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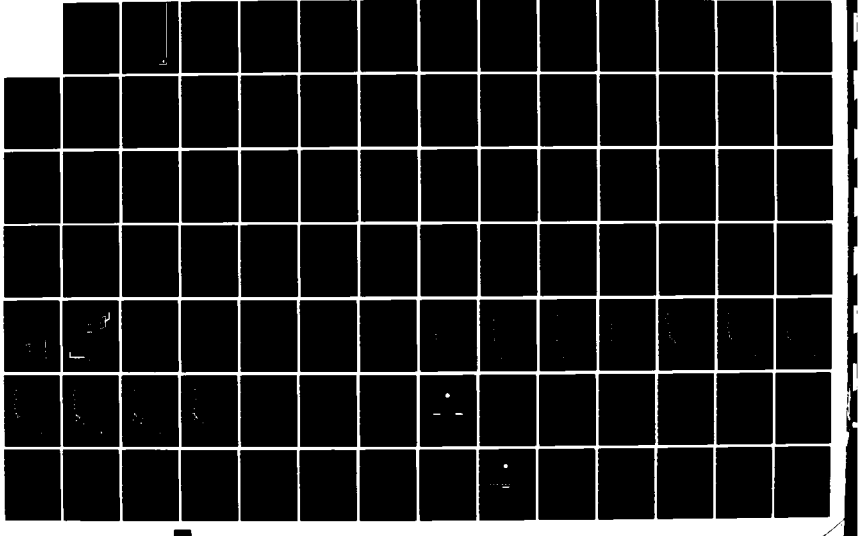

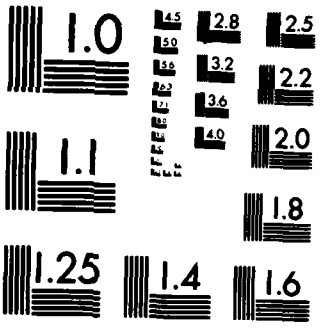

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DEVELOPMENT OF BEHAVIORAL TOXICOLOGY METHODOLOGY FOR INTERACTIVE EXPOSURE REGIMENS

FINAL REPORT
L06131-18

Maurline M. Preache
Patricia S. McGuire

December 1983

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This program was conducted to investigate methodology for determining effects on performance of exposure to inhaled substances alone or in combination with fatigue or heat stress. Carbon monoxide (CO) was used as a prototypic chemical and rats as the animal model. A series of preliminary experiments were conducted to establish the appropriate test parameters, exposure methodologies, and effects on carboxyhemoglobin. All studies were conducted utilizing operant schedules of reinforcement with food as the reinforcer. Performance on a variable ratio-fixed ratio schedule was disrupted during a 1-hr exposure to 1250 ppm CO and by forced

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swimming regardless of CO concentration. Lower CO concentrations had no effect. Performance on a fixed ratio-fixed ratio schedule was disrupted during 75 min exposures to CO at 700 ppm but not at 200 or 450 ppm. Forced swimming and a high environmental temperature (30.5 ~~degrees~~ C) also impaired performance on this schedule. There were no significant interactions for CO and swim stress; however, the combination of 450 ppm and heat stress produced a greater impairment than predicted by the separate effects of these conditions. Testing on a reaction time task indicated increased reaction times at 450 ppm and a trend in this direction at 700 ppm. Responding in this task was lowered by 700 and 450 ppm but heat affected neither reaction time nor responding.

EXECUTIVE SUMMARY

The ojective of this program was the development of behavioral methodologies which would be sensitive to disruption by inhaled compounds or physical stress conditions. The scope of the project included a literature investigation in relevant areas which provided the basis for selection of the behavioral methodologies, a series of preliminary investigations to insure standardization of exposure conditions and to determine appropriate parameters, and examination in rats of three schedules of reinforcement during exposure to carbon monoxide alone and in combination with fatigue stress and/or heat stress.

Preliminary studies were conducted to establish the appropriate test parameters, methods for inducing fatigue by forced swimming, methods for generation of homogenous CO distribution, and methods for generation of a consistent high environmental temperature (30.5 degrees C). Carboxyhemoglobin determinations were made at various times following carbon monoxide exposure alone and in combination with a period of forced swimming or heat stress. The schedules of reinforcement selected for investigation were a chained variable ratio-fixed ratio schedule, a chained fixed ratio-fixed ratio schedule and a reaction time task.

The effects of 1-hour exposures to CO in combination with fatigue were investigated for performance on the variable ratio-fixed ratio schedule of reinforcement. CO at a concentration of 1250 ppm but not at 200 or 700 ppm impaired performance on this schedule reducing responding to approximately 45% of baseline performance. Five exposures conducted at weekly interval had identical effects. Five consecutive daily exposures resulted in partial tolerance to the disruptive effects seen in the 1250 ppm group. When a period of forced swimming preceded the exposure session, performance was disrupted in all groups including the 0 ppm group.

The interaction of carbon monoxide with fatigue was examined in animals performing on a fixed ratio-fixed ratio schedule. In these studies carbon monoxide exposures were conducted for 75 minutes. Performance disruptions occurred at 700 ppm carbon monoxide and responding was virtually eliminated at 1250 ppm. The combination of CO exposure and fatigue produced greater effects than either condition alone but the effect was not synergistic.

Seventy-five minute exposures to carbon monoxide at concentrations of 0, 200, 450, and 700 ppm were combined with heat stress for investigation of effects on performance of the chained fixed ratio-fixed ratio schedule. In the absence of heat stress, 700 ppm carbon monoxide again reduced respnding whereas 200 and 450 ppm did not. Heat stress reduced responding in all

groups including the control group. Heat combined with 450 ppm carbon monoxide produced greater response reductions than predicted for either condition alone but these were limited to the last 30 minutes of exposure.

On the reaction time task, there was a significant increase in reaction time at 450 ppm CO. A similar trend was observed at 700 ppm and this concentration also produced and overall decrease in responding. Heat had no significant effect on responding or reaction time.

Carboxyhemoglobin levels were highest at the earliest time point considered, 2 minutes after termination of exposure. In the study in which swimming or heat stress was combined with carbon monoxide exposure, mean carboxyhemoglobin values for the groups exposed to 700 ppm ranged between 42%-47% and between 32%-38% for groups exposed to 450 ppm. Considered across all time points, carboxyhemoglobin for rats exposed to swimming or heat stress were statistically higher than those exposed to carbon monoxide alone but the maximum difference at any specific time point was small (6% or less).

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Uses of Laboratory Animals." prepared by the Committee on Care and use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

Due to the exploratory nature of the program, there was no attempt to rigidly conform to Good Laboratory Practices Regulations (Fed. Reg. 21 CFR Part 38, 1978). However, during the course of the program 14 inspections or program reviews were conducted by the Quality Assurance Unit and the Final Report was reviewed by the Supervisor of Quality Assurance.

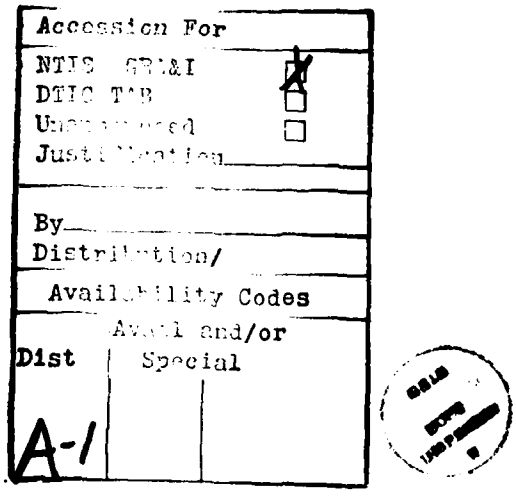

TABLE OF CONTENTS

Page

EXECUTIVE SUMMARY.....1

FOREWORD.....3

I. INTRODUCTION.....13

II. GENERAL METHODS.....15

A. Animals.....15

B. Test Material.....16

1. Handling of Test Material.....16
2. Purity of Test Material.....16

C. Inhalation Exposure System.....16

1. Air Supply, Preparation and Exhaust.....16
2. Inhalation Chambers.....17
3. Carbon Monoxide Generation and Analysis System.....18
4. Routine Procedure for Standardizing CO Analyzer.....19
5. Temperature and Humidity Recordings.....19

D. Generation of Heat Stress Condition.....19

E. General Methods for Swim Studies.....20

1. Swim Tanks.....20
2. Heating Units and Thermometers.....20
3. Hindlimb Extensor Apparatus.....20
4. Grip Strength Apparatus.....21
5. Operation of Strain Gauges.....21
6. Procedure for Operating the Hindlimb Extensor Apparatus.....21
7. Procedure for Operating the Fore- and Hindlimb Grip Strength Apparatus.....22
8. General Procedure for Swim Stress.....22

F. Operant Testing Equipment and Procedures.....22

1. Behavioral Chambers.....22
2. Computer.....22
3. Shaping Procedures for Operant Schedules.....23

III. METHODOLOGY DEVELOPMENT.....23

A. Standardization of Inhalation Exposure Conditions.....23

1. Determination of Time Factors and Stability of CO Concentrations.....23
2. Determination of Homogeneity of CO Distribution.....24

B. Determination of Values for Swim Fatigue.....26

1. Fatigue from Swimming as Measured by Fore- and Hindlimb Grip Strength.....26
2. Fatigue from Swimming as Measured by Hindlimb Extensor Thrust.....27
3. Evaluation of the Fore- and Hindlimb Grip Strength Procedure by the Use of a Positive Control Condition (Phenobarbital Treatment).....27
4. Additional Pilot Studies to Establish Swim Stress Conditions.....28

C. Standardization of Procedures for Generation of High Temperatures.....29

D. Pilot Study of Effects of Carbon Monoxide Alone and in Combination with Heat Stress on Performance on a Two-Lever Chain Fixed Ratio Fixed Ratio Schedule of Reinforcement.....30

E. Determination of COHb Levels at Different Times following CO Exposure.....31

F. Determination of COHb Levels after CO Exposures in Combination with Heat or Swim Stress.....33

IV. EXPERIMENTAL INVESTIGATIONS.....34

A. Effects of CO and Swim Stress on VR5-FR15 Performance.....34

1. Methods.....34
2. Results.....35

B. Effects of CO and Swim Stress on FR30-FR30 Performance.....36

1. Methods.....36
2. Results.....37

C. Effects of CO and Heat Stress on FR30-FR30 Performance.....38

1. Methods.....38
2. Results.....39

D. Effects of CO and Heat Stress on Reaction Time Task Performance.....40

1. Methods.....40
2. Results.....41

V. DISCUSSION.....42

VI. REFERENCES.....49

APPENDIX A Literature Review: Behavioral Toxicology Methodology.....109

APPENDIX B Literature Review: Carbon Monoxide Effects on Behavior.....135

APPENDIX C Literature Review: Physical, Physiological and Psychological Stressors.....169

APPENDIX D Literature Review: Extremes in Ambient Temperature as a Stress Condition and Interactions With Chemically Induced Toxicity.....179

APPENDIX E Animal Weights and Ages at Evaluation.....189

APPENDIX F Method For COHb Determination.....191

APPENDIX G Numerical Values For COHb Determinations...201

APPENDIX H Individual Animal's Data for Responses on VR5 Component of Chain VR5 FR15 Schedule...205

APPENDIX I Baseline Performance for FR30-FR30 Schedule.....231

APPENDIX J Tabular Summary and Statistical Analyses For FR30-FR30 Schedule of Reinforcement After Exposure to CO and/or Swim Stress....233

APPENDIX K Baseline Performance for FR30-FR30 Performance Prior to Heat Stress.....241

APPENDIX L Tabular Summary for FR30-FR30 Schedule of Reinforcement after Exposure to CO and/or Heat Stress.....243

APPENDIX M Tabular Summary for Number of Reinforcers Obtained and Number of Timeouts during Reaction Time Testing following CO and/or Heat Stress.....247

LIST OF FIGURES

Figure	Title	Page
1	Air supply, Preparation and Exhaust System	52
2	Inhalation Chamber with Associated Air Flow and Pressure Controls	53
3	Carbon Monoxide Generation, Sampling and Analysis Systems	54
4	Fore- and Hindlimb Grip Strength Apparatus	55
5	Schematic Representation of an Inhalation Chamber with the Six Behavioral Chambers in Place	56
6	Carbon Monoxide Concentrations as a Function of Time for Target Concentrations of 250 and 1000 ppm	57
7	Schematic Representation of a Behavioral Chamber	58
8	Temperatures at Seven Locations within Chamber 2 over Time within the Session	59
9	Temperatures at Seven Locations within Chamber 4 over Time within the Session	60
10	Temperatures at Seven Locations within Chamber 1 over Time within the Session	61
11	Temperatures at Seven Locations within Chamber 3 over time within the Session	62
12	Temperatures Read from Permanently Mounted Sensors in Three Inhalation Chambers (1,2, and 4) on Day 1 of the Experiment	63
13	Temperatures Read from Permanently Mounted Sensors in Three Inhalation Chambers (1, 2, and 4) on Day 2 of the Experiment	64
14	Temperatures Read from Permanently Mounted Sensors in Three Inhalation Chambers (1, 2, and 4) on Day 3 of the Experiment	65

15 Temperatures Read from Permanently Mounted Sensors in Four Inhalation Chambers on Day 4 of the Experiment.....66

16 Temperature Readings for Inhalation Chamber 1 from the Permanently Mounted Temperature Sensor over 4 Days of Testing.....67

17 Temperature Readings for Inhalation Chamber 2 from the Permanently Mounted Temperature Sensor over 4 Days of Testing.....68

18 Temperature Readings for Inhalation Chamber 4 from the Permanently Mounted Temperature Sensor over 4 Days of Testing.....69

19 Effects of CO in Combination with High Environmental Temperatures on Performance on a Chain FR30-FR30 Schedule.....70

20 Linear Regression Model for COHb Data with 95 Percent Confidence Intervals.....71

21 Percent Carboxyhemoglobin in Blood at Different Times after Exposure to Carbon Monoxide and/or Swim Stress or High Environmental Temperatures.....72

22 Intelligence Panel: Arrangement for Chain VR5-FR15.....73

23 Responses on FR15 Component of the Chain VR5-FR15 Schedule.....74

24 Concentration-Response Curve for VR5-FR15 Performance on the Initial Day of CO Exposure.....75

25 Effects of Five Consecutive Weekly Exposures to CO on VR5-FR15 Performance.....76

26 Comparison of the Number of Reinforcer Presentations on a VR5-FR15 Schedule Following CO Alone and CO in Combination with Swim Stress.....77

27 Effects of Five Consecutive Daily Exposures to CO on VR5-FR15 Performance.....78

28 Effects of CO and Swim Fatigue on Performance of a Chain FR30-FR30 Schedule of Reinforcement.....79

29 Time to First Response.....80

30 Time Course of Effects following CO and/or Swim Stress: Responses for Light Presentation.....81

31 Time Course of Effects following CO and/or Swim Stress: Responses for Food Presentation.....82

32 Time Course of Effects following CO and/or Swim Stress: Number of Reinforcers.....83

33 Effects of CO and Heat Stress on Performance on a Chain FR30-FR30 Schedule of Reinforcement.....84

34 Time Course of Effects following CO and/or Heat Stress: Responses for Light Presentation.....85

35 Time Course of Effects following CO and/or Heat Stress: Responses for Food Presentation.....86

36 Time Course of Effects following CO and/or Heat Stress: Number of Reinforcers.....87

37 Intelligence Panel: Arrangement for Reaction Time Task.....88

38 Time Course of Effects for the Reaction Time Task: Correct Lever Presses.....89

39 Mean Number of Reinforcers Obtained during 60-Minute Performance Sessions in the Reaction Time Task.....90

40 Time Course of Effects for the Reaction Time Task: Number of Reinforcers.....91

41 Mean Number of Timeouts Resulting from Premature Releases of the Lever during 60-Minute Performance Sessions in the Reaction Time Task.....92

