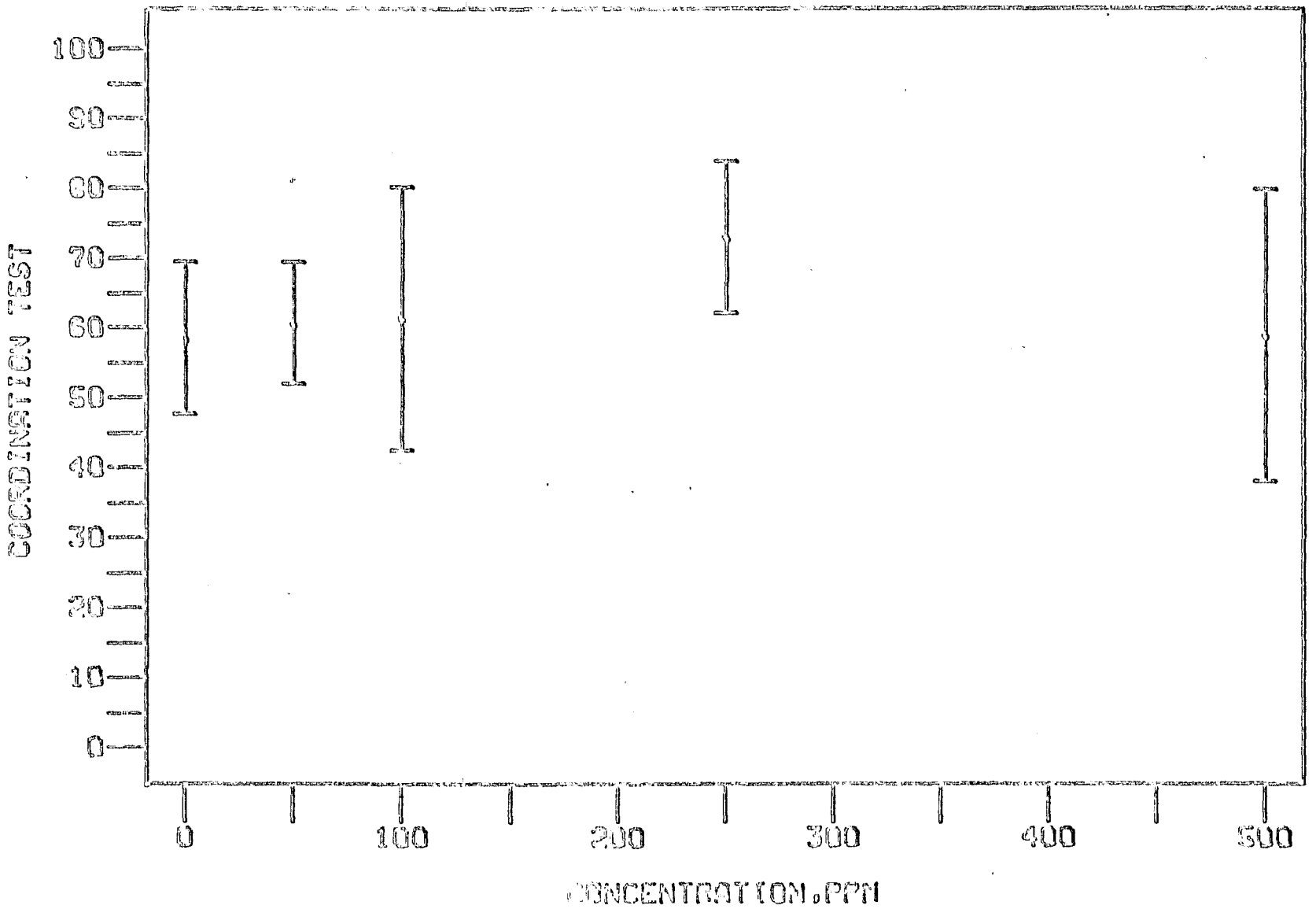


FIGURE 35

GROUP I

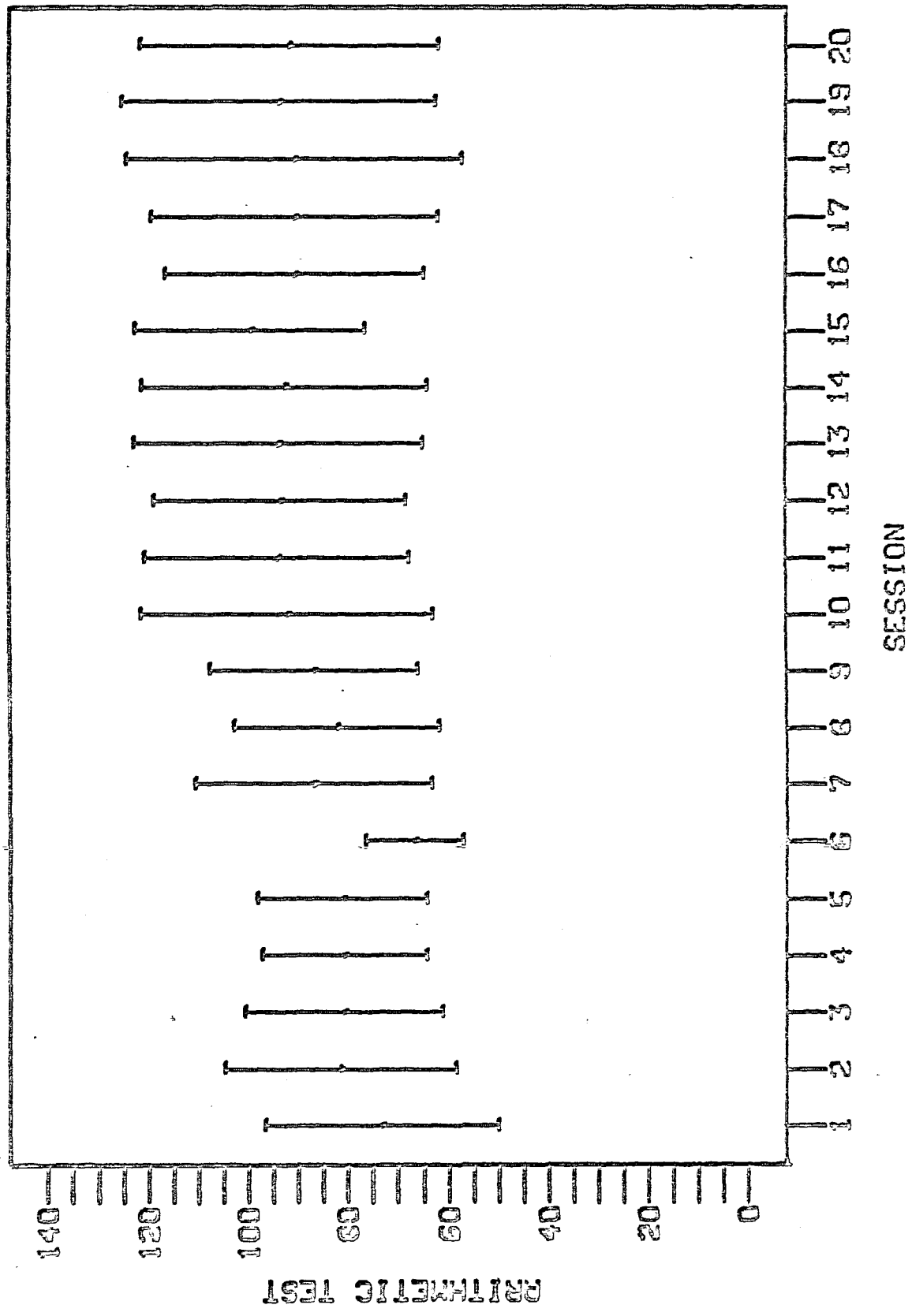


FIGURE 36

GROUP I

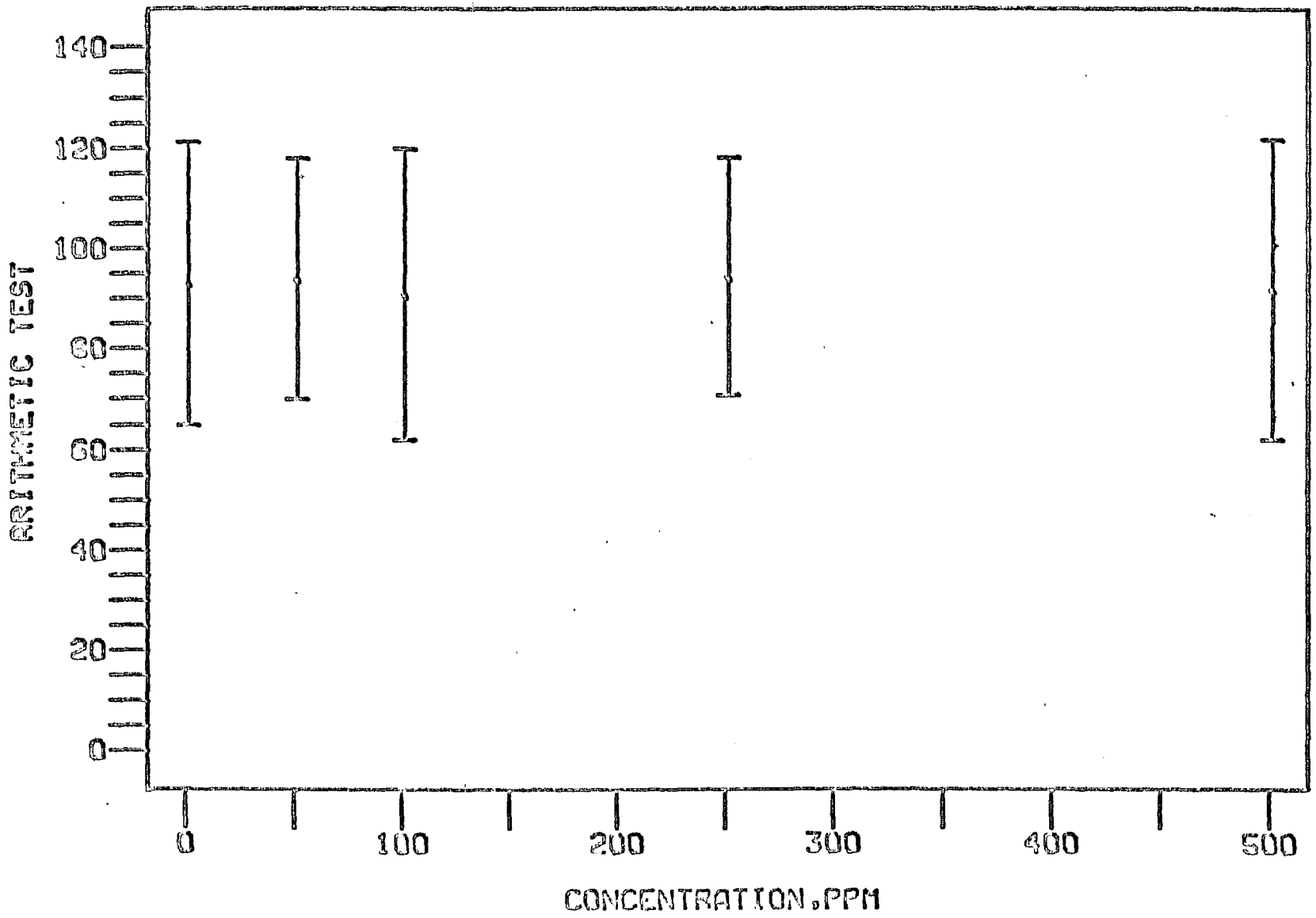


FIGURE 37

GROUP II

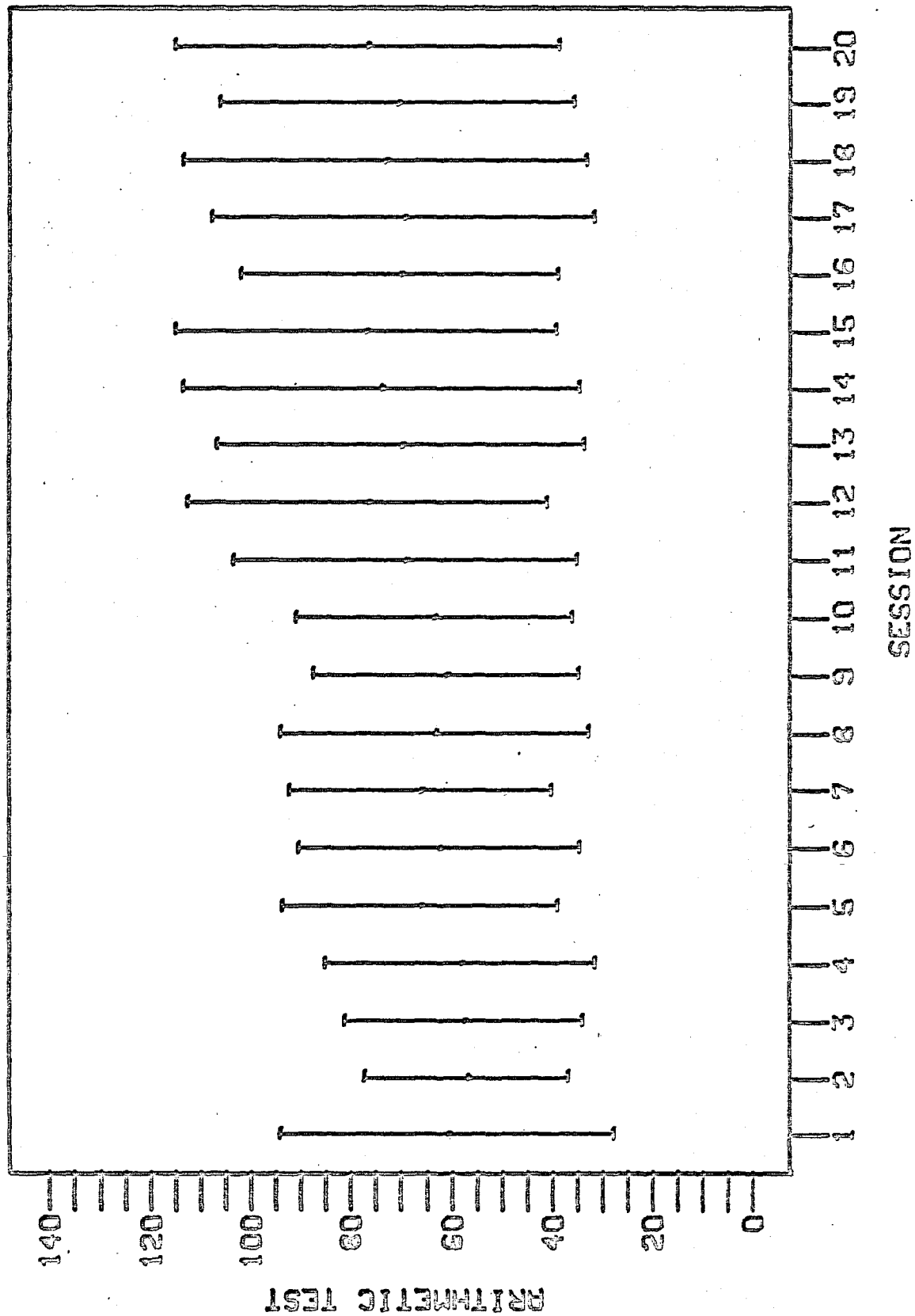


FIGURE 38

GROUP II

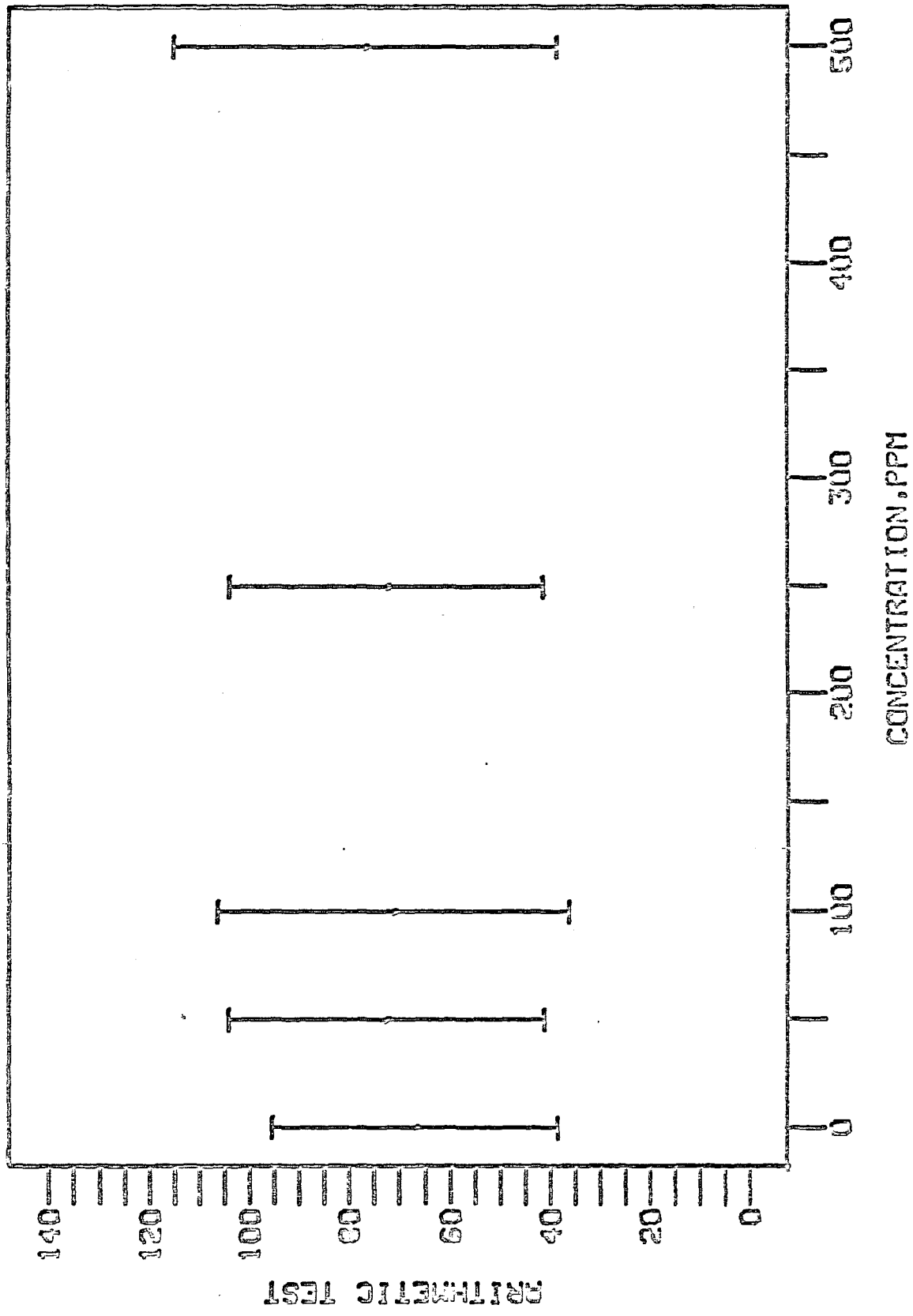


FIGURE 39

GROUP I

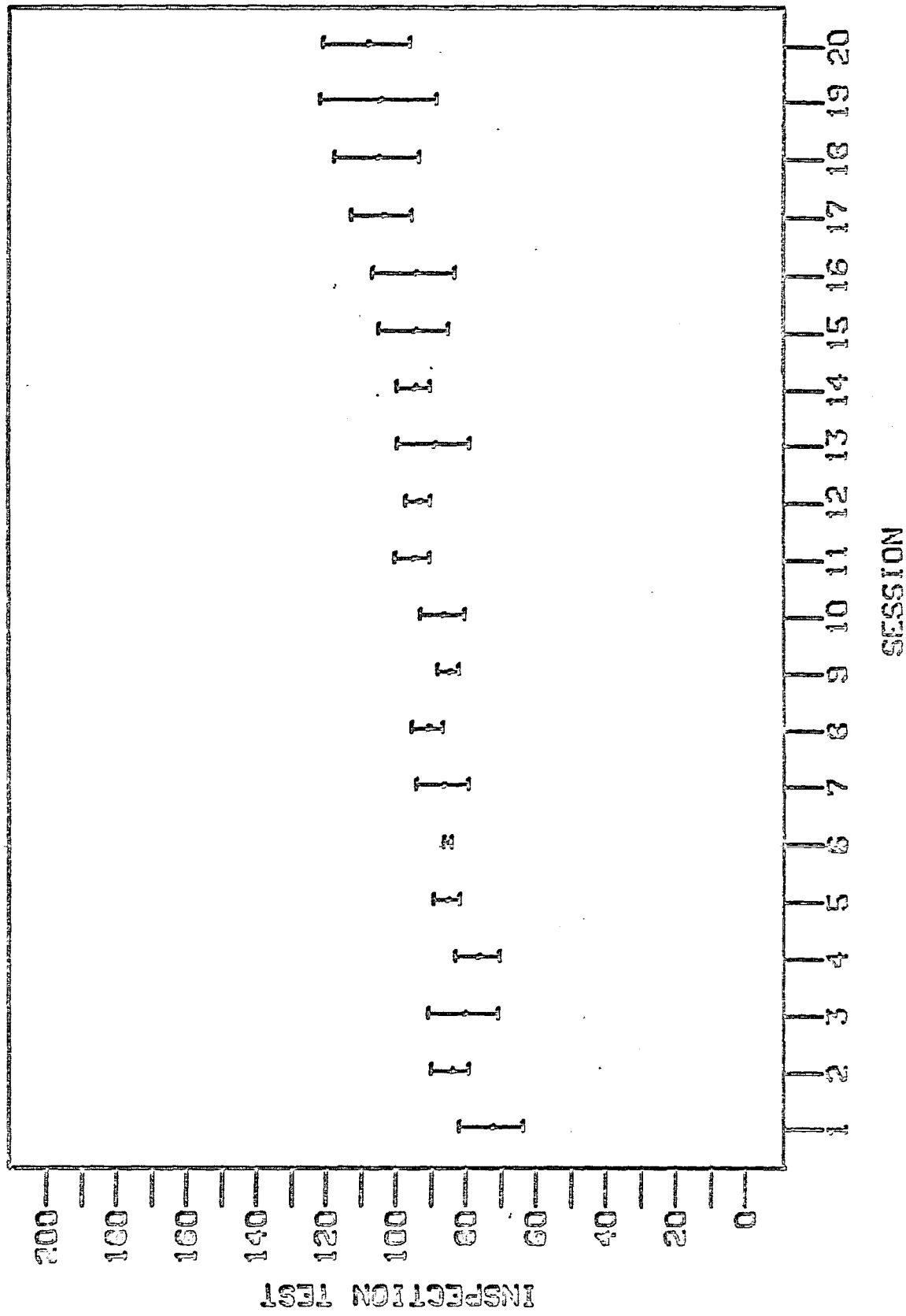


FIGURE 40

GROUP I