LIST OF TABLES

Table	Title	Page
1	Carbon Monoxide Concentration at Different Probe Locations within the Inhalation Chamber	94
2	Carbon Monoxide Concentration during Sessions	95
3	Fore- and Hindlimb Grip Strength for Rats following Forced Swimming	96
4	Effect of Swim Stress on Hindlimb Extensor Response	97
5	Fore- and Hindlimb Grip Strength for Rats Following Forced Swimming with 10 g Weights	98
6	Effects of Swim Stress under Varying Conditions on VR10-FR30 Schedule Performance	99
7	Effects of Heat Stress on Rectal Temperatures	100
8	Distribution of Animals to Exposure Conditions for Pilot Heat Stress Experiment	101
9	Experimental Design Summary - Allocation of Animals for COHb Determinations	102
10	Duration of Swimming for Individual Animals Prior to Testing of VR5 - FR15 Performance	103
11	Distribution of Animals to Swimming and CO Exposure Conditions for a Single Replicate	104
12	Distribution of Animals to Heat Stress and CO Exposure Conditions for a Single Replicate	105
13	Distribution of Animals for the Reaction Time Task	106
14	Mean Values for Measures of Performance in the Reaction Time Task	107

1. INTRODUCTION

Disturbances in central nervous system functioning prior to the onset of other signs of toxicity have been recognized as a critical effect of exposure to many chemicals. The sequelae of disturbance of central nervous system functioning are manifest as behavioral changes which frequently result in disturbance of performance. This is an area of special concern to the military where exposure to toxic fumes may present a serious threat in situations where intact or heightened functioning of the nervous system is critical. Exposure of military personnel to toxic fumes, in situations where other stressful conditions are present, poses an even greater threat to central nervous system function. Central nervous system effects of a compound following even brief exposures in a compromised organism may produce behavioral disruptions which will prevent appropriate behavior and may result in life threatening situations.

The assessment of higher nervous system function through the use of animal models presents a challenging problem. With many neurotoxic agents the initial symptoms are subjective complaints and/or subtle behavioral changes. Behavioral change as the first indicator of neurotoxicity may allow early detection of toxicity and prevention of irreversible sequelae of continued exposure.

One approach to evaluating the behavioral effects of exposure to toxic chemicals has been the use of operant conditioning techniques. This methodology has been well developed and successfully applied in both pharmacology and toxicology.

The overall objective of this program was to develop and validate an animal model methodology which can be used as an aid to assessing the risks of adverse effects on performance of military personnel from chemicals and stressors to which they may be exposed.

Carbon monoxide was chosen as the prototypic chemical because of the availability of a large data base which allowed meaningful comparisons. In addition, carbon monoxide is a noxious gas found in its pure form in multiple situations and released as a by product of the combustion of many compounds. The exposure scenario in military operations is typically short term, with relatively high concentration levels. This exposure regimen has not been extensively used in laboratory experiments. The first phase of this contract involved literature reviews in the areas of behavioral methods, the behavioral effects of carbon monoxide, physical and psychological stress and temperature stress (see Appendices A through D). These reviews were used as the basis for selecting methodological approaches to investigating the effects of CO combined with physical stresses.

Three behavioral schedules were chosen for investigation of the effects of carbon monoxide and two stressors were selected. The behavioral schedules were a two lever chained variable ratio-fixed ratio schedule, a two lever chained fixed ratio-fixed ratio schedule and a reaction time task.

The use of ratio schedules was based on previous work found in the literature which suggested that ratio schedules were more sensitive to disruptions by carbon monoxide than schedules which produce lower rates of responding. A chain schedule allowed assessment of differential effects as a function of behavior maintained by conditioned or primary reinforcers. Preliminary studies on this program indicated that low ratio values were less sensitive than high ratio values in both components of the schedule. Thus, high ratio value schedules were incorporated into later studies. The choice of a reaction time task was based on data suggesting that reaction time is affected in humans following CO exposure and recognizition that this aspect of performance may be especially important to military personnel.

The two stressors selected were physical stress using swimming fatigue and high temperature stress. While a large body of literature is available on various stressors, little work has been done on the interaction of stressors and chemical agents.

The selection of swim fatigue as a physical stress required preliminary evaluation of the appropriate parameters. These included observational data to determine the length of time rats could swim with various weightings before exhaustion, modifications in design of the swim tank and attempts to use behavioral methods to assess levels of fatigue. Thus, a series of experiments were included to evaluate swim fatigue methodology.

For heat stress, it was necessary to design appropriate methodology for establishing the high temperature conditions. Inhalation chamber standardization experiments were conducted and a pilot investigation of the effects of heat on animals performing on a schedule of reinforcement was conducted.

The effects of carbon monoxide alone and in combination with swim stress were examined on the variable ratio-fixed ratio and fixed ratio fixed-ratio schedules. The effects of carbon monoxide alone and in combination with heat stress were examined on the fixed ratio fixed-ratio schedules and reaction time task.

A prerequisite of laboratory inhalation chamber experiments is verification that the distribution of the exposure chemical is equal throughout the inhalation chamber. This distribution is a function of the flow rate of both the chemical and the air into the chamber and can be affected by the configuration of the chamber and the components therein. Theoretical determination of

flow rates required to yield a specific concentration of a test material in the chamber air are possible but empirical verification is essential to determine that a uniform distribution of the nominal concentration has been achieved. Carbon monoxide (CO) is an odorless, colorless gas with physical characteristics such that it mixes readily with air. For the purpose of further experiments, it was first necessary to determine that despite these inherent characteristics of the gas, there were no impediments to equal distribution throughout the inhalation system that would be used in future studies. Chamber homogeneity studies were conducted before initiation of animal exposures.

Finally, determinations of carboxyhemoglobin levels at various time points following the exposures were made. This allowed verification that the animals were being adequately exposed and determination of the relationship between carboxyhemoglobin levels and the extent of behavioral disruption.

II. GENERAL METHODS

A. Animals

For all experiments, the animals were male Sprague-Dawley rats (CD, Charles River Breeding Laboratories, Inc., Portage, MI) weighing 250 to 300 grams upon arrival from the supplier. A table of animals weights and ages for individual experiments is given in Appendix E. The animals were quarantined for a period of at least one week. During quarantine they were given unrestricted access to tap water and food (Wayne Lab Blox, Scientific Animal Feed, Arlington Heights, IL). The rats were housed either individually or two animals/cage in 9 in x 10 1/2 in x 8 in (147 sq in) plastic cages with stainless steel wire bar tops. The individual housing was initiated with animals used after the CO-swim stress interaction experiment entitled "Effects of Carbon Monoxide Alone and in Combination With Swim Stress on Performance on a Two-Lever Chain Fixed Ratio - Fixed Ratio Schedule of Reinforcement" because of large variability in the weights of animals on deprivation schedules when animals were housed two/cage. A layer of Ab-sorb-dri® covered the bottom of the cage. Each animal was numbered with a study-unique number which was shown on an ear tag. When animals were housed two/cage, one animal had the other ear punched with a single hole to discriminate between the two animals in the event they both lost their ear tags. The animal rooms were maintained at 22 ± 2 degrees C with a 14:10 hour light/dark cycle. Standard animal care included weekly cage and water bottle changes for animals housed individually and twice weekly cage and water bottle changes for animals housed two/cage. Daily inspections of the animals were made to check for any problems with their health status or with the food and water supplies.

B. Test Material

The test material was carbon monoxide (MW 28.01) and was obtained from Matheson, Joliet, IL.

1. Handling of Test Material

a. Storage Conditions: CO was stored in cylinders at ambient room temperature (23 degrees ± 2 degrees) in the room where exposures were conducted.

b. Special Handling Procedures: The CO tanks were secured to the wall by restraining chains.

2. Purity of Test Material

The carbon monoxide grade was Matheson Purity (99.99% minimum, the sum of N_2, O_2, CO_2, H_2, THC as CH_4, and H_2O < 100 ppm). The standards were CO in air with CO concentrations of 450 ppm, 900 ppm and 0.45% CO. Standards were supplied by Matheson (Joliet, IL). All standards had a preparation tolerance of ± 10% and a certification accuracy of ± 2%. The purity analysis of the CO standards specified by the manufacturer were accepted.

C. Inhalation Exposure System

The major components of the inhalation exposure system were the air supply, preparation, and exhaust system; the inhalation chamber with associated air flow and pressure controls; and the carbon monoxide generation, sampling, and analysis systems. For the first year of the program, the system was equipped with three 1 cu meter inhalation chambers. Thereafter, a fourth chamber was used for the experiments.

1. Air Supply, Preparation and Exhaust (Figure 1)

The air supply for the exposure system was preconditioned building air that was pulled through the system by two blowers each of which are composed of a 14-in diameter fan driven by a three horsepower motor (Baldor Electric Company, Ft. Smith, AK, Catalog No. VWM-3158). Four motor (Honeywell, Minneapolis MN, Type M435A-11162) controlled dampers within the ductwork permitted operation of either or both blowers. Simultaneous operation of both blowers provided a potential system pressure equivalent to 4-5 inches of water through the four chambers.

Incoming air was initially passed through a 60% cotton prefilter and downstream from this was filtered through a 99% HEPA filter and then through a bed of charcoal. The HEPA filter and charcoal were located in 24 x 24 x 6 in stainless steel housings that were equipped with magnehelics (Dwyer Instruments, Michigan City, IN) which were monitored to determine when filter changes were required.

A blast coil heater (Industrial Engineering and Equipment, St. Louis, MO, Type XUB) was located in and adjacent to the incoming ductwork and insulating material was added to the exterior of the ductwork at this location. The heater was included in the system to provide for increased ambient temperature as a stress condition. A Universal Power humidifier (Model 97, Auto-Flow Company, Detroit, MI) was located downstream to the heater. Regulation of the heater and humidifier was by a solid state thermostat and a humidistat (Type 174H, Honeywell) for which the sensors were located in the ducts for the main incoming airstream prior to its diffusion to the various inhalation chambers. In addition, for generation of high temperatures, each chamber was wrapped with approximately 200 ft of 1/2 in wide heating tape (7 watts/foot), spaced at intervals of approximately 2-1/2 in, and the exterior of the chamber was covered with 1/2-in styrofoam insulating material.

The ductwork between the cotton prefilter, the HEPA filter and the charcoal filter was 8-in flexible hosing. All ductwork from the charcoal filter to the diffuser was a combination of both galvanized steel and flexible hosing with all couplings being flexible hosing and all other ductwork galvanized steel. After passage through the inhalation chambers, the air from multiple chambers was collected and exhausted through galvanized steel ducts to the outside at a point 6 ft above the roof of the building.

2. Inhalation Chambers (Figures 1 and 2)

Each inhalation chamber was 1 cu meter in volume with a 36-in cube type body. The top and bottom were pyramidal shaped cones. The chamber door was 34 x 34 in with a wire reinforced glass window (24 x 24 in). One side of the chamber also had a 24 x 24 in wire reinforced glass window and three equidistant sampling ports were located on the opposite side. For standardization experiments, flexible copper tubing probes were inserted through the sampling ports. The probes were of adequate length to sample all points in the chamber. Components of the chamber other than the windows and gaskets were of stainless steel construction. The gaskets were constructed of sponge rubber.

Clean air entered the chamber through a valve, located in a removable assembly at the top of the chamber, which provided for regulation of the pressure within the chamber. The valve regulating the pressure within the chamber was a damper box composed of two stainless steel plates, the positioning of which was controlled by a screw-type damper. The gas input ports were located downstream from this, and an orifice plate was located further downstream but prior to entry of the air line into the chamber head. The pressure drop across this plate was measured

for each chamber by a magnehelic (Dwyer, Catalog No. 2001) which was calibrated with a mass flow meter to verify curves for pressure to flow rate conversion. A second magnehelic (Catalog No. 2010) with one tap line located at the top of the chamber head and the other downstream to the valve through which air was exhausted measured the total potential draw through the chamber. A third magnehelic (Catalog No. 2001C) for which the tap line was also located at the top of the chamber head measured the negative pressure of chamber interior in relation to room pressure. Air was exhausted near the bottom of the inhalation chamber through a valve which regulated the air flow rate. A damper box similar to that described above regulated exhausted air flow. In addition, ball valves downstream from this provided for additional control of exhausted air. The chamber also included a valve controlled opening at the bottom of the lower cone for water drainage.

The three magnehelics which measured pressure within the chamber were calibrated with a mass flow meter prior to the initiation of standardization experiments. There was little drift in the magnehelic readings over time and, thereafter calibrations with the mass flow meter were conducted at approximately six month intervals.

The target total flow rate of air into the 1000 liter chamber was 18 cfm (500 l/min). From this the t_{99}, time required to bring the concentration to 99% of the nominal value, was calculated using the formula:

t_{99} = K x a/b, where K is a constant:
For t_{99} K = 4.605 (value from MacFarland, 1926)
a is the volume of the chamber (35.2 cu ft)
b is the total flow through the chamber (18 cfm), the
t_{99} = 4.605 x 35.2 cfm/18 cfm
= 9.0 min.

3. Carbon Monoxide Generation and Analysis System (Figure 3)

The carbon monoxide test environments were generated from tank carbon monoxide with a single tank serving up to four chambers. The flow from the tank was controlled through a two-stage regulator (Matheson No. 76311). Flow to each chamber was individually controlled by flow meters (Matheson, No. 7632T) located for each chamber upstream from the point where carbon monoxide entered through the gas input port. This allowed the concentrations in the four chambers to be controlled independently.

For sampling CO concentrations within the chambers, 1/8 in inner diameter flexible copper tubing was used. The probes were long enough to be positioned at all points in the chamber. During normal usages, the probe was positioned in the center of the top

shelf at the level of the animals' heads. The samples were pulled through ports located in the side of the inhalation chambers by an air pump (Arthur H. Thomas, Model No. 1050-A10) through a gas purifier (Alltech Associates, Model 8128) with indicating drierite (J.T.Baker Chemical Co.) for the removal of moisture. Copper tubing was used for connections between sampling points, the gas purifier, and the carbon monoxide analyzer. Analysis was by a Beckman Model 864 infrared analyzer for which calibration curves in the ranges of 0-5000, 0-1000, and 0-500 ppm were available. This instrument has an accuracy of ± 1% full scale, a span drift of ± 1% of full scale in 24 hr, and a zero drift of ± 1% full scale in 24 hr. The flow rate for the CO analyzer was 500-1000 cc/min. A separate flow path from the calibration gas supply to the analytical instrument was established for calibration of the infrared analyzer. Flow of the calibration gas was controlled by a manually operated valve and the rate regulated by a type 602 two stage regulator (Matheson).

4. Routine Procedure for Standardizing CO Analyzer

For all experiments, prior to the use of CO in the inhalation chambers, the CO analyzer was calibrated both immediately before and immediately after the use of CO in the inhalation chambers. This was accomplished by calibrating the CO analyzer with air and with CO calibration gases of approximately the upper limit for all scales on the analyzer which were used during the exposure.

5. Temperature and Humidity Recordings

Initally temperature and humidity recordings were made using a wet/dry bulb (Taylor Comfort Guide Hygrometer). Subsequently, each chamber was equipped with General Eastern temperature and humidity sensors (Model 411) and a transmitter (Model 455) for which the output was fed to a multichannel recorder (Esterline Angus, Model MS430-0-81-82-0202-65-25). As the General Eastern humidity sensors yield values substantially different from the wet/dry bulb, temperature and humidity were recorded from both the sensors and the wet/dry bulb for the remainder of the program.

D. Generation of Heat Stress Conditions

For all experiments requiring high temperatures, the following procedures were used to generate the appropriate temperature in the inhalation chambers. The air supply system, heater, heating tapes and humidifier were turned on and the chambers were allowed to warm. This initial phase typically required 30-40 minutes depending on the desired temperature. When the desired temperature was obtained, the air supply system, heater, and humidifier were turned off. Animals were then placed in the behavioral chambers; this required approximately 5 minutes. The

inhalation chamber doors were then closed and the air supply, heater, and humidifier started. The heating tapes were turned on and off at intermittant time periods to maintain the desired temperature.

E. General Methods For Swim Studies

The following equipment was used for swim stress experiments and/or experiments designed to quantify the extent of fatigue that resulted from different periods of forced swimming. The standard equipment included: swim tanks and associated heating elements, hindlimb extensor response apparatus, fore- and hindlimb grip strength apparatus and strain gauges which were used for both the hindlimb extensor response apparatus and the fore- and hindlimb grip strength response apparatus.

1. Swim Tanks

The swim tanks were 40 gal plastic cylindrical containers 21-inches in diameter and 27-inches high. Each tank was filled to a depth of 16-inches and provided a water capacity of 23.9 gal. The water temperature was maintained at 23 degrees C ± 1 degree.

2. Heating Units and Thermometers

Each swim tank was equipped with a heating unit and a standard thermometer. The heating units were Ebo-Jagir (El Segundo, CA) automatic aquarium heaters which were thermostatically controlled and waterproof. The 200 watt units have the capacity to heat 60 gallons and up with an accuracy of plus/minus 0.7 degrees C. The thermometers were stainless steel aquarium thermometers (Rolf C. Hagen Co, MA, 02048, A-1203) with a temperature range -2 to 40 degrees C.

3. Hindlimb Extensor Apparatus

The hindlimb extensor apparatus was constructed according to the specifications of Cabe and Tilson (1978). Attached to a push-pull recording strain gauge (Chatillon Model DPP; J.A. King and Co., Box 21225, Greensboro, NC, 27420), was a T-bar constructed by inserting a 5" x 1/8" diameter (12.7 x 0.003 cm) brass rod through a hole near one end of a 3" (7.6 cm) hexagonal threaded aluminum standoff. The strain gauge was rigidly mounted at a 45 degree angle by means of pre-tapped holes in the body of the instrument, such that the T-bar was parallel to the edge of a 9" x 12" (22.9 x 30.5 cm) platform covered by a sheet of coarse sandpaper. The sandpaper sheet was attached with spring clips, so it could be replaced when soiled. The bar was (arbitrarily) 13" (33 cm) above the bench top and the platform was typically positioned 1 1/2" (3.8 cm) higher than and 3" (7.6 cm) laterally displaced from the T-bar. This bar to platform gap size was

derived through pilot testing in this laboratory. The apparatus was constructed of plexiglas with the platform horizontally and vertically adjustable by means of slots and screws.

4. Grip Strength Apparatus

The grip strength apparatus was constructed to the specifications of Meyer et al (1979). The apparatus was mounted on a 26 in long by 9 in wide plexiglas base (Figure 4). Mounted 6 inches above the base was a pedestal shaped like a plus sign which held an 11.5 x 9 in platform. Across the length of the platform were two adjustable 3 in high L-shaped guides which formed a trough. Two vertically adjustable plexiglas pedestals positioned at either end of the base held the strain gauges. Both gauges were push-pull strain gauges (Chatillon, Models DPP-1.0 kg and DPP-2.5 kg, J.A. King and Co., Box 21225, Greensboro, NC, 27420). For measuring forelimb grip strength, the strain gauge was equipped with a 3 in equilateral triangular brass ring (1/8 in. diameter) soldered onto a hexagonal aluminum standoff, which threaded onto an extension arm supplied with the strain gauge. The grasping portion was aligned parallel to the surface of the trough and 3/4 in from the edge of the plexiglass platform. Attached to the strain gauge for measuring hindlimb grip strength was a T-bar constructed by inserting a 5 in by 1/8 in diameter brass rod through a hole near one end of a 3 in hexagonal threaded aluminum standoff. The T-bar was parallel to and 1/2 in from the edge of the platform. The strain gauges were mounted securely on the plexiglas bases by means of pretapped holes in the bodies of the instruments. The pieces of the plus-shaped pedestal and the adjustable pedestals that hold the gauges were constructed of 1/2 in plexiglas; all other components are 1/4 in plexiglas.

5. Operation of Strain Gauges

Each strain gauge was calibrated daily before the experiments by recording the meter deflection as the instrument was loaded with weights. For use with the hindlimb extensor apparatus the weight covered the range of the meter (25 g to 2.5 kg). For use with the fore- and hindlimb grip strength apparatus, a strain gauge covering a meter range of 25 g to 1.0 kg was used and appropriately calibrated.

6. Procedure for Operating the Hindlimb Extensor Apparatus

The procedure for the use of the hindlimb extensor apparatus outlined by Cabe and Tilson (1978) was followed. The strain gauge was zeroed and set to record meter deflections produced by pushing forces. The animal was held in the experimenter's right hand around the thorax, under the shoulder girdle. The rat's tail was taken in the left hand and the plantar surfaces of the rat's hind feet placed symmetrically on the bar. The antebrachia were then placed on the sandpaper covered platform, and the

animal was released from the experimenter's grip. A sharp air puff was then administered to the rat's rump, by mouth from about six inches away. Each animal was given three successive trials. The intertrial interval was the time needed to record the data and zero the meter for the next trial.

7. Procedure for Operating the Fore- and Hindlimb Grip Strength Apparatus

The procedure for the use of the fore- and hindlimb extensor apparatus outlined by Meyer et al (1979) was followed. The strain gauge was zeroed and set in the record mode. The animal was placed in the trough with the forepaws inside the triangular grasping ring. With one hand, the animal was grasped about 3/4 of the way up the tail toward the base. The animal was steadily pulled by the tail away from the ring until the grip was broken. Pulling was continued until the hindlimbs grasped the T-bar. A trial was completed when the grip of the hindlimbs was broken. Each animal was given three successive trials. The intertrial interval was the time needed to record the data and zero both meters for the next trial (typically less than 30 sec.).

8. General Procedure for Swim Stress

For all swim studies, the appropriate weight was attached approximately 0.5 cm from the base of the animal's tail. The rat was then placed in the swim chamber and swum for the appropriate time. Following completion of swimming the animal was removed from the water, the weight removed, and excess water wiped from the animal's body. The behavioral test was then initiated.

F. Operant Testing Equipment and Procedures

1. Behavioral Chambers

The behavioral chambers were modified operant chambers 9-1/2 in wide x 9-1/2 in long x 13 in high. The top, back and left walls were constructed of slotted stainless steel. The door was initially constructed of slotted plexiglas but was subsequently replaced with a slotted stainless steel door. The right wall contained the modular behavioral components (Coulbourn Instruments, Columbus, OH) which were manipulated appropriately depending on the experiment in progress. The behavioral chambers were arranged in the inhalation chamber as shown in Figure 5.

2. Computer

Two PDP 8/a computers (Digital Equipment Corporation) equipped with 32K word software memory were used for controlling behavioral experiments. Associated with each system was a VT 100 Digital terminal; a line printer (Digital Decwriter III) for hard copy printing was shared between the two systems. The

behavioral chambers were interfaced to the computers by a PDP8 Computer Interface Panel and Card (GC Controls, Greene, NY 13778). Programming of behavioral experiments was accomplished using the SKED Software System (State Sytems, Incorporated).

3. Shaping Procedures for Operant Schedules

For all behavioral studies employing a chain two lever schedule, the animals were initially shaped by reinforcing successive approximations to a lever press response (Ferster and Skinner, 1957). Each animal was then given one additional session on an FR-1 schedule which terminated after 100 food deliveries. Similarly, animals trained on the reaction time task were shaped to hold down the lever by reinforcing successive approximations to the response and successively increasing of the hold-down time required to obtain reinforcement. The specific procedures used in the various schedules are discussed in Section IV.

III. METHODOLOGY DEVELOPMENT

Prior to the initiation of the behavioral experiments, a series of preliminary experiments was necessary. These included experiments to standardize the inhalation exposure and heat stress conditions, experiments to determine the appropriate parameters for swim stress, and experiments to assess carboxyhemoglobin (COHb) levels at different time points after exposure to the conditions used in the behavioral experiments.

A. Standardization of Inhalation Exposure Conditions

1. Determination of Time Factors and Stability of CO Concentrations

The purpose of this experiment was to validate empirically the time required to bring the inhalation chamber to the desired concentration, to determine the stability of that concentration over time, and to validate the time required for chamber clearance. A single point in the center of the chamber was used for sampling. The use of continuous sampling from a single point allowed the determination that the flow rate was correct and appropriate and that a constant carbon monoxide concentration could be maintained. The stability of the CO concentration was monitored for 1 hour. Following the CO monitoring the chamber was cleared, i.e. the CO supply to the chamber was stopped and recording of the air samples continued until the chamber CO levels returned to pre-exposure levels. The concentration was recorded at 1 min intervals for the first 15 minutes of the session, at 5 min intervals for the remainder of the 1 hour session and at 1 min intervals during chamber clearance. For this experiment a high CO concentration (1000 ppm) and a low concentration (250 ppm) were used for determination in one

chamber. The second and third chambers were tested similarly at one concentration each with the chamber for high and low concentrations randomly assigned. Humidity and temperature were recorded from a wet/dry bulb, with recordings taken at 10 minute intervals during the exposure periods. The total flow rate of air into the 1000 liter chamber was 18 cfm (500 l/min). From this the t_{99}, time required to bring the concentration to 99% of the nominal value, was calculated by the method of McFarland (1976).

Results. During the first 10 min of the session, CO concentrations showed a rapid increase with levels reaching the ultimately stable concentration within 10 min after the onset of CO flow (Figure 6). The concentrations remained stable over the balance of the 60 min period during which CO was being added to the chamber. For chamber clearance it took approximately 10 min after the cessation of CO flow for the concentration to return to the pre-exposure level (0 ppm).

2. Determination of Homogeneity of CO Distribution

The purpose of this experiment was to check the distribution of CO when six behavioral chambers were in place within the inhalation chamber and an adult rat was present in each.

A balanced incomplete block design was used. Three points were sampled for two 10-min periods during each of ten 60-min sessions. The design was as follows:

	Session									
Sample Point	1	2	3	4	5	6	7	8	9	10
1	X	X	X	X	X					
2	X	X				X	X	X		
3			X	X		X	X		X	
4			X		X	X		X		X
5	X				X		X		X	X
6		X		X				X	X	X

The probes were positioned near the right wall of the behavioral testing chambers which contained the stimulus-response modules at approximately the level of the animal's head. There was some variability in the exact location of the probes both within and across sessions, however, they were consistently located within the area shown in Figure 7.

The behavioral chambers were positioned as they were during subsequent behavioral experiments (See Figure 5). Each was completely equipped with behavioral modules including

feeders. Only the connection cables were not in the chamber. The order of the sessions and the order of sampling within the sessions were randomized. The complete design was tested using two CO concentrations (250 and 1000 ppm) in one inhalation chamber. The second and third inhalation chambers were tested at a single concentration.

For humidity and temperature readings, a wet/dry bulb was suspended above the top shelf, slightly off center. The positioning of the wet/dry bulb was restricted because of the placement of the behavioral chambers and the requirement that it was readable through the front of the chamber.

For each standardization session the procedure was:

a. The CO analyzer was zeroed and calibrated for the appropriate range (See Section II. C.4).

b. After positioning the probes and placing a rat in each behavioral chamber, the inhalation system was turned on.

c. At t_0 , the CO was turned on; the flowmeter regulating CO flow was adjusted to the appropriate setting as determined by the calibration chart or previous empirical checks. Readings were taken for temperature and humidity and the reading on the CO analyzer recorded.

d. At 10-min intervals during the 60-min session, readings were taken for temperature and humidity and for CO at one of the probe locations. The probe to be sampled was randomly selected prior to the start of the experiment.

e. When the 60-min session was completed, the CO was shut off and the calibration of the CO analyzer was reverified.

Results: The results of this experiment showed that there was little variability in CO concentrations as a function of probe location (Table 1) and over time within the session (Table 2). A four factor mixed-model analysis of variance was conducted to test the null hypothesis of within chamber spatial and temporal homogeneity. Results of the analysis revealed that the null hypothesis of no between time or location differences could not be rejected ($F = 0.48$; $df = 9/195$, and $F = 0.36$; $df = 5/195$; respectively). This temporal and spatial homogeneity was consistent across chambers ($F = 0.46$; $df = 9/195$, and $F = 0.44$, $df = 5/195$) and concentration ($F = 0.44$, $df = 9/195$ and $F = .32$, $df = 5/195$.

When the data of Experiment 2 were examined for consistency between inhalation chambers, it was apparent that at a target concentration of 250 ppm CO, the concentration was slightly high in Chamber 1 and slightly low in Chamber 2. The amount of CO introduced into the chamber was based on a CO flow rate calculated to yield a specific concentration with an air flow of 500 ppm through the chamber. The air flow rate for each chamber was calibrated prior to the beginning of the standardization experiments. However, there were some inherent variability in the flows through the different chambers. For purpose of this experiment, i.e., to show uniformity and stability over time, it was not considered appropriate to adjust CO flow during the session in order to better achieve the target concentration.

These data show that for the system and the procedures under consideration, CO concentrations within the range investigated were reliably achieved within 10 min from the onset of CO flow. CO concentrations remained stable over at least a 1-hr session and following discontinuation of CO flow, chamber clearance required less than 10 min. The distribution of CO when sampled at points of interest, i.e., at the level of the animal's head in behavioral apparatuses spaced at different locations within the chamber, was uniform.

B. Determination of Values for Swim Fatigue

Prior to the initiation of behavioral studies of the interaction of CO and swim stress, observations were made with rats swimming in water of varying depths and with different amounts of weighting. From these preliminary observations, it was decided that a water depth of 16 in was adequate. There appeared to be a large amount of variability in the length of time animals could continue to swim with various weightings. For this reason, three studies were undertaken using different weightings and different periods of forced swimming followed by a behavioral test (fore- and hindlimb grip strength or hindlimb extensor response) to quantitatively assess fatigue and subsequently some preliminary observations were made on the effect of swim stress on schedule performance.

1. Fatigue from Swimming as Measured by Fore- and Hindlimb Grip Strength

The first experiment was conducted to assess the effects of swimming duration on fatigue in rats forced to swim with a 5 g weight as measured by fore- and hindlimb grip strength.

Forty-eight, food deprived male rats were randomly assigned to one of the following swim conditions

a. 0 minutes - no swimming but animals were placed into the swim chamber and immediately removed.
b. 10 minutes - forced swimming
c. 20 minutes - forced swimming
d. 40 minutes - forced swimming.