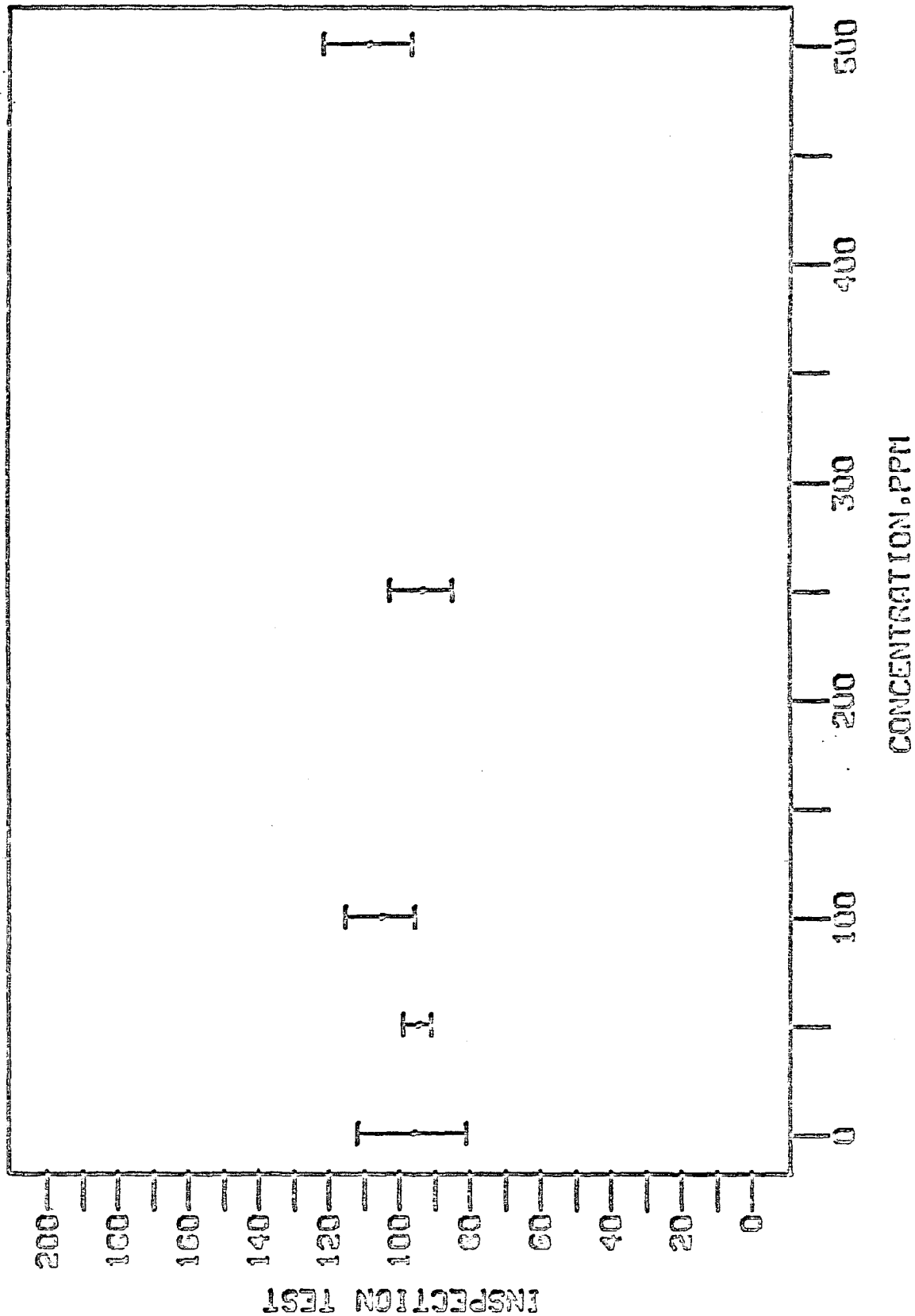


FIGURE 41

GROUP II

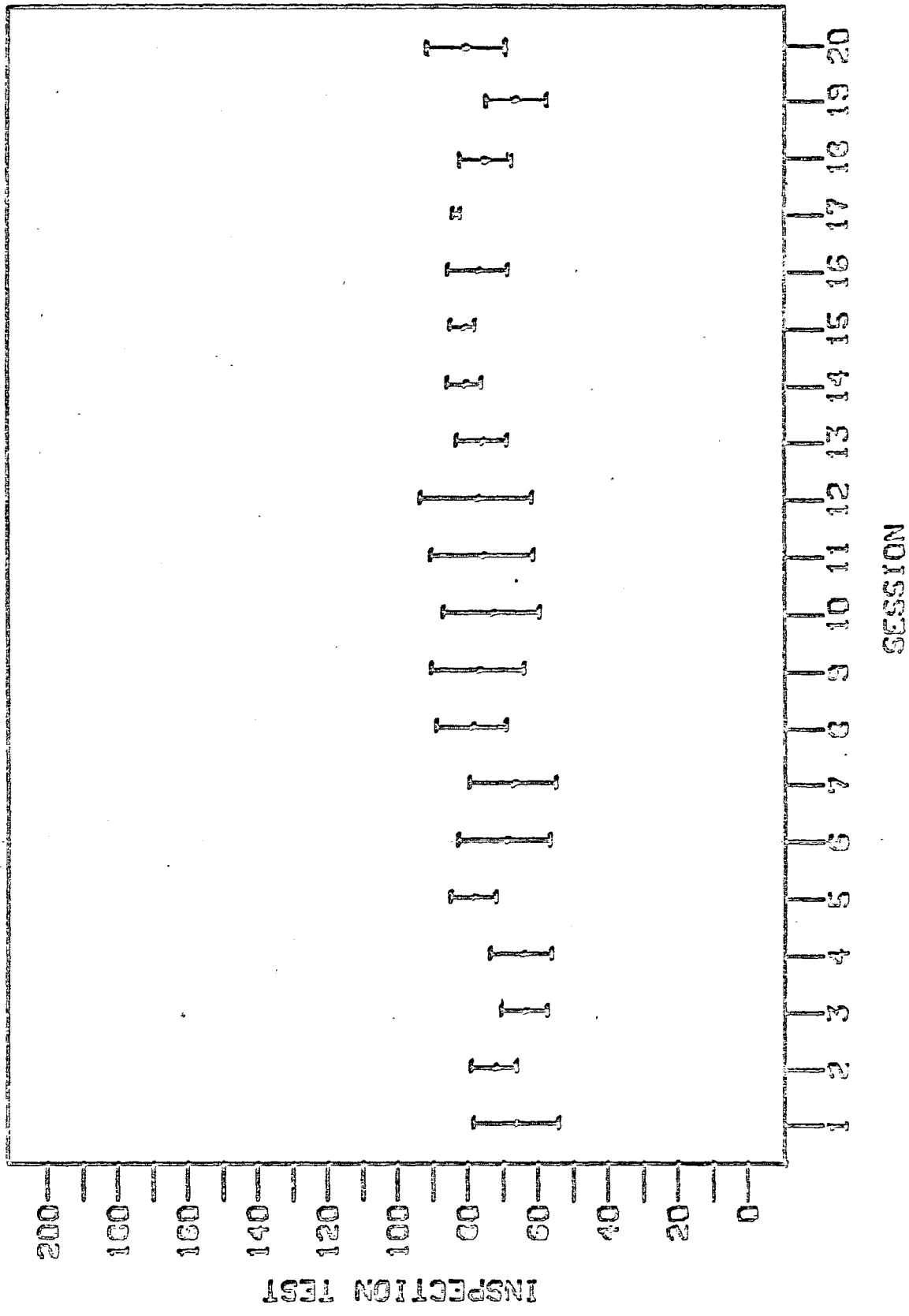


FIGURE 42

GROUP II

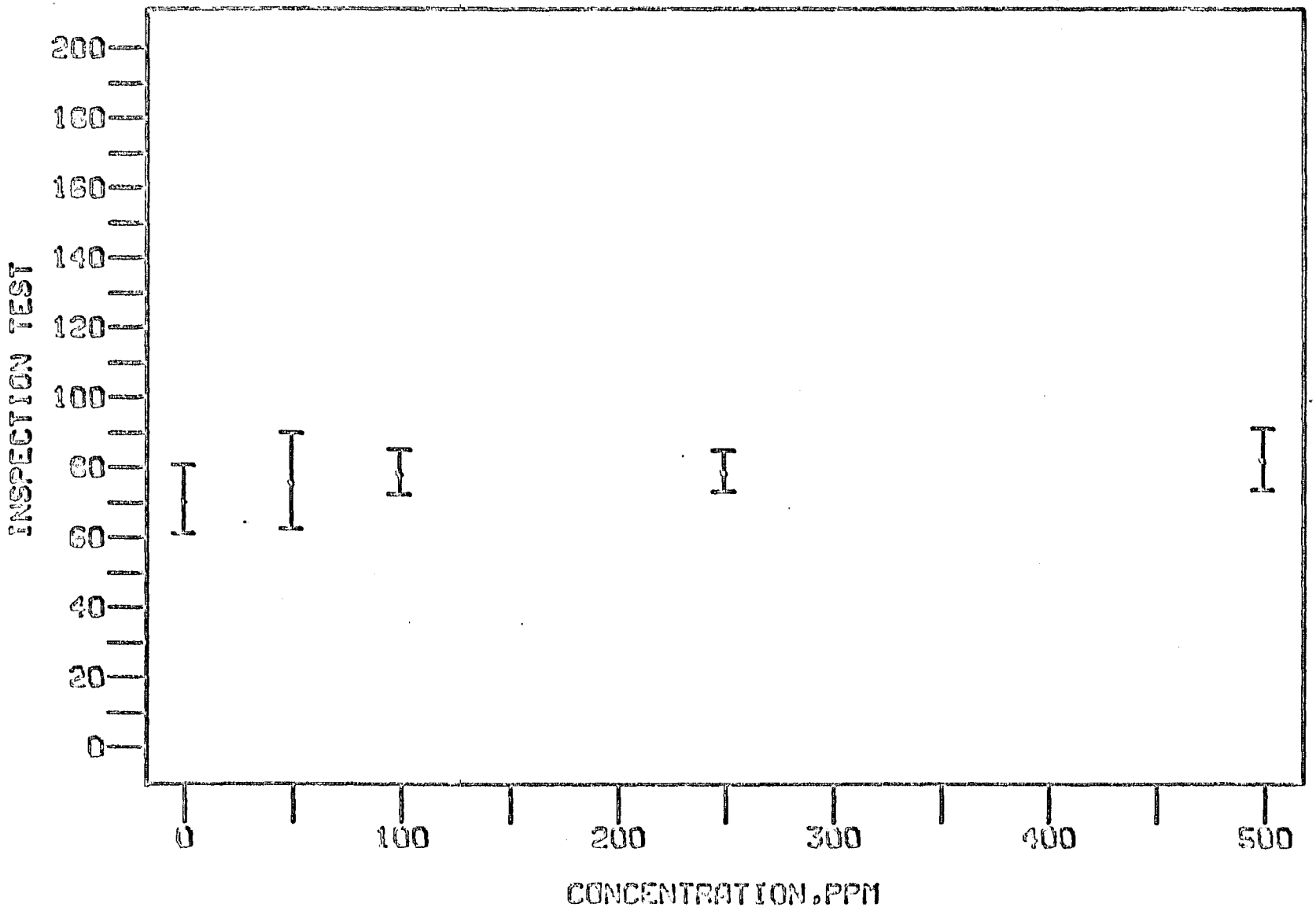


FIGURE 43

RELATIONSHIP OF POST-EXPOSURE CARBOXYHEMOGLOBIN LEVELS

TO MAGNITUDE OF 7½-HOUR EXPOSURE TO METHYLENE CHLORIDE

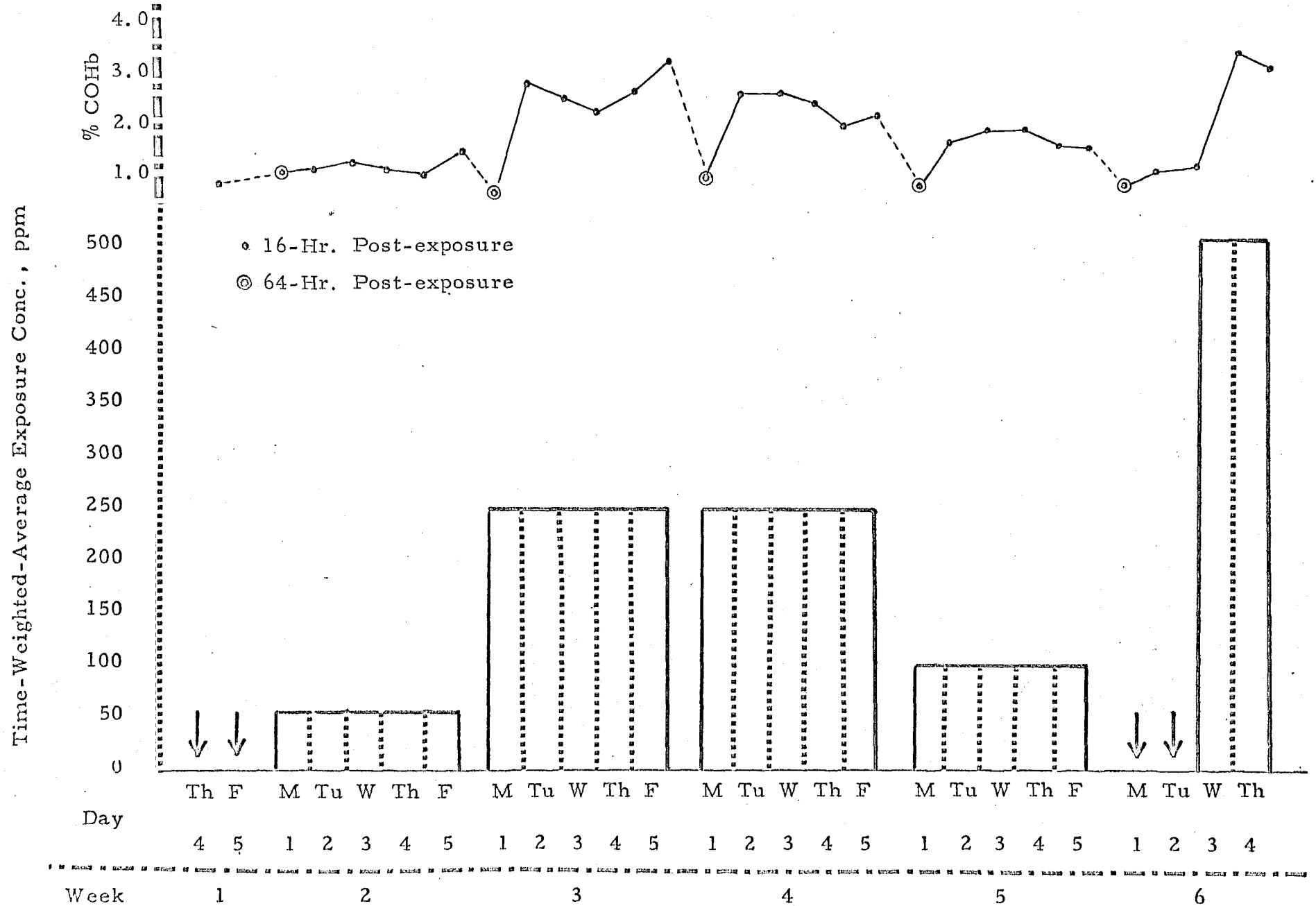


FIGURE 44

AVERAGE CARBOXYHEMOGLOBIN LEVELS OF NON-SMOKERS