The fore- and hindlimb grip responses were minimally affected or not all as a function of the swim conditions used in this experiment (Table 3). Single factor analysis of variance indicated no significant differences among the four groups (Forelimb: $F = 1.77$, df 3/43; Hindlimb: $F = 1.33$, df + 3/43); $p>0.05$.)

2. Fatigue from Swimming as Measured by Hindlimb Extensor Thrust

This experiment was conducted to determine if forcing animals to swim with a 10 g weight would affect the hindlimb extensor response.

Twenty-four animals were assigned to one of two conditions:

a. 0 minutes - no swimming but animals were placed into the swim chamber and immediately removed
b. 20 minutes - forced swimming

The animals used in this study were taken from the animals used previously to assess the effects of forced swimming on fore- and hindlimb grip strength. The twelve animals that had no swimming experience in the previous experiment (i.e. controls) were used for the 20 minute swim test for this experiment. The twelve animals that swam for 20 minutes in the previous study were used as controls for this study.

Results: The animals that swam for 20 min with a 10 g weight had lower hindlimb extensor scores than those that were not forced to swim (Table 4) ($F = 5.37$, df = 1/22, $p<0.05$). However, there was large variability within both groups and the effect appeared to be due to three animals which made few or no responses.

3. Evaluation of the Fore- and Hindlimb Grip Strength Procedure by the Use of a Positive Control Condition (Phenobarbital Treatment)

The objective of this experiment was to determine whether animals forced to swim with a 10 g weight for periods of 10 or 20 min would show an effect in the fore- and hindlimb

grip strength test and to evaluate our test procedures by the inclusion of a group that had recived phenobarbital, a treatment that others (Meyer et al, 1979) had used as a positive control condition for this test.

Forty-eight rats were randomly assigned to one of the following conditions:

a. 0 minutes - no swimming but animals were placed into the swim chamber and immediately removed.
b. 10 minutes - forced swimming
c. 20 minutes - forced swimming
d. 60 mg/kg phenobarbital - the drug was dissolved in distilled water and injected IP 30 minutes prior to testing. These animals received no swimming but were placed into the swim tank and immediately removed.

As can be seen in Table 5, neither fore- nor hindlimb grip strength showed an effect following forced swimming with a 10 g weight. Analysis of variance indicated no significant differences on either measure among the groups that swam 0, 10, or 20 min. The phenobarbital treated group was compared to the 0 minutes group and showed a significant decrease on both measures. These data essentially replicate those reported by Meyer et al (1979) for grip strength following treatment with phenobarbital.

4. Additional Pilot Studies to Establish Swim Stress Conditions

From the above data it appeared the most promising combination for swim fatigue was a 10 g weight combined with swimming for 20 min and the first CO-swim stress interaction study, which assessed the effects of swim stress on VR-FR performance (Section IV.A), was conducted with this combination. During that experiment, it became apparent that these conditions were too severe. Therefore, additional pilot studies were conducted and utilized performance on an operant schedule as the endpoint.

The first of these studies utilized animals from a previous experiment that had been trained on a VR-FR schedule. Four animals were tested at each of three conditions:

10 min forced swimming - 7 g weight
15 min forced swimming - 5 g weight
20 min forced swimming - 5 g weight

Under all three conditions, at least two of the four animals failed to respond and for the others there was no consistency with respect to the magnitude of effect (Table 6).

The second of these pilot studies utilized animals that had been trained on an FR-FR schedule of reinforcement but had been eliminated from use in an interaction study during randomization or due to poorer performance. Performance was evaluated following 20, 40, or 60 min swimming with a 3 g weight or 30 or 40 min with a 4 g weight. At least three animals were evaluated at each condition. Of these combinations, a 4 g weight with a 30 min swim period appeared to best achieve the objective of reducing, without totally eliminating, responding and this combination was used for the CO-swim stress interaction study of FR30-FR30 performance (Section IV.B)

C. Standardization of Procedures for Generation of High Temperatures

An experiment was conducted to evaluate the differences in temperatures for the four inhalation chambers over time and at different locations in the chambers. For this experiment, the chambers were loaded with six animal behavioral testing chambers as they were in standard usage but no animals were present. The experiment was conducted in four trials on each of 4 days. On each trial one of the four inhalation chambers contained a thermometer in each of its six behavioral testing chambers. Temperatures for the other chambers were read from the permanently mounted sensor in each chamber. Each chamber was sampled at approximately 5 min intervals for the first 15 min and at 15 min intervals for the remainder of the 90 min period. Heating tapes were turned on and off according to a schedule which was selected based on the results of the preliminary studies and the first day of the experiment. This schedule is indicated in the data presentation. The heater was set at 35 degrees C for both the prewarming and 90-min test periods.

Results: Figures 8 through 11 show for individual inhalation chambers, the temperatures achieved at different time points at locations in the six behavioral testing chambers and at the location of the permanently mounted sensor. Behavioral testing chambers located on the lower shelves of the inhalation chambers were slower to heat up. However, within approximately 15 min after the doors were reclosed, simulating the start of a test session, the range of temperatures at the seven different locations was typically 3 degrees C or less and remained so throughout the session.

Temperatures, read from the permanently mounted sensors, in different inhalation chambers on a given test day are shown in Figures 12 through 15. After the warmup period, chamber 4 tended to have lower temperatures than the other chambers for all 4 days considered but again the differences were small; the maximum difference was approximately 2 degrees C Figures 16 through 18 indicate that over the different days of the experiment, there were only small differences in the temperatures achieved for a given inhalation chamber.

These data indicated that by using a warmup period, the variability between inhalation chambers and locations within an inhalation chamber could be controlled within reasonable limits. They also indicated that a slightly different regimen for warming chambers was required if a temperature of 35 degrees C was to be achieved and maintained. Based on further pilot experiments described below, it was decided to use temperatures lower than 35 degrees C for the heat stress experiments. For these the prewarming time and on-off periods for the heating tapes were adjusted as required to maintain the appropriate temperatue.

D. Pilot Study of Effects of Carbon Monoxide Alone and In Combination with Heat Stress on Performance on a Two-Lever Chain Fixed Ratio Fixed Ratio Schedule of Reinforcement

Prior to the conduct of a pilot study of the investigation of heat stress on performance on a two-lever chain fixed ratio fixed ratio schedule of reinforcement, a group of rats was exposed to 35.0 degrees C. Gross observations were made during the heat exposure and rectal temperatures were taken prior to and immediately following the exposure. In general, the animals displayed little activity during the heat exposure. Because of the limited activity the animals displayed, a second group of animals was exposed to a lower temperature, 32.2 degrees C. These animals appeared somewhat more active during early minutes of the exposure but by the end of the period activity appeared less than normal. Rectal temperatures for both groups showed little change (Table 7). Because the animals appeared to tolerate the lower heat but still appeared somewhat disrupted, the pilot study was conducted using both 32.2 and 29.5 degrees C to determine the extent of disruption at these temperatures.

The animals used in this pilot study had been previously exposed to CO in a determination of the effects of CO and swim stress on performance of an FR30-FR30 schedule. Baseline performance on the chain FR30-FR30 was re-established. Following stabilization of baseline performance, the effects of carbon monoxide and heat stress on performance on the chain FR30-FR30 schedule was investigated. For this investigation animals were assigned

to CO conditions based on their past exposure history: nine controls from earlier experiments were used as controls; ten animals previously exposed to 200 ppm were exposed to 450 ppm, and ten animals from the 700 ppm group were re-exposed to that concentration. All animals were exposed to either 29.5 degrees C or 32.2 degrees C heat stress condition. The experimental design is summarized in Table 8. CO levels were randomly allocated to chambers and animals allocated to boxes within chambers. Each animal was exposed to either 0, 450, or 700 ppm CO at normal ambient temperature (24 degrees centigrade) and to the CO concentration in combination with an environmental temperature of 29.5 degrees centigrade or 32.2 degrees centigrade over the two weeks of the experiment. The CO exposures were of 75 min duration and began immediately after the animals were placed in chambers that had been prewarmed as described in Section II.D.

Results:. The results of this experiment are shown in Figure 19. At 0 ppm CO, a temperature of 29.5 degrees C had no effect on any of the performance measures examined. The combination of 29.5 degrees C and carbon monoxide decreased all measures to about 70% of baseline for 450 ppm and 40% of baseline for 700 ppm CO. This was in contrast to no effect at 450 ppm and a decrease to 60% of baseline at 700 ppm under ambient temperature conditions. Exposure to 32.2 degrees C decreased performance in all groups. With 0 ppm, 32.2 degrees C decreased performance to 50% of baseline. The combination of 32.2 degrees C and 450 or 700 ppm CO decreased performance to approximately 40% and 28% of baseline, respectively.

Based on these results it was decided that the final study of the effects of heat stress interactions with carbon monoxide on FR30-FR30 performance should be conducted at 30.5 degrees C. The 32.2 degrees C environmental temperatures produced greater effects in the 0 ppm group than was desirable for an interaction study. A temperature of 29.5 degrees centigrade appeared to have shifted the dose-responses curve for CO only slightly. Therefore, it seemed appropriate to conduct the final study at a temperature somewhere in between these two.

E. Determination of COHb Levels at Different Times Following CO Exposure

The purpose of this study was to obtain information concerning (COHb) levels following 1-hour exposures to CO at concentrations, 700 and 1250 ppm, that were used in behavioral studies. Thus, the behavioral effects could be evaluated with some idea of the animals physiological state at the end of the exposure period. COHb determinations were

made prior to exposure and 10 min, 1 hr, and 3 hrs after exposure.

Prior to these determinations the rats were given sufficient experience on a fixed ratio schedule of reinforcement to provide a background of ongoing responding during the CO exposure sessions. This procedure was included to make the conditions similar to those used in behavioral studies. This aim was not achieved since, in general, cannulated animals undergoing serial blood collections failed to perform on the schedule.

Following training on the fixed ratio schedule, the rats were cannulated with catheters in the carotid artery. Cannulations were performed 1 to 2 days prior to the scheduled exposures and blood collection for COHb determinations. A total of 12 cannulated rats were used for COHb determinations. All rats received 1 hr exposures to CO, six animals at 700 ppm and six at 1250 ppm and blood samples were collected from each rat at all time points considered. The animals were placed in the chamber and the CO flow started. CO flow was discontinued after 60 min. The animals were left in the chamber for an additional 10 min to allow chamber clearance and were then removed. Blood samples for COHb determinations were collected prior to the start of CO exposure, immediately upon removal of the animals from the chambers (10 min after offset of CO flow), and 1 and 3 hrs later.

Two inhalation chambers each holding six behavioral testing chambers were used for the experiments. Carbon monoxide concentrations were randomly assigned to the chamber. To limit the spacing of sample collection at each time point, the experiment was conducted in three replicates of four animals each (two at each CO concentration). So that the chamber loading was comparable to that used in the chained VR-FR study described below, three uncannulated animals (or a total of five animals) were in place in each chamber during the exposure sessions. At each concentration one animal was tested in each of the six behavioral testing chambers within an inhalation chamber. The decision as to which location within an inhalation chamber was tested in the first replicate and which in subsequent replicates was decided by a random selection process.

At each of the time points outline above, a heparinized 1 ml syringe was used to withdraw a small blood sample from the artery. Samples were immediately placed on ice and duplicate 3 ul aliquot were analyzed by the method of Rodkey et al (1979) (Appendix F).

Results: Figure 20 presents a linear regression plot for the COHb data with 95% confidence intervals. Numerical values for COHb are provided in Appendix G. At the earliest time point considered (10 minutes after the end of the exposure), mean COHb values of approximately 26 and 34% were obtained for 700 and 1250 ppm CO, respectively. COHb values were essentially 0 by 130 min after the CO flow was stopped. As can be seen from the figure there was some variability in the time at which the first postexposure samples were taken and no samples were taken earlier than 10 min after the stopping of CO flow as this design incorporated a 10 min period for chamber clearance following exposures. When the sampling time was more than 2 min after the scheduled time, the actual sampling time was used in the generation of the regression curves. Because of the planned 10 min clearance time and the at times delayed sample collection, these data do not permit a determination of COHb values immediately following exposure when the maximum values would be expected. Data from Rubin and Montgomery (1971) show a 20% drop (from approximately 62% to 42%) in COHb levels during the first 15 min after the end of a 4 hr exposure to 1000 ppm CO. From their data it would be predicted that the maximum COHb levels from the exposures reported here would be in the range of 50%.

F. Determination of COHb Levels after CO Exposures in Combination with Heat or Swim Stress

The experimental design is summarized in Table 9. A total of 60 rats were used for COHb determinations. Six exposure conditions were examined: 450 ppm CO, 700 ppm CO, swim stress + 450 ppm CO, swim stress + 700 ppm CO, heat stress and 450 ppm CO, and heat stress and 700 ppm CO. The swim stress condition consisted of a 30 minute period of forced swimming with a 4 g tail weight prior to the CO exposure. For the heat stress condition a temperature of 30.5 degres centigrade was employed.

For all exposures, the animals were placed in the chamber and the CO flow started. CO flow was discontinued after 75 minutes. For the heat stress condition, the chambers were prewarmed as described in Section II.D before loading them with animals. The animals remained in the chamber for 2 min after cessation of CO flow to decrease chamber CO levels and were then rapidly removed. Blood samples were taken immediately after the exposure, and at 15 minutes and 30 minutes after the exposure. For each condition three animals were sampled at each time point. Six animals were used as controls.

At each of the time points outlined above, animals were anesthetized with an i.p. injection of 2.25 mg Brevital®. A heparinized 3 ml syringe was used to withdraw a blood sample from the descending aorta. Samples were immediately placed on ice and duplicate 3 ul aliquots were analyzed for COHb by the method of Rodkey et al. (1979).

Results: COHb values for 450 ppm and 700 ppm CO at 2, 15, and 30 minutes after exposure are given in Figure 21 and numerical values are provided in Appendix G, Table G-2. The maximum COHb values were obtained at the 2 min post-exposure sampling time. These values ranged from 42-47% COHb for 700 ppm and 32-38% for 450 ppm CO. Three factor analyses of variance were performed for the COHb data utilizing CO concentration, stress condition (heat or swim stress), and time as the factors. These analyses indicated a significant effect for CO concentration, heat stress and swim stress but no significant interactions among these conditions. In practical terms, the differences between a given concentration of CO in the absence of stress and that observed when CO was combined with heat or forced swimming was small (6% or less). COHb levels significantly decreased over time for all conditions examined ($p < 0.05$). Comparisons of the rate of decrease for 450 and 700 ppm indicated no significant difference in the rates for the two concentrations. When the curves for CO alone were compared to those for which CO was combined with either heat or swim stress, with respect to rate of decrease of COHb over time, there were again no significant differences.

IV. EXPERIMENTAL INVESTIGATIONS

A. Effects of CO and Swim Stress on VR 5-FR 15 Performance

1. Methods

The first series of experiments employed a chain two lever variable ratio 5-fixed ratio 15 schedule of food reinforcement. For these experiments, the behavioral chambers were configured as shown in Figure 22. Following shaping the animals were put on a schedule which required one response on the left lever, which resulted in a light above the lever going on. A response on the second lever resulted in food presentation. The number of responses required on the second lever was gradually increased to 15. Appropriate increases in the FR value were determined for each animal individually. When this performance was established the response requirement on the left lever was increased first to VR 2.5, then to a VR 5.