EXPOSED TO THREE CONCENTRATIONS OF METHYLENE CHLORIDE

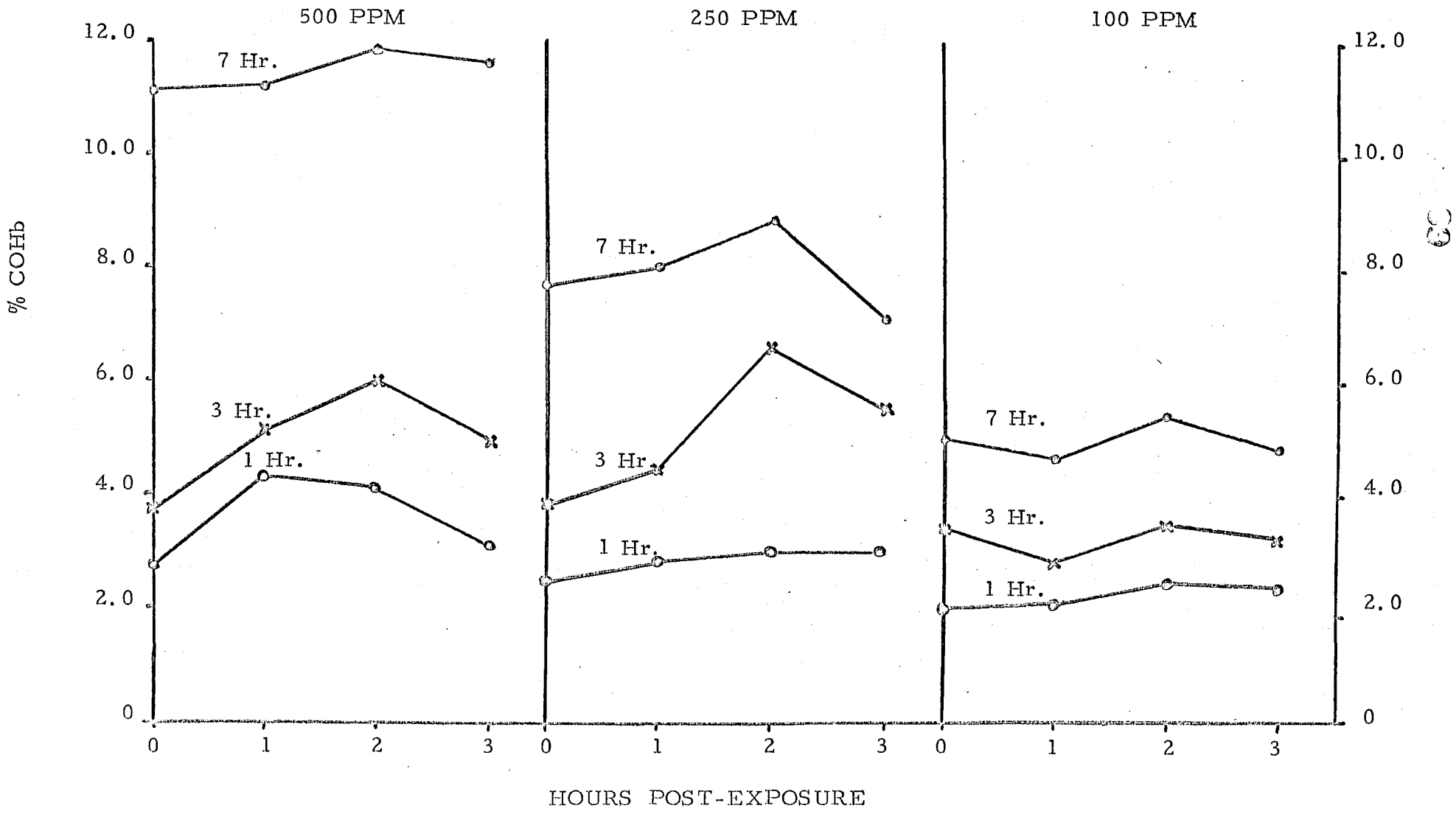


FIGURE 45

CARBOXYHEMOGLOBIN VALUES FROM A SUBJECT EXPOSED FOR THREE HOURS TO THREE CONCENTRATIONS OF METHYLENE CHLORIDE ON THREE SEPARATE DAYS

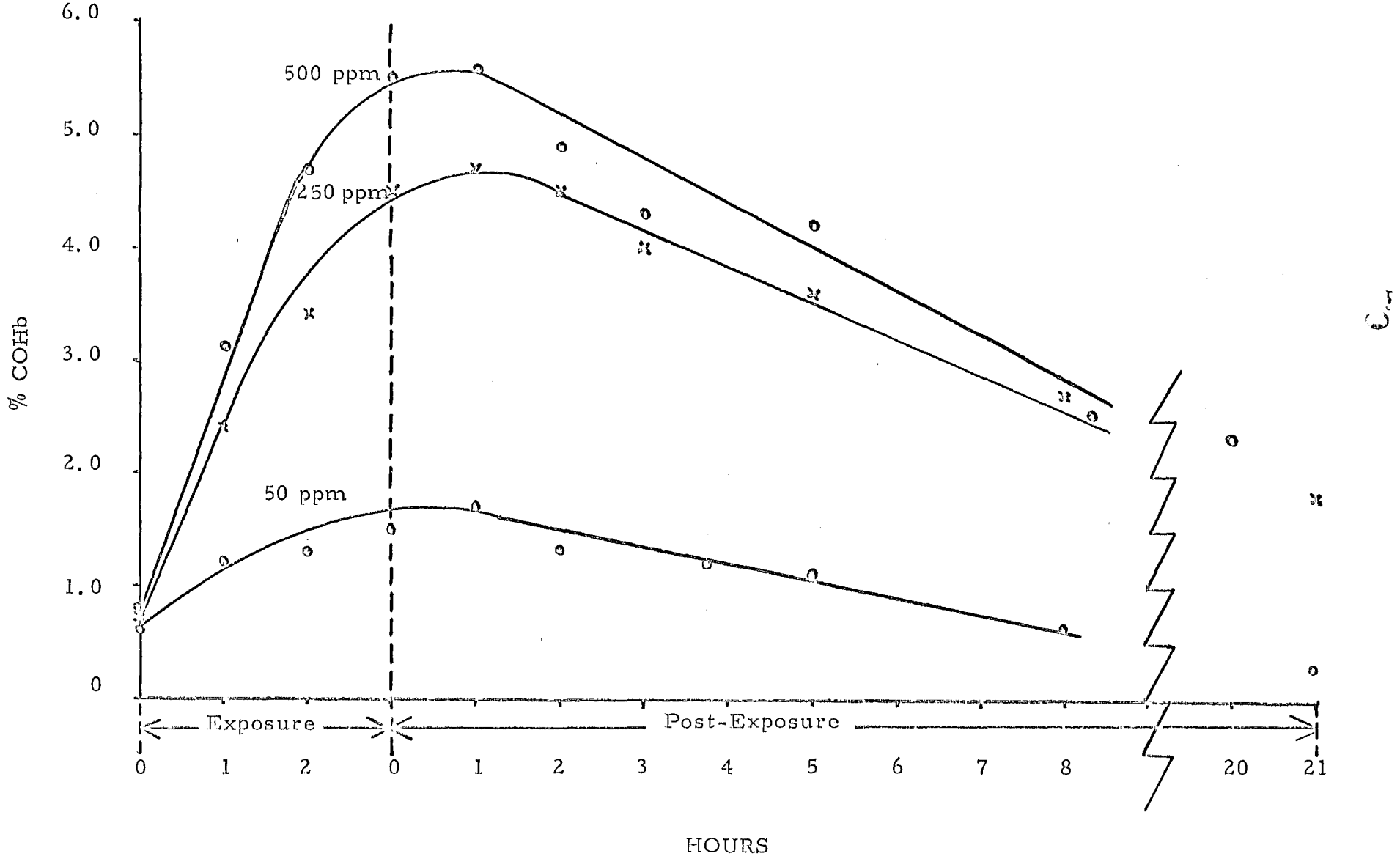


TABLE I

METHYLENE CHLORIDE

TIME-WEIGHTED-AVERAGE VAPOR CONCENTRATION

Week	Day of Week	Proposed Conc., ppm	7½-Hour GROUP I PPM	± S.D.	3-Hour GROUP II PPM	± S.D.	1-Hour GROUP III PPM	± S.D.	Subjects Absent
1	4	0	0		0		0		None
	5	0	0		0		0		None
2	1	50 Stable	49.8	2.3	49.6	2.4	47.4	3.0	None
	2		48.7	4.6	50.2	2.2	47.1	4.1	None
	3		50.0	2.6	49.9	1.6	49.6	2.2	None
	4		50.0	1.4	50.1	1.2	49.9	0.6	None
	5		50.1	2.1	49.2	1.9	50.3	2.3	1 - Gp. III
3	1	250 Stable	248.3	15.5	250.3	9.0	248.1	10.0	None
	2		249.9	8.6	251.4	9.0	251.7	9.9	None
	3		250.0	9.4	248.9	12.1	243.4	3.1	None
	4		250.0	7.6	249.9	7.0	251.8	9.3	None
	5		249.8	9.5	249.7	7.8	249.7	4.8	None
4	1	250 Fluctuating	249.2	50.3	249.2	52.3	248.7	47.0	None
	2		247.1	45.3	247.6	50.7	248.6	45.6	None
	3		250.1	52.2	250.9	50.9	254.2	52.8	None
	4		250.1	51.0	249.0	51.7	252.6	48.1	None
	5		250.1	51.2	250.3	47.1	247.8	49.1	None
5	1	100 Stable	99.7	4.1	99.4	2.2	101.9	4.3	None
	2		100.0	4.2	99.0	3.2	100.2	2.6	None
	3		100.1	9.4	100.4	6.4	103.4	9.4	None
	4		99.0	5.1	100.6	4.1	99.8	1.5	None
	5		100.5	5.8	100.2	3.0	101.2	4.2	None
6	1	0	0		0		0		None
	2		0		0		0		None
	3	500 Stable	499.5	15.9	494.2	10.7	495.3	30.1	1 - Gp. I
	4		497.1	11.7	494.1	9.5	498.5	8.4	None