Following stabilization of baseline performance, carbon monoxide exposures were conducted. The effects of carbon monoxide alone and in combination with swim stress on the VR 5-FR 15 schedule were investigated. Independent groups of five or six animals were used to study the effects of different concentrations of carbon monoxide. The animals were assigned to exposure conditions using a stratified randomization procedure. CO concentrations of 0 (N=5), 200 (N=6), 700 (N=5) and 1250 ppm (N=5) were investigated. The animals were initially exposed to carbon monoxide 1 hour per week for five consecutive weeks. Performance was evaluated during each of five exposure sessions. The sixth week, a period of forced swimming preceeded the carbon monoxide exposure. The animals were weighted with a 10 g weight and a period of 20 minutes of forced swimming was scheduled. One month later, baseline performance was reestablished and the animals behavior was evaluated during each of 5 consecutive daily exposures to carbon monoxide.

Two-factor, mixed model analyses of variance were utilized to evaluate the effects of CO concentration, days or weeks of exposure, and forced swimming on performance as measured by the number of reinforcers obtained. As the response measures yielded performance patterns comparable to those shown by reinforcers, separate analyses were not performed on these variables for this schedule. The computer storage of the data for this schedule was incomplete and therefore analysis was based on entries recorded by hand at the end of each session.

2. Results

Behavior on this schedule required approximately 30 days to stabilize. There was a wide range of variability across animals in performance. Prior to the initiation of CO exposures, responses on the lever for light presentation ranged from 565 to 2561; responses on the lever for food presentation ranged from 2622 to 8094. The number of reinforcers obtained ranged from 64 to 417. Figure 23 shows baseline data for individual animals prior to the first CO exposure. A complete table of baseline values for individual animals is included in Appendix H.

The concentration response curve for the first exposure to carbon monoxide is shown in Figure 24. Data shown are for three measures: responses on the variable ratio component of the schedule, i.e., responses on the lever for light presentation; responses on the fixed ratio component or responses for reinforcer presentations; and the number of reinforced responses. All data are plotted as percent baseline, with baseline defined as the mean of the three days pre-exposure. There was no significant effect on any

measures until 1250 ppm CO when all measures decreased to approximately 45% of control.

When the rats were given four additional exposures to the same concentrations of CO spaced at weekly intervals, comparable results were obtained across all 5 weeks (Figure 25). For all five exposures 1250 ppm CO produced a reduction in performance, as evaluated by all three measures, to approximately 45% of baseline; no other concentration had an effect. In the sixth week, forced swimming for 20 min was scheduled prior to exposure but in many cases the animals were unable to continue for this length of time. The actual time each animal swam is shown in Table 10. Forcing the animals to swim produced a decrease in performance in all groups including the control (0 ppm CO) group for which performance, as measured by the number of reinforcers obtained (Figure 26), was at approximately 30% of baseline on the day of forced swimming. Similar effects were seen on the other measures (Appendix H). When combined with swimming, CO at 1250 ppm reduced the number of reinforcers to approximately 7% of baseline as compared to the 47% of baseline performance observed for 1250 ppm CO alone. For groups exposed to the two lower concentrations, performance on the day of swimming was equivalent to or better than that of the 0 ppm group after swimming.

When the animals were subsequently given a series of five exposures to CO spaced at 1-day intervals, there was still no effect at the lower concentrations and the initial reduction in performance by 1250 ppm CO was progressively attenuated (Figure 27). On the last day of the five daily exposures, the number of reinforcers obtained was at approximately 70% of baseline in contrast to the 41% seen on the first of these five exposures. The attenuation of the effect of CO with repeated daily exposure was confirmed by analysis of variance in which both factors (CO concentration and days of exposure) yielded significant F ratios ($p < 0.01$).

B. Effects of CO and Swim Stress on FR30-FR30 Performance

1. Methods

Following shaping, the animals were put on a schedule which required one response on the left lever, which resulted in a light going on above the lever. A response on the second (right) lever resulted in food presentation. The number of responses required on each lever was gradually increased to 30 (chain FR30 - FR30). Following stabilization of baseline, the animals were rank ordered and the top 48 animals assigned to exposure conditions using a stratified

randomization procedure. After group assignment additional sessions were conducted to allow the animals to habituate to the behavioral test chambers to which they were assigned.

The effects of carbon monoxide alone and in combination with swim stress on performance on the chain FR30-FR30 schedule were investigated. Independent groups of animals were used to study the effects of three concentrations of carbon monoxide, 200, 700 and 1250 ppm. To evaluate the effects of swim stress and its interaction with carbon monoxide, each animal was exposed to the specified concentration of CO both in the absence of and following a period of forced swimming. Three sessions were required to complete one replicate of six animals/CO exposure group with each animal being forced to swim prior to one of the three sessions in which it was tested. The allocation of animals to exposure condition and swimming condition for a single replicate is illustrated in Table 11. Two such replicates were conducted to yield 12 animals at each CO concentration.

The swim condition consisted of forced swimming for 30 min with a 4 g tail weight. An exposure session was timed from the onset of CO and lasted 75 min. The performance session was initiated 15 min after the onset of CO and continued for 60 min.

The data for this shedule were analyzed by a multivariate mixed model analysis of variance to identify effects of CO concentration, swim stress, and concentration x swim stress interactions for a series of behavioral parameters. Subsequent univariate analysis was performed to identify the behavioral parameters yielding significant effects. A statistical trend analysis was performed to evaluate the time course of effects utilizing a multivariate analysis of variance for repeated measures.

2. Results

The animals were trained on this schedule for approximately 2 1/2 months before exposures were conducted. Summary tables for baseline performance prior to each exposure are given in Appendix I and tabular summaries and statistical analyses for exposure periods are in Appendix J.

Carbon monoxide at 700 and 1250 ppm reduced responding in both components of the FR30 - FR30 schedule and resulted in a corresponding decrease in the number of reinforcers obtained (Figure 28). At 700 ppm the reduction in responding and reinforcers was to approximately 45% of baseline, whereas, 1250 ppm CO reduced responding and reinforcers to 8-9% of baseline. The effects of CO were virtually the same using responses in either component of

the schedule or the number of reinforcers obtained as the measure of performance.

Forced swimming reduced responding and the number of reinforcers to 80 - 90% of baseline values in rats not exposed to CO (0 ppm group). The combination of CO exposure and forced swimming resulted in fewer responses and fewer reinforcers than either condition alone (Figure 28). For groups exposed to 200, 700, and 1250 ppm, the number of reinforcers was reduced to 73%, 21%, and 0% of baseline. Equivalent effects were observed for the two response measures of performance. The absence of a significant interaction between CO exposure and forced swimming indicates that the effects of the two treatments were additive.

To determine whether there was a delay in the time to initiate responding following CO and CO in combination with swim, the time to the first response was examined. This variable indicated a significant effect for CO at 1250 ppm and a significant effect of forced swimming ($p < 0.01$). The effect of these conditions was to increase the time to the first response (Figure 29).

Examining the time course of effects on responding on the lever for food indicated that 1250 ppm CO significantly decreased responding during the first 10 min of the performance session (15-25 min after the start of exposure) and abolished responding thereafter. Responding was not significantly altered by 700 ppm CO until 35-45 min after the start of exposure (Figure 30). A comparable pattern of effects was also obtained for responses on the lever for light presentation (Figure 31) and on number of reinforcers (Figure 32).

For rats not exposed to CO, responding for food tended to decrease slightly or stay the same over time within the session (Figure 30). After swimming, responding was substantially reduced (approximately 50%), however, responses increased over time within the session and by the end of the session the reduction was only 20%. Thus, as might be expected, forcing the animals to swim had the greatest effect during the early part of the session when fatigue would be most severe.

C. Effects of CO and Heat Stress on FR30-FR30 Performance

1. Methods

The chain FR30-FR30 used in this study and the procedure for training and selection of animals is identical to that described in section IV.-B.1. Following stabilization of

baseline, rats performing on the FR30-FR30 schedule of reinforcement were exposed to carbon monoxide alone and in combination with heat stress. Heat stress was defined as a chamber temperature of 30.5 degrees C. Groups of 12 animals each were exposed to one of four CO concentrations (0, 200, 450 or 700 ppm) alone and in combination with 30.5 degrees C. The exposures were conducted for two consecutive weeks. The allocation of animals to exposure condition and heat is shown in Table 12. Half the animals in each group received the heat exposure in the first week and the remainder in the second week. For this experiment the chambers were prewarmed according to the following procedures. The air supply system, heater, heating tapes and humidifier were turned on and the chambers allowed to warm. When the chamber had reached the desired temperature, the heater, heating tapes and blower were turned off and the animals placed in the behavioral chambers. The CO was turned on and the temperature maintained at 30.5 degrees C for the duration of the session. A 15-minute period of exposure to CO and the heat stress preceeded the beginning of the behavioral session. Performance on the FR30-FR30 schedule was assessed for an additional 60 minutes during exposure to both CO and/or heat stress.

The statistical analysis for this schedule was the same as that used for evaluating the effects of CO and swim stress on FR30-FR30 performance except that the second factor was heat stress.

2. Results

Summary tables for baseline performance prior to each exposure are given in Appendix K and tabular summaries for exposure periods are in Appendix L.

Considering first total session performance, the multivariate analysis indicated significant overall effects for both CO concentration ($p < 0.003$) and heat stress ($p < 0.0001$).

The univariate analysis indicated that the CO effects were limited to the high dose and that the variables affected included responses on the lever for light presentation, responses for food, and reinforcers (Figure 33, $p < 0.0001$)) all of which were decreased by 700 ppm CO. Expressed as a percentage of baseline performance, the reduction achieved by 700 ppm CO was to approximately 45%.

Heat stress also resulted in decrements of these three measures of performance (Figure 33, $p < 0.0001$ for responses on the lever for light, responses for food, and reinforcers). For the group exposed to 0 ppm CO, the

reduction by high environmental temperature was to approximately 55% of baseline performance. Heat stress potentiated the effects of CO. At 450 ppm performance during the heat exposure was approximately 43% of baseline and at 700 ppm approximately 30% of baseline values. However, heat did not differentially affect the control and CO-exposed groups, i.e, there was not a significant interaction between these conditions when total sessions performance was considered.

The time course for effects of CO and heat on responses on the lever for light (Figure 34), responses on the lever for food (Figure 35), and for reinforcers (Figure 36) again suggest comparable effects for the three measures of performance. The time course analysis indicated that the effects of CO and of heat become more pronounced over time within the exposure session. At 700 ppm animals exposed to heat had virtually quit responding by 55 minutes into the exposure session and a comparable cessation of responding was also present at this concentration for animals not exposed to heat. Heat alone (0 ppm group) resulted in linear decreases in responding over time but there was still responding present at the end of 75 min of exposure. In contrast to the total session performance, the time course analysis indicated a significant interaction between CO and heat stress ($p < 0.001$). The nature of this interaction was a decrease in performance of the 450 ppm group during the last 30 min of exposure to heat.

D. Effects of CO and Heat Stress on Reaction Time Task Performance

1. Methods

The purpose of this study was to investigate the effects of two concentrations of carbon monoxide in combination with high temperature (30.5 degrees C) on a reaction time task. This task required that the animal depress a lever in the presence of a stimulus light onset and continue holding the lever down until the presentation of a second stimulus, the onset of a series of three lights. Lever release following the presentation of the second stimulus was followed by food presentation. Early lever releases, i.e. prior to the onset of the three lights resulted in a time out. Presentation of the first stimulus (S1) occurred on a variable ratio 5-sec schedule. The time required for lever depression, time from onset of S1 until onset of S2 (three lights) occurred on a variable ratio 2-sec schedule (0.5 to 3.5 sec). The terminal time out value was 80 seconds. Reaction time was defined as the length of time following the onset of the second stimulus until the lever was released.

For this schedule the chambers were configured as shown in Figure 37. The animals were hand shaped to depress the lever and then release it in order to obtain food reinforcement. During shaping the animals were required to hold the lever down for a duration of .25 sec. During shaping both the houselight and S1 remained on continuously. When the animals had acquired the response they were given experience on a schedule which required that the lever be held down for gradually increasing durations. Hold down times were progressively increased on an individual basis to at least 2.5 sec. The animals were then exposed to a modification of the terminal schedule, with the timeout values being progressively increased to the terminal value of 80 sec.

Following stabilization of baseline performance, the effects of carbon monoxide and heat stress on performance on the reaction time task were investigated. Using a stratified randomization procedure animals were assigned to one of three conditions: 0 ppm, 450 ppm, or 700 ppm CO. Each animal was exposed to either 0, 450 or 700 ppm CO at normal ambient temperature (20-24 degrees C) and to the CO concentration in combination with an environmental temperature of 30.5 degrees C. The experiment was conducted for two consecutive weeks. The experimental design is shown in Table 13. The procedure for generating the heat stress has been described above.

2. Results

Among the tests considered, the reaction time task was the most difficult to train. The animals were trained over a period of approximately 6 months. Of 60 animals that initiated training, 42 never achieved stability of performance adequate to permit testing. Baseline performance for several of the animals utilized in testing reflected larger shifts in baseline performance between the first and second test week than had been characteristic of the other tests (Appendix M). In that these occurred in both control and CO-exposed rats, it is likely that these shifts further indicate a lack of adequate baseline stability.

Exposure to 450 ppm CO caused a significant increase in reaction time as compared to times observed in the air control group (Table 14). Although a trend in the same direction was observed in the 700 ppm group, the effect was not statistically significant.

Other variables considered in the analysis of the data for the reaction time test included the number of correct lever presses (depression in the presence of S1), the number of

reinforcers or correct lever releases (releases of the lever in the presence of S2), and the number of timeout periods resulting from premature release of the lever. All of these variables reflected significant effects of CO concentration either in terms of total session performance or as changes in performance over time within the sessions. The pattern of these effects suggest that they result from a general decrease in responding. Considering the total session, correct lever presses were reduced only by 700 ppm CO ($p < 0.007$, Table 14). The analysis of the different 10 min segments of the session indicated that the suppression of correct lever presses by 700 ppm was during the last 30 min of session for 700 ppm CO and also indicated response suppression, restricted the final 10 min of performance, for 450 ppm CO ($p < 0.004$, Figure 38). Reinforcers obtained was not signifcantly altered by CO based on total session analysis (Figure 39), however, the time trend analysis indicated a significant suppression for 450 ppm for the last 10 min segment ($p < 0.006$) and for 700 ppm for the last 20 min of the session ($p < 0.0001$) (Figure 40). The decrease in timeouts also reflects this response suppression in that both the analysis of the total session ($p < 0.0003$, Figure 41) and the time trend analysis indicated decreased timeouts with the decrease being significant for 700 ppm for the last 40 min of the session ($p < 0.02$ for the third segment, $p < 0.0001$ for the final three segments).

Regardless of the performance measure considered, there were no significant effects of heat identified by reaction time testing nor did heat interact with CO to alter performance of this task. Animals exposed to 0 or 700 ppm CO tended to obtain more reinforcers during heat exposure than when tested at ambient temperature (Figures 39 and 40), however, this trend did not achieve statistical significance.

V. DISCUSSION

Of the schedules considered, the FR30-FR30 was the most sensitive to the disruptive effects of carbon monoxide. When CO was combined with stress the nature of the stressor was an important determinant of the pattern and extent of disruption. Fatigue stress, induced by forced swimming, produced a pattern of disruption which suggested an all or none phenomenon, with a progressive recovery occurring during the session when combined with low levels of CO. In contrast, heat stress produced a progressive decrease in responding over the course of the session which was greater when combined with CO.

The initial selection of ratio schedules was based on a review of the literature which suggested that the most

disruptive effects of carbon monoxide occur on high rate schedules while schedules which engender a low response rate remain intact until very high CO exposures. The use of a chained schedule was selected because the nature of this schedule is such that due to its temporal and spatial relationship to primary reinforcement the first component may be differentially affected as a consequence of chemical insult. However, for CO and the stressors considered, the two components of the schedules were affected comparably.

The first schedule selected for investigation was a variable ratio-fixed ratio schedule using two levers. Only at 1250 ppm CO was performance on this schedule affected. Several possibilities were considered as a basis for the failure of 700 ppm CO to disrupt performance in this experiment. The first possibility was that at this concentration a 1-hr exposure was too short to result in disruption. On this basis, later experiments included a 75-min exposure period with the first 15 min of exposure occurring prior to assessment of performance. However, in these later experiments, performance was decreased within 25 min after the start of exposure to 700 ppm CO which contradicts the interpretation that the length of exposure was the critical factor.

Another factor which may have contributed to the lack of effects at the lower concentrations on the variable ratio 5-fixed ratio 15 schedule was that the ratio values for the two components may have been too low to provide a sensitive measure. It was on this basis that the chain schedule was modified to include higher response requirements. As noted above, a fixed ratio requirement of 30 responses was used in both components. Performance on the chain FR30-FR30 schedule was disrupted during exposure to 700 ppm CO and virtually abolished at 1250 ppm. Thus, modification to include a higher response requirement increased the disruption observed and suggested that a more meaningful selection of doses would include a dose between 200 and 700 ppm. Thus, for subsequent experiments a CO concentration of 450 ppm was added. This concentration, which for a 75-minute exposure produced COHb levels of 30% 2 min after exposure termination, had no effect on performance of the chain fixed ratio-fixed ratio schedule. COHb levels for 700 ppm CO were on the order of 40%, a value that is consistent with those reported by Ator and coworkers (1976) and Montgomery and Rubin (1971). From these data, it would appear that COHb levels in excess of 30% are required for disruption of FR30-FR30 performance, however, the experiments did not include a direct assessment of the correlation between COHb and performance decrement over time.

Reaction time was of interest in that findings using human subjects suggested a disruption in vigilance as a function of exposure to CO (Beard and Grandstaff, 1970; Fodor and Winneke, 1972). The task selected was based on a reaction time procedure used previously by Stebbin and Lanson (1962). Those investigators first conditioned rats to depress a telegraph key in the presence of two neon lights (S1). During subsequent discrimination training, onset of the lights was contingent upon 15 sec of no responding in its absence. In the presence of S1, a key press of greater than 0.5 sec duration produced a 4000 cps tone. Key release during both light and tone was reinforced. Following reinforcement, the 15 sec response free interval began again. Although based on these procedures, the design used in the above experiment differed in several respects. To avoid the animals developing patterns of responding based on elapsed time as opposed to the stimulus presentations, S1 was presented on a variable interval schedule. The length of time the lever was required to be held down was also varied and, ranging from 0.5 to 3.5 sec, was longer than the time used by Stebbin and Lanson (1962). Early releases resulted in a timeout period.

Shaping of the reaction time task proved difficult and required extensive time investment. An important factor might have been the stimuli used. Due to the arrangement of chambers within the inhalation chamber, lights were used as stimuli for both S1 and S2, i.e., as cues for lever depression and release. The use of a light stimulus and a tone stimulus may have been more effective. Despite these difficulties and extensive between and within animal variability, the reaction times reflected an effect (increase) of CO at 450 ppm and both concentrations considered reduced responding near the end of the session. The lack of a clear concentration-response relationship with respect to the effects of CO on reaction time raises a question concerning the reliability of this phenomenon.

Due to the extensive training involved in behavioral studies employing schedules of reinforcement, typically repeated measures designs are used. This presents a potential problem with tolerance development. Using a fixed consecutive number schedule, Ator and Merigan (1980) reported complete recovery of response rates when rats were exposed to 700 ppm CO for 75-min over five consecutive days. After a two week CO free period performance was again sensitive to CO. Under the one hour exposure conditions used in the studies described above it was found that five consecutive weekly exposures to 1250 ppm CO produced consistent decreases in performance across the five weeks. Five consecutive daily exposures, administered one month later, however, did result in partial tolerance development.

Although in contrast to Ator and Merigan's finding, tolerance was not complete after five days of exposure, additional exposures would be predicted to result in complete tolerance.

Attempts to use stress as an independent variable in behavioral studies have been limited. Following review of the literature two stressors were selected: forced swimming, which can be considered a method for inducing fatigue, and heat stress, which represents a naturally occuring phenomenon which can interact with prevailing conditions to cause severe stress to the animal.

Physiological investigations of the effects of exercise on rodents have shown that rats can swim for 50 hours in water near body temperature (Richter, 1957). Rats forced to swim in cold water show a rapid decrease in body temperature and exhaustion occurs when body temperature falls to approximately 26 degrees C (Dawson et al., 1968). Due to the physiological and metabolic changes which occur as consequence of swimming in hot or cold water, factors which exhaust an animal under these conditions may not be those associated with muscular fatigue (Baker and Horvath, 1964). In the present study cold water was not used because this produces an extreme stress on the animal and the effort was to produce muscular fatigue without excessive psychological stress.

The conditions and duration of swimming which could result in a fatigued but not incapacitated animal were not available in the literature and thus, the parameters for swim stress were determined in a number of pilot investigations prior to the use of this procedure in testing for interactions with carbon monoxide. In an attempt to behaviorally quantify the extent of fatigue, two behavioral tests were employed following various swim conditions, the hindlimb extensor response (Cabe and Tilson, 1978) and the fore- and hindlimb grip strength measure (Meyer et al., 1979). These tests had been used successfully to assess the effects of toxic agents and it was anticipated that the nature of the measures was such that they may be sensitive to muscle fatigue following swimming. Although the hindlimb extensor response showed significant differences between controls and animals that had been forced to swim, the variability in the test raised questions as to the validity of the results. This test requires the delivery of an airpuff stimulus to the animal and there was a difficulty in repeatedly delivering a consistent airpuff stimulus without developing a mechanical procedure for delivery of the stimulus.

In contrast, the fore-and hindlimb grip strength does not require the administration of an air puff stimulus to elicit the response. A second advantage of this test is that it measures both fore-and hindlimb grip strength. If weighted and forced to swim in a confined area, rats tend to use their forepaws extensively. This is in contrast to the normal swimmming pattern for adult rodents in which the hindlimbs are used almost exclusively except during attempts to escape from the water. Therefore, a measure of fatigue in terms of both forelimb and hindlimb strength seemed more appropriate. Efforts to show a quantitative relationship between duration of swim time and fatigue as measured by the fore- and hindlimb grip strength test were unsuccessful. This could not be attributed to inappropriate execution of the test since for a phenobarbital control group results comparable to those reported previously for this drug were replicated (Meyer et al., 1979). This suggested that the fore-and hindlimb grip strength test was not a good measure of muscular fatigue following forced swimming.

Unfortunately, variability across animals was apparent during swimming. One source of variability across animals was a difference in responsiveness of the animals upon placement in the swim chambers. Occasionally, an animal would panic and it was necessary to remove the animal from the water to prevent drowning. A similar phenomenon has been reported by Richter (1957) and it was suggested that this was related to the emotional state of the animal. One possibility for alleviating this problem would be to pretrain the animals. This was not used because of the performance improvement in swimming which occurs as a result of training would be expected to slow down the development of fatigue. Thus, the time investment would become greater both because of training time and the necessity of having the animals swim for a longer period prior to testing.

Another method for inducing fatigue in rats is the use of treadmill running. McMaster and Carney (1983) have shown enhanced sensitivity to organophosphates and psychomotor stimulants using this procedure. In the initial selection of a method for inducing fatigue, treadmill running was not selected because of the necessity of using shock as a stimulus to maintain running.

When a period of forced swimming occurred prior to the exposure there was a decrease in responding in all groups. This was a generalalized effect in that all measures showed comparable decreases. The effects of forced swimming appear to represent a threshold phenomenon. Where animals were affected the typical result was a complete suppression of responding. The pattern of responding following swim stress showed a recovery of responding beginning approximately 20

minutes into the exposure in the controls and the 200 ppm CO group. This could represent a recovery from the fatigue effects of the swimming. Another possibility is that immediately following swimming the animals engage in grooming behavior which interferes with responding. When grooming decreases, an increase in responding then becomes apparent.

Increasing the environmental temperature produced a pronounced effect on performance on the FR30-FR30 schedule. This was manifest as a decrease in responding both alone and in combination with CO. The pattern of disruption, however, differed from that seen with swim stress. There was a progressive decrease in responding over time, which showed a dose dependent relationship. In contrast, heat stress did not significantly affect the reaction time task.

Increasing environmental temperature produces compensatory changes in the animal which may be physiological or behavioral. One example of this is the spreading of saliva over the animals body as a means of cooling. This behavior would interfere with lever pressing and as the discomfort incurred as a result of the heat increases, this behavior might become predominant, thus disrupting performance. Alternatively, heat may have decreased appetite (Hinde, 1970) making food a less effective reinforcer. Regardless of the mechanism for these effects it should be noted that they occurred at an environmental temperature that did not alter rectal temperature.

Explanations based on alternative behaviors for alleviating the discomfort from heat exposure do not adequately account for the larger effects seen when CO is combined with heat. These effects may be due to modifications in intake, absorbtion, distribution, or excretion of CO. There was no extensive effort in the present studies to determine the underlying mechanism for the CO-heat interaction. Heat produced significant but relatively small changes in the levels of COHb present following the exposure, however, since increases were not apparent at the earliest time point considered it is unlikely that these account for the interaction. The mechanism accountable for these changes is unquestionably an area for further research.

The lack of effect of heat stress on the reaction time task could be attributed to several factors. This task has not been extensively investigated and the design used in the present study is somewhat novel. Further behavioral investigations, including the use of different stimuli, would be required to establish the sensitivity and the applicability of the reaction time task. There was a suggestion of an increase in responding on this schedule

under heat only conditions. A similar effect of heat, that is an increase in response rate, has been reported by McGuire and Annau (1980) in rats performing on an avoidance schedule. During high temperatures, responding was increased and consequently shocks received decreased. This rate increasing effect under conditions of high temperatures deserves further investigation.

Based on the findings of this reasearch several areas can be suggested for further investigation. As mentioned above, heat stress appears to be an important factor in determination of responsiveness to CO and, most likely, other gases as well. The consistency of this and the underlying mechanism deserve attention. The approach to be used with schedules of reinforcement, due to the time factor in training animals, would most efficiently involve more extensive testing of the animals once trained. This of course also creates potential problems relative to residual and cumulative effects of the exposure conditions. While fixed ratio schedules appear to be most sensitive and have the additional advantage of being easily shaped, further research on novel schedules like reaction time might ultimately provide more sensitive measures. More extensive use of repeated measures designs might, in part, compensate for the time investment involved in training.

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FIGURES

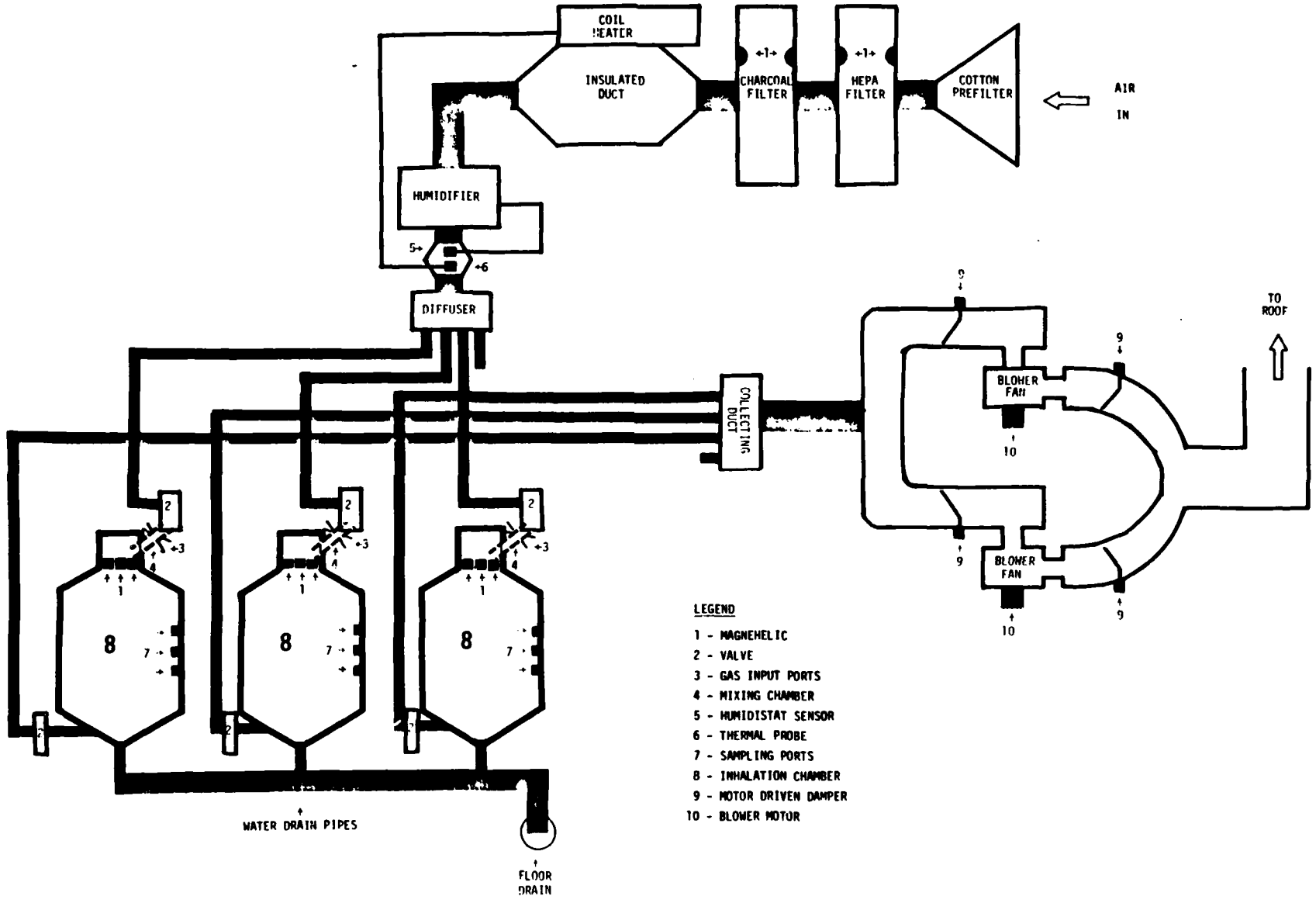


Figure 1. Air Supply, Preparation and Exhaust System.