TABLE II

BLOOD COHb % SATURATION[a] OF HUMANS EXPOSED DAILY FOR 7½ HOURS TO CH_2Cl_2

	DAY 1					DAY 2	DAY 3					DAY 4	DAY 5			
	pre-exp.	7½ hr. exp.	1 hr. post exp.	2 hr. post exp.	3 hr. post exp.	pre-exp.	pre-exp.	7½ hr. exp.	1 hr. post exp.	2 hr. post exp.	3 hr. post exp.	pre-exp.	pre-exp.	7½ hr. exp.	1 hr. post exp.	16 hr. post exp.
WEEK 2 (50 ppm)																
3 non-smokers	0.9[b]	2.4	2.3	NA	NA	1.0	1.1	2.5[b]	2.9	NA	NA	1.0	1.0	2.5	2.1	1.4
1 lt. smoker	1.4	2.9	2.5	NA	NA	1.3	1.5	3.1	2.7	NA	NA	1.3	1.2	2.8	2.5	1.9
WEEK 3 (250 ppm)																
3 non-smokers	0.6	9.4	9.2	10.3[b]	10.0[b]	2.7	2.4	7.4	7.8	8.9	7.0	2.2	2.5	9.2	9.6	3.1
1 lt. smoker	0.7	7.4	8.5	10.3[b]	9.6	3.0	2.7	8.7	8.6	7.9	7.1	2.8	3.1	10.1	10.6	4.5
WEEK 4 (250 ppm)																
3 non-smokers	0.9	8.9	8.5	7.6	8.9	2.5	2.5	8.3	8.2	8.7[b]	7.3	2.3	1.9	9.3	9.5	2.1
1 lt. smoker	0.8	7.5	7.6	NA	NA	3.0	3.3	9.4	8.9	7.5	10.1	3.5	2.7	9.9	9.4	3.4
WEEK 5 (100 ppm)																
3 non-smokers	0.8	5.0	4.9	6.1[b]	5.9[b]	1.6	1.9	5.0	4.7	5.4	4.8	1.9	1.6	5.2	5.7	1.6
1 lt. smoker	1.5	5.3	5.0	7.1	5.8	1.9	2.6	6.1	5.3	6.4	-	2.1	2.5	5.3	6.0	3.1
WEEK 6 (0 & 500 ppm)																
3 non-smokers	0.8	1.0[b]	0.7	NA	NA	1.1	1.2[b]	10.7[b]	11.4[b]	11.7[b]	11.4[b]	3.4[b]	3.1	NO EXPOSURE		
1 lt. smoker	1.5	1.1	1.3	NA	NA	1.6	1.4	11.9	11.0	12.2	12.1	4.2	5.3	NO EXPOSURE		

[a] All pre-exposure, 7½ hr. exposure and 1 hr. post-exposure values represent blood analyses by CO-Oximeter. 2 hr. and 3 hr. post-exposure values calculated from CO concentration by G. C. in breath samples.

[b] Average of values from 2 non-smokers. Other values are averaged from 3 non-smokers.

NA Value not available.

TABLE III

BLOOD COHb % SATURATION OF HUMANS EXPOSED DAILY FOR 3 HOURS TO CH_2Cl_2

	DAY 1					DAY 2	DAY 3					DAY 4	DAY 5			
	pre-exp.	3 hr. exp.	1 hr. post-exp.	2 hr. post-exp.	3 hr. post-exp.	pre-exp.	pre-exp.	3 hr. exp.	1 hr. post-exp.	2 hr. post-exp.	3 hr. post-exp.	pre-exp.	pre-exp.	3 hr. exp.	1 hr. post-exp.	21 hr. post-exp.
WEEK 2 (50 ppm)																
non-smoker	0.8	1.6	1.2	NA	NA	0.5	0.8	NA	1.4	NA	NA	1.7[a]	0.6	3.0[a]	1.2	1.7[a]
N-S Garageman	1.2	1.9	1.5	NA	NA	1.0	0.9	1.2	1.2	NA	NA	1.3	0.8	1.4	1.3	2.1
heavy smoker	7.0	5.5	5.0	NA	NA	6.3	6.5	5.7	4.7	NA	NA	6.1	6.2	5.1	4.2	7.4
WEEK 3 (250 ppm)																
non-smoker	0.9	5.8[a]	3.6	5.3[a]	5.3[a]	2.2[a]	NA	NA	4.6	6.8[a]	6.6[a]	2.5[a]	1.1	8.3[a]	5.0	NA
N-S Garageman	0.7	3.0	3.3	3.7[a]	NA	2.0	1.7	3.8	4.4	6.4[a]	4.4[a]	1.9	1.9	4.1	4.8	2.2
heavy smoker	7.6	8.6	8.0	11.9[a]	11.9[a]	8.0	6.1	8.6	8.1	11.0[a]	6.0[a]	9.0	8.7	9.5	9.9	7.8
WEEK 4 (250 ppm)																
non-smoker	0.8	7.1[a]	4.3	4.9[a]	5.3[a]	1.9[a]	NA	5.3[a]	4.0	5.1[a]	NA	NA	1.2	NA	3.9	NA
N-S Garageman	1.3	3.4	4.0	4.4[a]	3.0[a]	2.2	1.9	3.4	4.3	4.9[a]	NA	2.0	1.2	3.2	3.6	1.0
heavy smoker	8.6	8.9	8.7	6.8[a]	9.4[a]	9.0	8.2	8.2	8.2	8.7[a]	10.6[a]	8.2	7.5	8.3	8.6	7.7
WEEK 5 (100 ppm)																
non-smoker	0.5	3.7[a]	NA	3.5[a]	3.0[a]	NA	NA	3.9[a]	2.8	3.2[a]	3.2[a]	NA	1.0	2.0[a]	3.0	1.7[a]
N-S Garageman	1.7	2.3	2.2	3.2[a]	NA	1.4	2.3	2.7	2.8	3.7[a]	6.0[a]	3.3	1.0	1.7	2.5	0.9
heavy smoker	7.9	7.3	7.0	9.4[a]	6.6[a]	7.0	9.3	8.0	7.1	9.8[a]	9.8[a]	6.3	7.0	6.9	7.1	8.5
WEEK 6 (0 & 500 ppm)																
non-smoker	1.0	NA	1.2	0.7[a]	1.5[a]	NA	NA	NA	5.6	6.0[a]	7.3[a]	2.2	1.6 (24 hr. post)			
N-S Garageman	1.2	0.9	0.9	2.2[a]	2.5[a]	1.6	1.2	3.7	4.6	6.0[a]	NA	2.5	2.7 (19 hr. post)		NO EXPOSURE	
heavy smoker	6.2	5.1	4.9	6.0[a]	4.6[a]	7.3	7.0	8.6	8.6	9.2[a]	11.9[a]	7.1	8.5 (24 hr. post)			

NA Value Not Available

[a] Value determined indirectly from CO concentration in breath. Other values obtained directly on CO-Oximeter.

TABLE IV

BLOOD COHb % SATURATION OF HUMANS EXPOSED DAILY FOR 1 HR. TO CH_2Cl_2

	DAY 1					DAY 2	DAY 3					DAY 4	DAY 5			
	pre-exp.	1 hr. exp.	1 hr. post-exp.	2 hr. post-exp.	3 hr. post-exp.	pre-exp.	pre-exp.	1 hr. exp.	1 hr. post-exp.	2 hr. post-exp.	3 hr. post exp.	pre-exp.	pre-exp.	1 hr. exp.	1 hr. post-exp.	23 hr. post-exp.
WEEK 2 (50 ppm)																
non-smoker	0.9	1.0	0.9	NA	NA	0.8	0.8	1.1	1.3	NA	NA	1.0	0.6	NA	NA	NA
N-S ind. wrkr	1.3	1.4	1.3	NA	NA	2.1	2.2	2.1	1.9	NA	NA	2.5	2.0	1.9	1.6	1.9
lt. smoker	2.9	2.7	2.1	NA	NA	4.2	2.5	2.3	2.3	NA	NA	2.6	2.7	2.2	2.1	2.4
WEEK 3 (250 ppm)																
non-smoker	1.1	2.3	2.8	3.7[a]	3.2[a]	1.7	1.2	2.0	2.4	3.2[a]	3.2[a]	1.7	1.4	2.9	3.0	1.9
N-S ind. wrkr	1.2	2.0	2.1	NA	1.2[a]	2.3	2.2	3.2	3.2	2.7[a]	NA	2.7	2.1	3.2	3.4	1.9
lt. smoker	2.1	2.7	3.1	3.7[a]	5.3[a]	3.0	1.5	2.3	2.8	5.1[a]	NA	2.3	3.2	4.0	4.6	4.6
WEEK 4 (250 ppm)																
non-smoker	0.8	1.6	2.0	3.2[a]	2.5[a]	1.5	1.6	2.1	2.5	NA	2.7[a]	1.1	1.1	1.7	2.0	1.2
N-S ind. wrkr	2.1	2.4	2.8	2.5[a]	2.7[a]	2.4	2.0	2.6	2.6	1.7[a]	3.2[a]	2.4	1.3	2.2	2.6	1.8
lt. smoker	2.4	2.7	3.3	3.7[a]	3.5[a]	NA	2.6	3.3	3.6	5.8[a]	4.9[a]	2.9	2.9	4.0	4.1	2.6
WEEK 5 (100 ppm)																
non-smoker	0.8	1.2	1.3	1.7[a]	2.0[a]	1.4	1.1	1.7	1.8	2.7[a]	2.0[a]	1.0	2.5	3.2	2.9	1.0
N-S ind. wrkr	1.8	2.0	1.7	2.7[a]	1.5[a]	2.0	1.9	2.3	2.3	2.2[a]	2.7[a]	2.1	1.4	1.8	1.8	0.9
lt. smoker	1.1	2.0	1.9	2.2[a]	1.7[a]	3.0	2.3	2.7	2.8	3.0[a]	3.0[a]	2.7	2.9	3.5	3.3	3.0
WEEK 6 (0 & 500 ppm)																
non-smoker	0.9	0.7	0.9	1.2[a]	0.9[a]	0.9	1.6	2.6	4.4	4.9[a]	3.5[a]	1.3	2.2 (23 hr. post)			
N-S ind. wrkr	2.3	1.9	1.8	1.7[a]	1.5[a]	2.2	2.1	2.8	4.2	3.2[a]	2.7[a]	2.8	2.1 (27 hr. post) NO EXPOSURE			
lt. smoker	2.0	1.6	1.5	6.6[a]	2.2[a]	2.2	NA	4.1	6.1	5.8[a]	5.8[a]	2.5	4.8 (25 hr. post)			