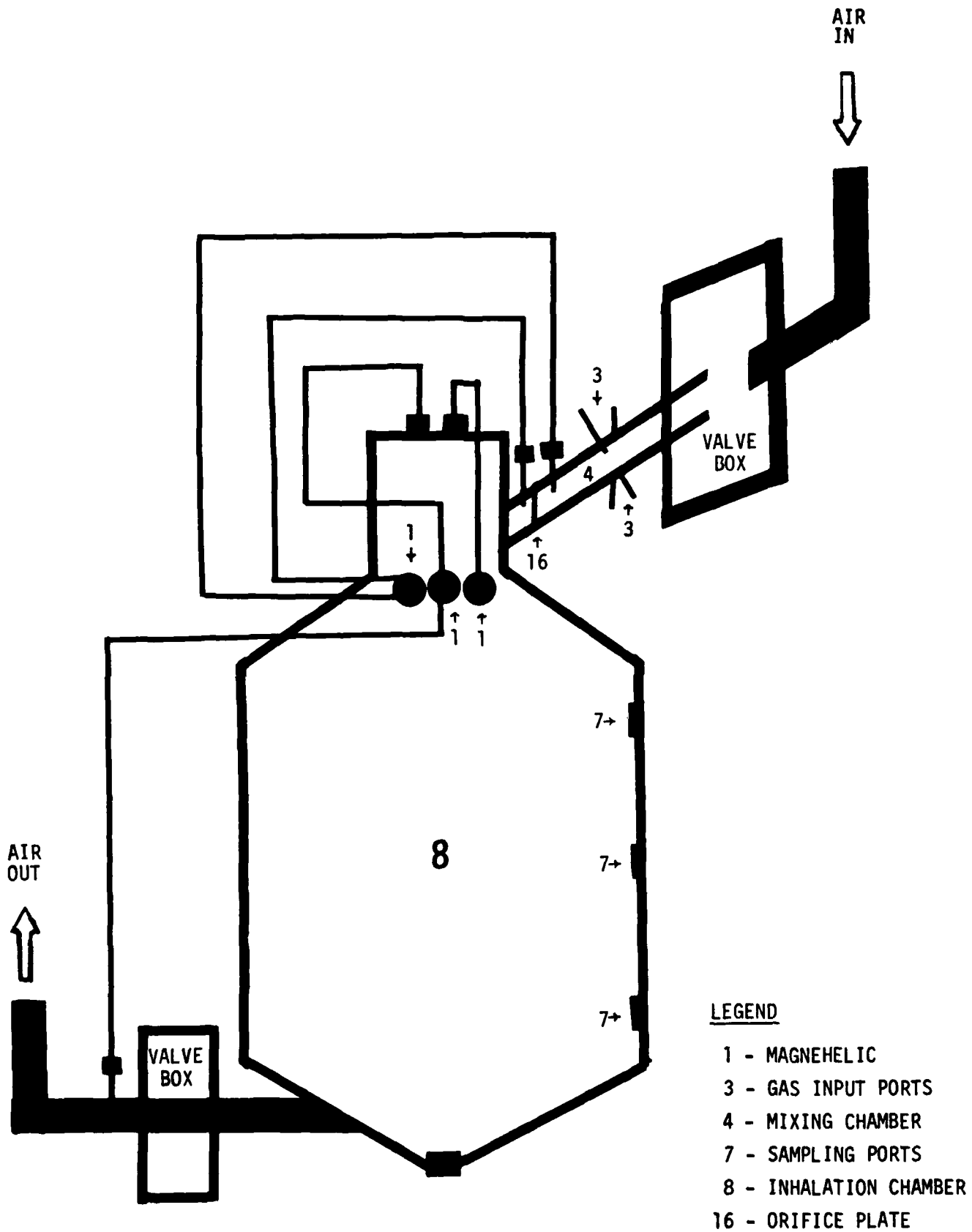


Figure 2. Inhalation Chamber with Associated Air Flow and Pressure Controls.

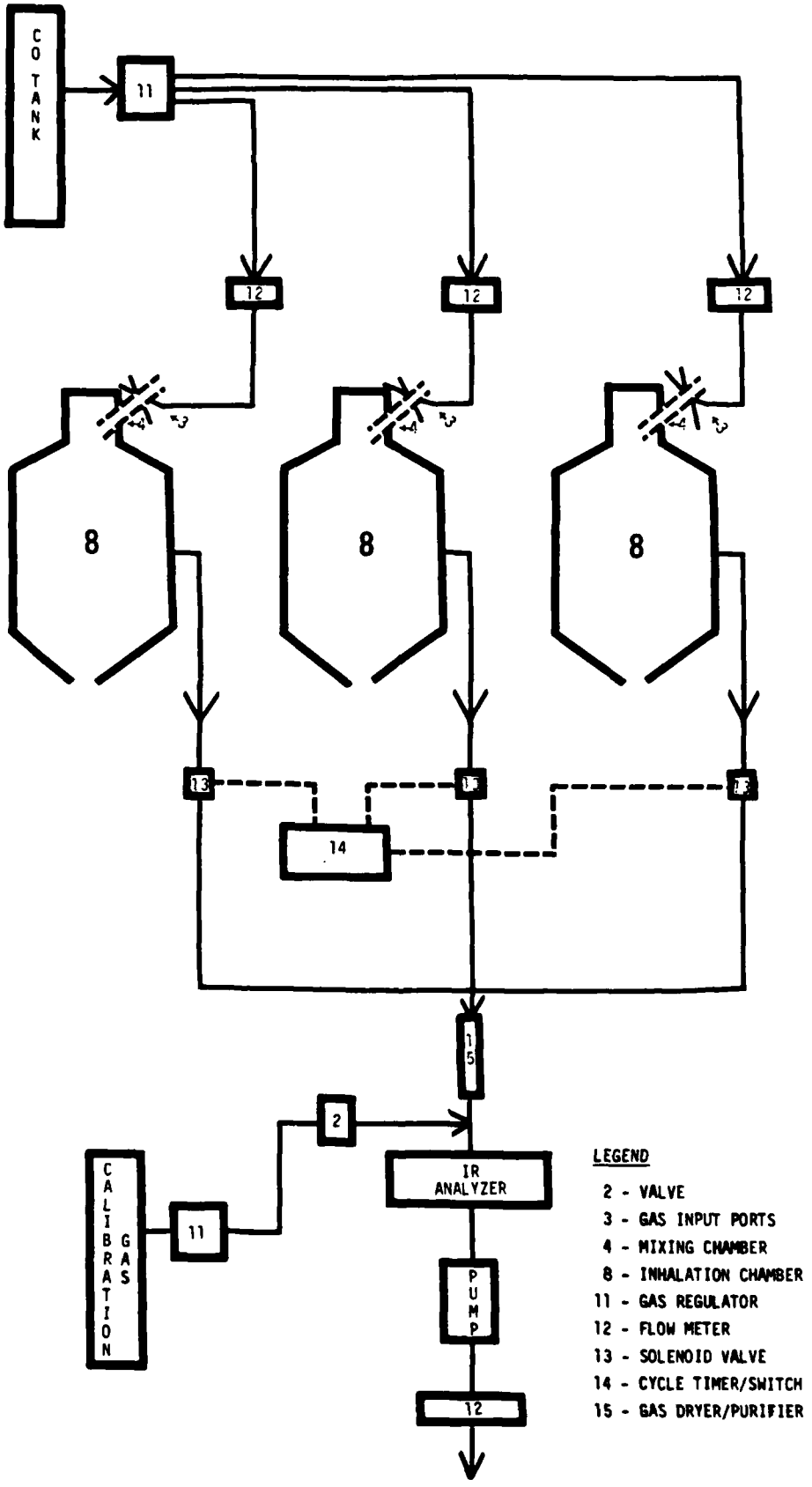


Figure 3. Carbon Monoxide Generation, Sampling and Analysis Systems.

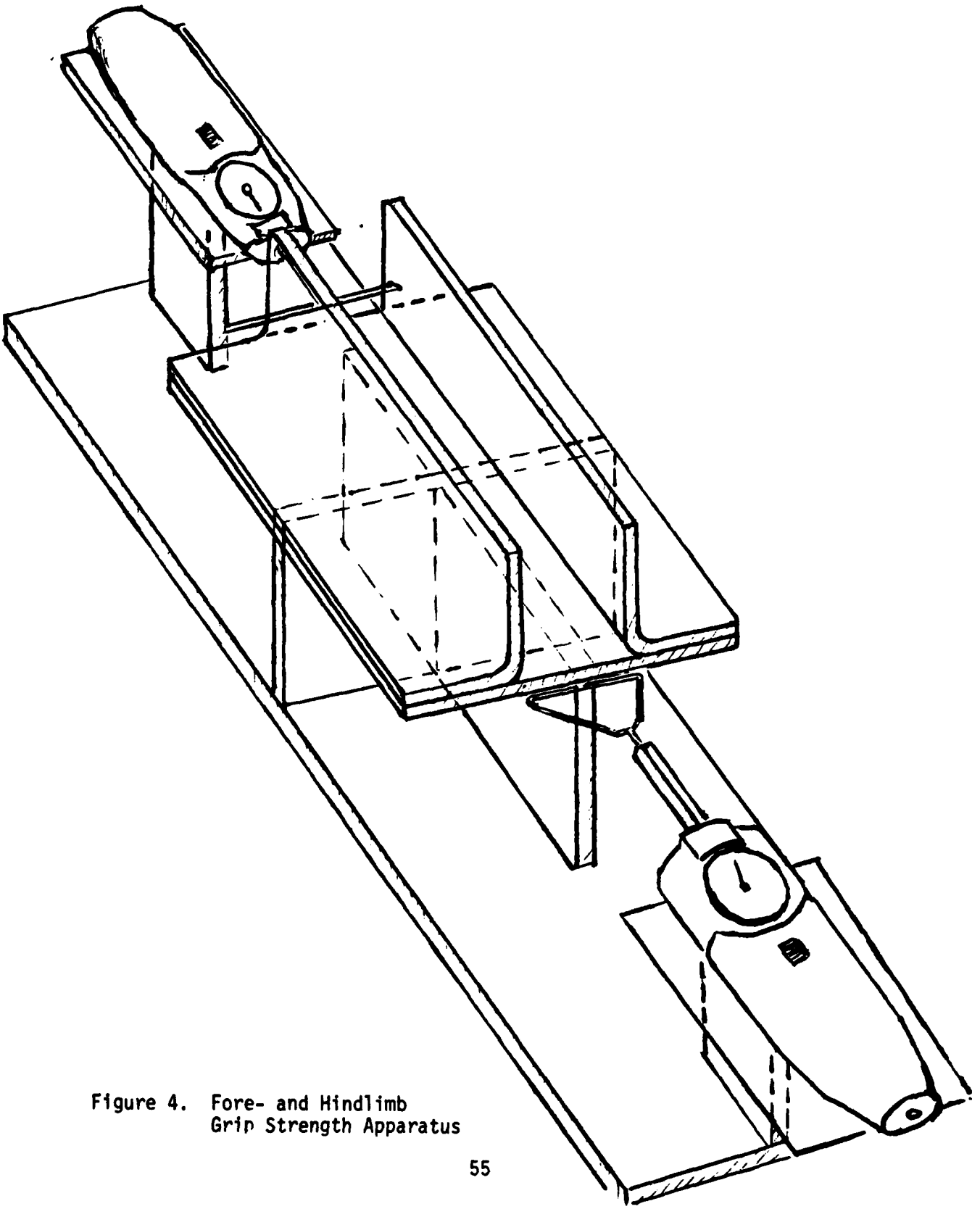
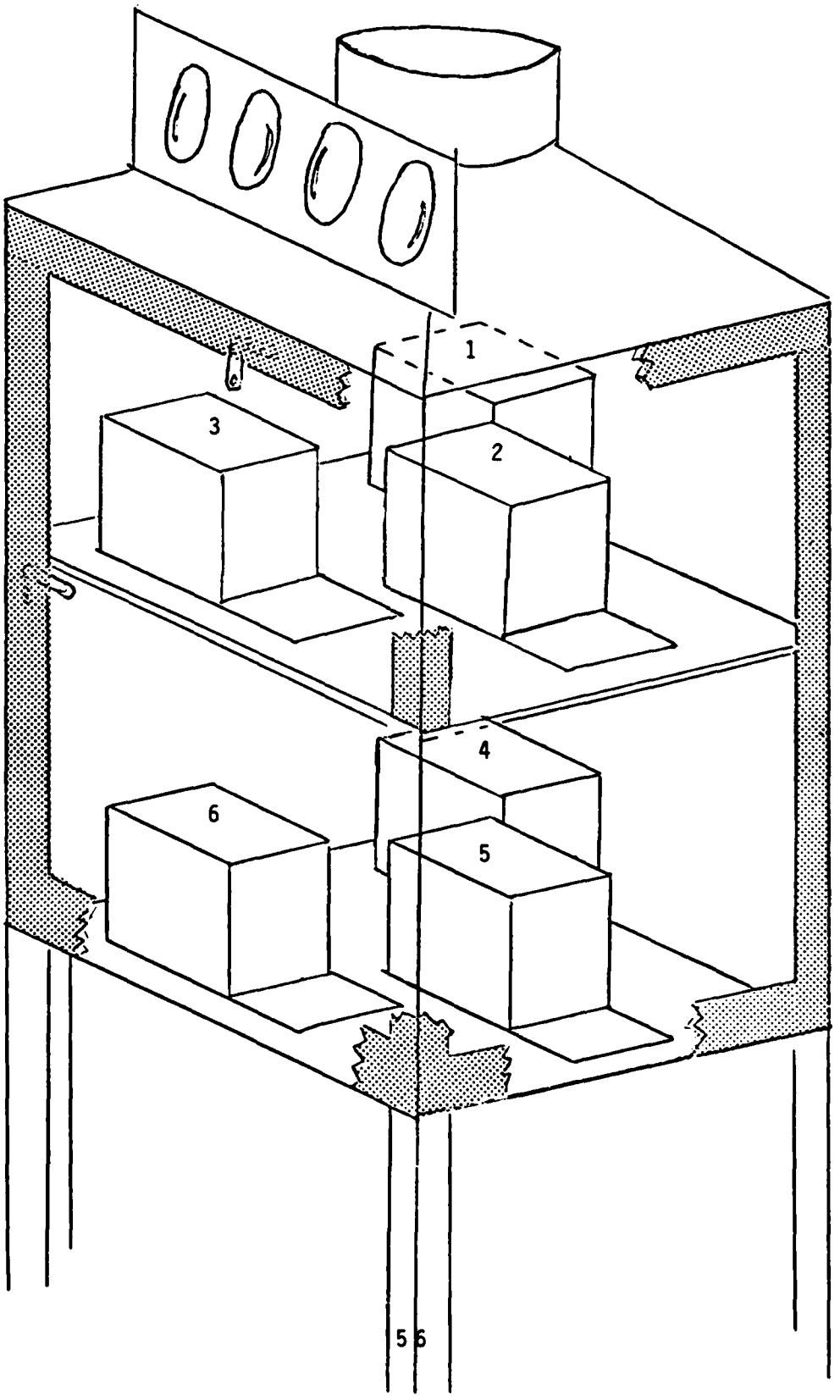


Figure 4. Fore- and Hindlimb Grip Strength Apparatus

Figure 5: Schematic representation of an inhalation chamber with the six behavioral chambers in place.