NA Value Not Available

[a] Value determined indirectly from CO concentration in breath. Other values obtained directly in CO-Oximeter.

TABLE V

ALERTNESS TEST

Paired t Values For Comparison of Control Vs. Exposure Test Scores

Group I df 3

Concentration ppm	% Correct	RXT	
50	.304	.958	Monday
100	-.642	2.312	
250	-.240	2.143	
250	-.058	3.181*	
50	-2.232	1.944	Wednesday
100	1.084	2.913	
250	- .031	1.720	
250	2.733	1.614	
50	.614	2.988	Friday
100	-.751	7.340 **	
250	-.284	2.409	
250	-.713	2.693	

* Significant at P = .05

** Significant at P = .01

TABLE VI

ALERTNESS TEST

Paired t Values for Comparison of Control Vs. Exposure Test Scores
Group II df 2

Concentration ppm	% Correct	RXT	
50	.878	- .210	Monday
100	.773	-1.135	
250	2.352	-1.262	
250	2.224	- .849	
50	1.051	.485	Wednesday
100	1.995	-1.459	
250	1.560	-1.403	
250	2.728	- .480	
50	15.254 **	- .756	Friday
100	.578	.524	
250	2.200	- .922	
250	9.430 *	-1.248	

* Significant at P = .05
** Significant at P = .01

TABLE VII

CORRELATION OF BEHAVIORAL TEST SESSION NUMBER AND CH_2Cl_2 EXPOSURE

Session No.	Day of Week	CH_2Cl_2 Concentration, PPM
1 - 9		0 (training)
10	4	0
11	2	50
12	4	50
13	2	250
14	4	250
15	2	250 (fluctuating)
16	4	250 (fluctuating)
17	2	100
18	4	100
19	2	0
20	4	500

TABLE VIII

PAIRED t VALUES FOR COMPARISON OF
CONTROL VERSUS EXPOSURE TEST SCORES

GROUP I

Test		T - Th 50 ppm t	df	T - Th 100 ppm t	df	T - Th 250 ppm t	df	Th 500 ppm t	df
Marquette Test:	E/S	.687	3	.052	3	.01	3	.797	3
Sound Stimulus	|E-S|	- .181	3	-1.000	3	- .747	3	.399	3
	RxT	.494	3	- .811	3	- .363	3	- .487	3
Light Stimulus	E/S	-2.114	3	-2.112	3	-2.118	3	-1.108	3
	|E-S|	2.277	3	.352	3	1.548	3	- .966	3
	RxT	4.852*	3	3.326*	3	3.770*	3	.046	3
10 Second Estimation		.321	3	- .594	3	- .626	3	.813	3
30 Second Estimation		2.961	3	-3.212	3	-1.156	3	.857	3
Arithmetic Test		- .333	3	.528	3	- .08	3	.233	3
Coordination Test		- .775	3	- .724	3	-1.743	3	.407	3
Inspection Test		.403	3	-2.772	3	.997	3	-9.101	3 **

* Significant at P = .05
** Significant at P = .01

TABLE IX

PAIRED t VALUES FOR COMPARISON OF
CONTROL VERSUS EXPOSURE TEST SCORES

GROUP II

Test		T - Th 50 ppm t	df	T - Th 100 ppm t	df	T - Th 250 ppm t	df	Th 500 ppm t	df
Marquette Test:	E/S	.477	2	- .045	2	- .02	2	- .053	2
Sound Stimulus	|E-S|	1.239	2	1.852	2	- .024	2	- .905	2
	RxT	.59	2	-2.031	2	-1.292	2	.308	2
Light Stimulus	E/S	.407	2	- .832	2	- .915	2	1.039	2
	|E-S|	4.413	2	2.240	2	.855	2	1.993	2
	RxT	1.403	2	.301	2	.752	2	- .571	2
10 Second Estimation		1.348	2	- .598	2	1.400	2	-1.068	2
30 Second Estimation		.699	2	- .677	2	.115	2	- .643	2
Arithmetic Test		-1.960	2	- .819	2	-1.957	2	-2.298	2
Coordination Test		- .577	2	- .839	2	-10.777**	2	- .023	2
Inspection Test		- .871	2	-2.644	2	-1.394	2	-3.120	2

* Significant at P = .05
** Significant at P = .01

TABLE X

DIFFERENCE IN COHb % SATURATION BEFORE AND AFTER

$7\frac{1}{2}$ HOURS EXPOSURE TO CH_2Cl_2

Exposure Conc., ppm	Δ[a] COHb % Day 1 N.S.[b]	Day 1 Lt.S.[c]	Day 3 N.S.[b]	Day 3 Lt.S.[c]	Day 5 N.S.[b]	Day 5 Lt.S.[c]
0	0.2	-0.2				
50	1.5	1.5	1.8	1.6	1.5	1.6
100	4.2	4.5	3.1	3.5	3.6	3.5
250	8.8	7.8	5.4	6.0	7.1	7.5
250[d]	8.0	7.2	5.8	6.1	7.6	7.2
500	10.2	10.5				

[a] Values all positive unless noted. Pre-exposure value subtracted from higher of $7\frac{1}{2}$-hour exposure or 1-hour post-exposure value.

[b] Non-Smokers, average of three. Average pre-exposure values ranged from 0.6 to 2.5

[c] Light Smoker. Pre-exposure values ranged from 0.7 to 3.3.

[d] Exposures to fluctuating concentrations with daily time-weighted-averages of 250 ppm.

TABLE XI

DIFFERENCE IN COHb % SATURATION BEFORE AND AFTER 3 HOURS EXPOSURE TO CH_2Cl_2

Exposure Conc., ppm	Δ[a] COHb % Day 1 N.S.[b]	Day 1 Garageman[c]	Day 1 H.S.[d]	Day 3 N.S.[b]	Day 3 Garageman[c]	Day 3 H.S.[d]	Day 5 N.S.[b]	Day 5 Garageman[c]	Day 5 H.S.[d]
0	0.2	-0.3	-1.1						
50	0.6	0.7	-1.5	0.6	0.3	-0.8	0.6	0.6	-1.1
100	3.2	0.6	-0.6	N.A.[e]	0.5	-1.3	2.0	1.5	-0.1
250	4.1	2.6	1.0	N.A.	2.1	2.5	7.2	2.9	1.2
250[f]	6.3	2.7	0.2	N.A.	2.4	no change	2.7	2.4	1.1
500	N.A.	3.4	1.6						

[a] Values all positive unless noted. Pre-exposure value subtracted from higher of 3-hour exposure or 1-hour post-exposure value.

[b] Non-Smoker. Pre-exposure values ranged from 0.5 to 1.1.

[c] Non-Smoker who worked in a garage 2 - 3 evenings per week. Pre-exposure values ranged from 0.7 to 2.3.

[d] Heavy Smoker. Pre-exposure values ranged from 6.1 to 9.3.

[e] Values not available (N.A.).

[f] Exposures to fluctuating concentrations with daily time-weighted-averages of 250 ppm.