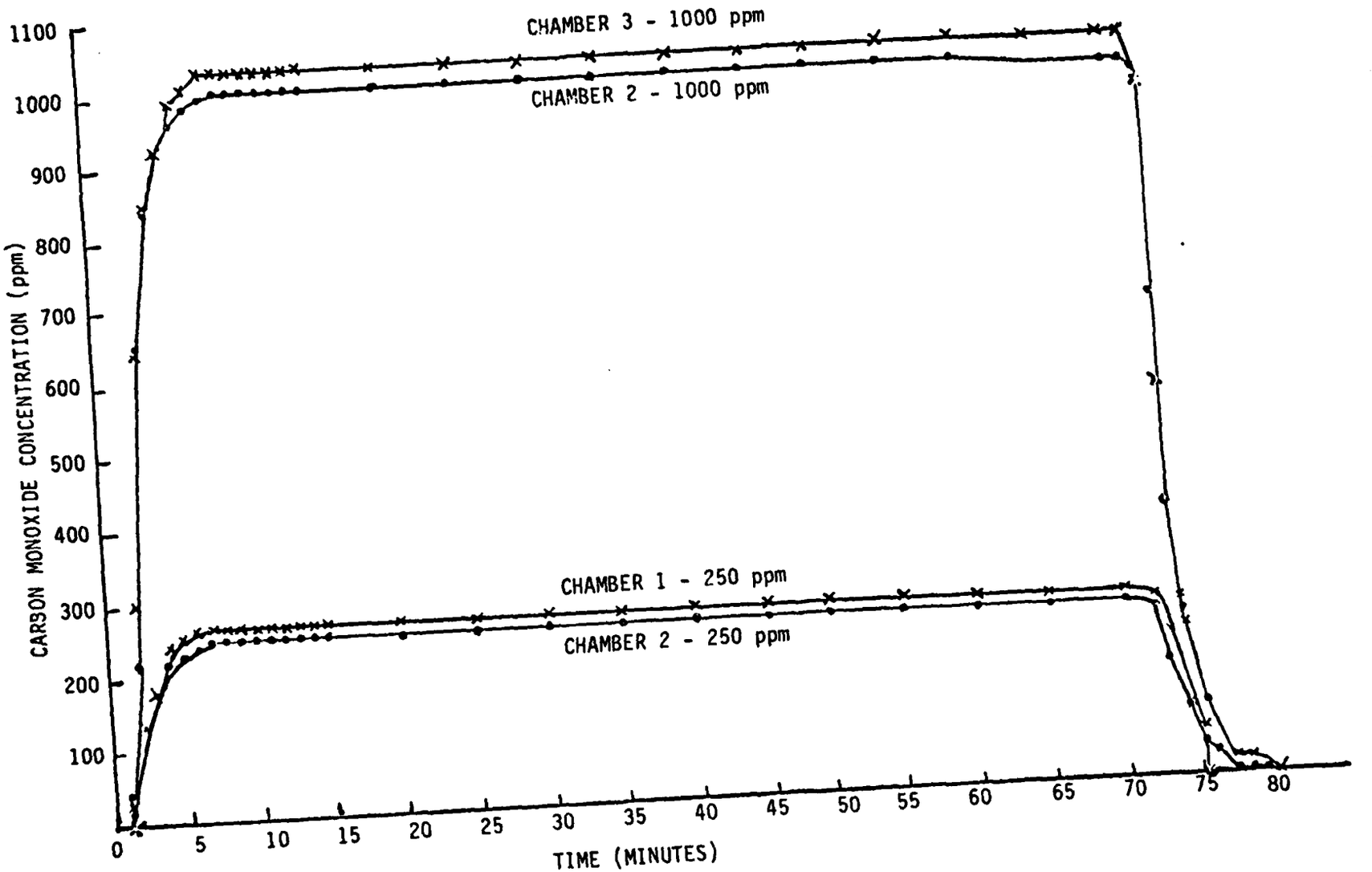


Figure 6 : Carbon monoxide concentrations as a function of time for target concentrations of 250 and 1000 ppm.

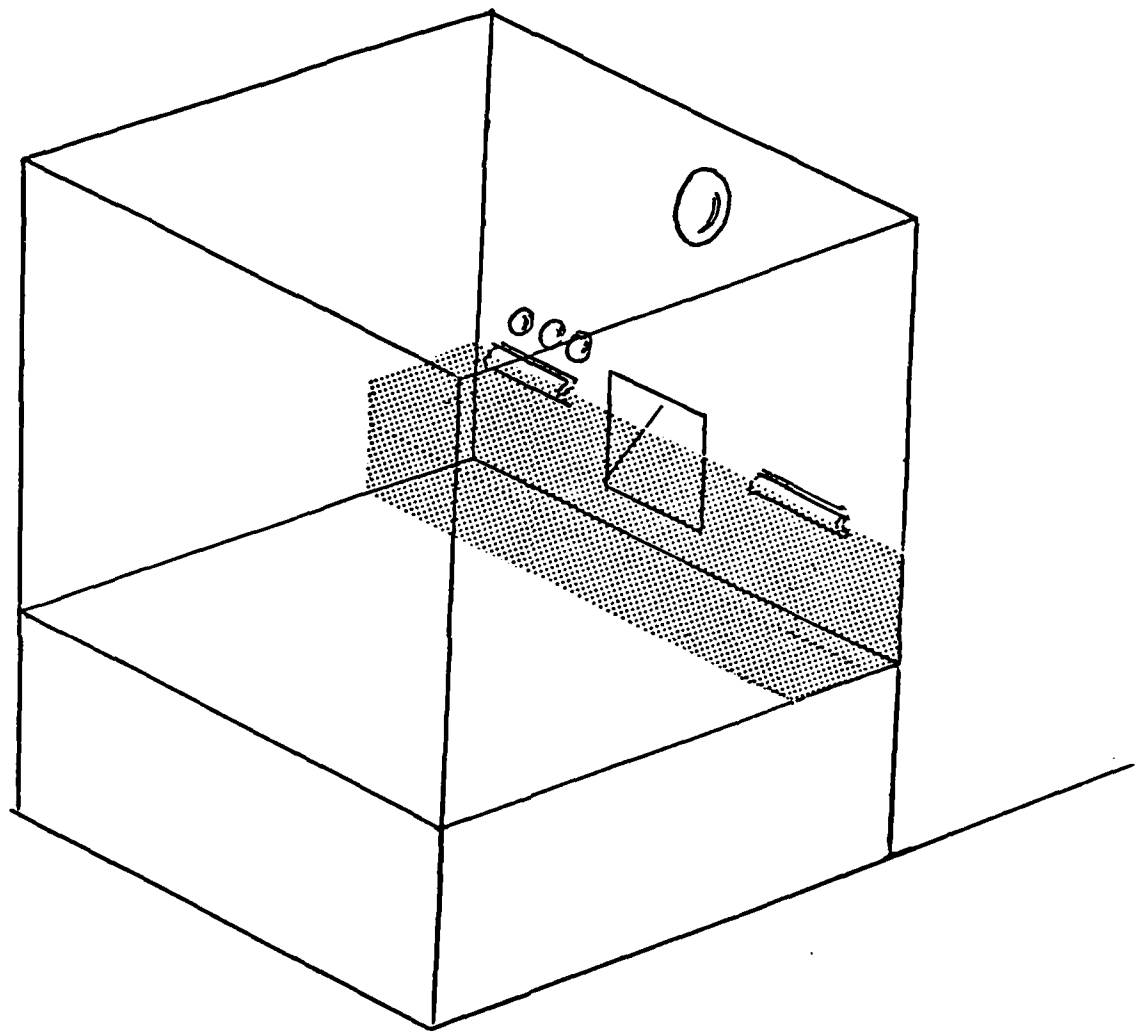


Figure 7: Schematic representation of a behavioral chamber. Shaded portion indicates area within which the sampling probes were located.

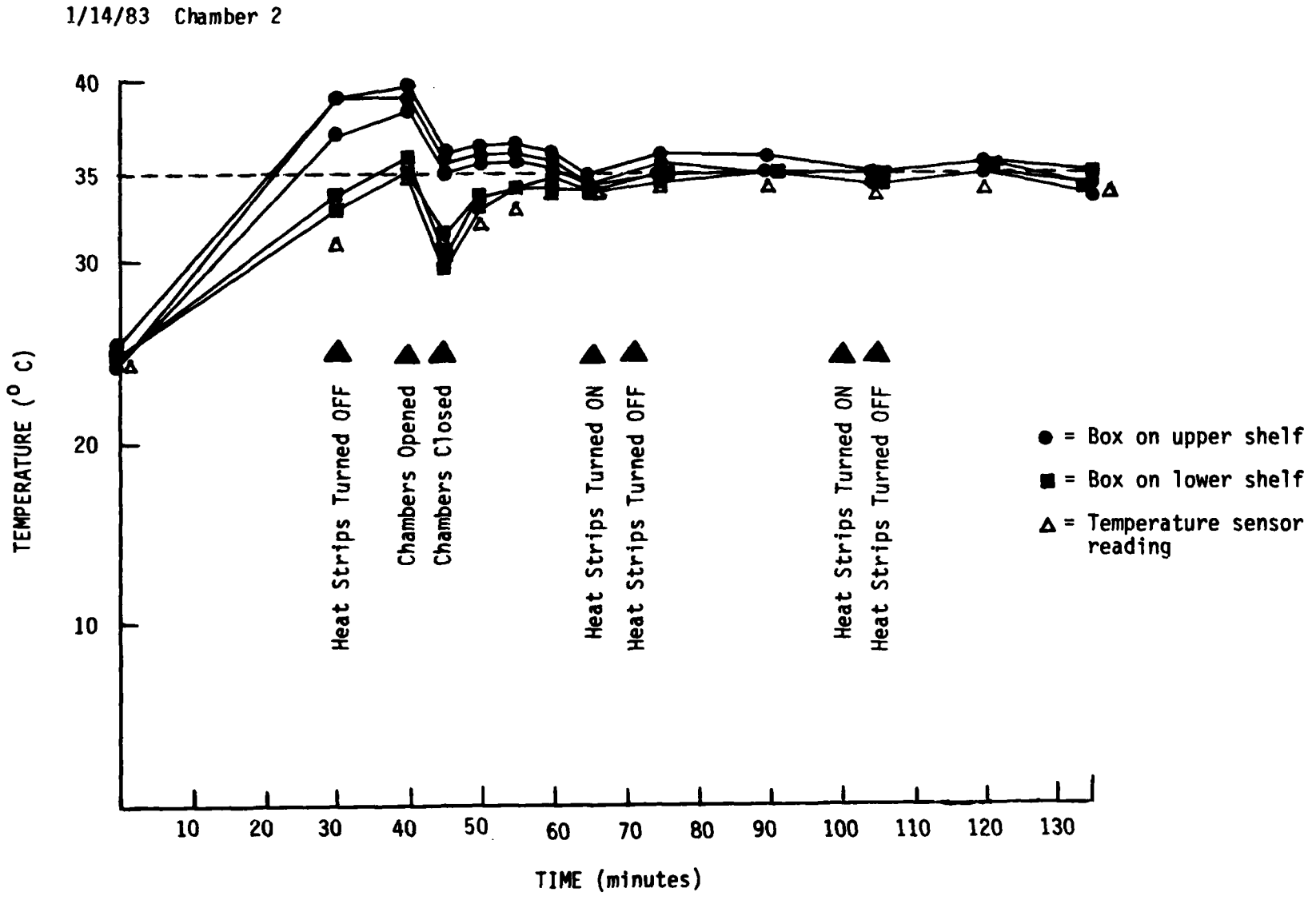


Fig. 8 . Temperatures at Seven Locations within Chamber 2 over Time Within the Session. The seven locations were one in each of the six behavioral testing chambers plus one at the site of the permanently mounted sensor.

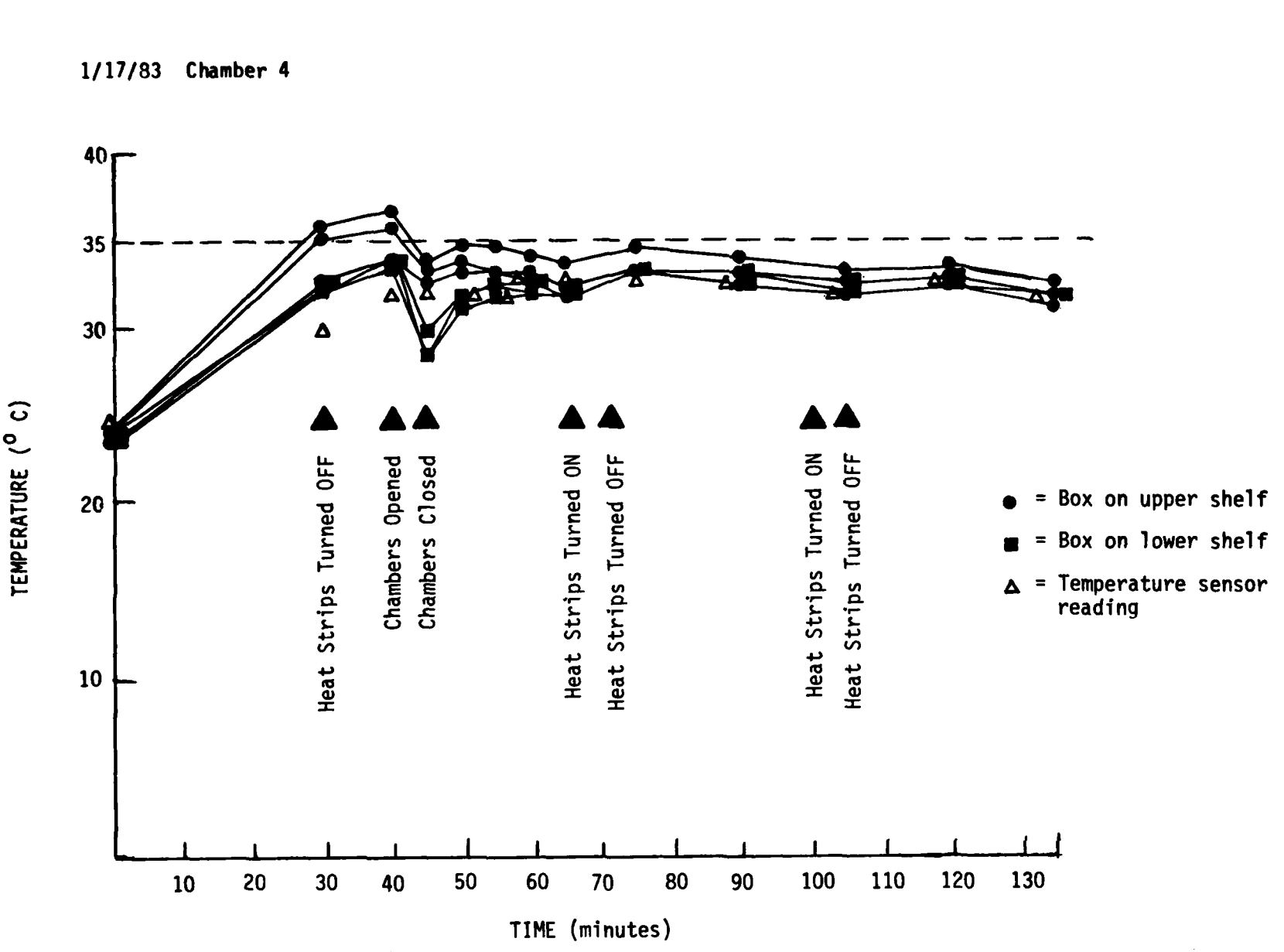


Fig. 9 . Temperatures at Seven Locations within Chamber 4 over Time Within the Session. The seven locations were one in each of the six behavioral testing chambers plus one at the site of the permanently mounted sensor.

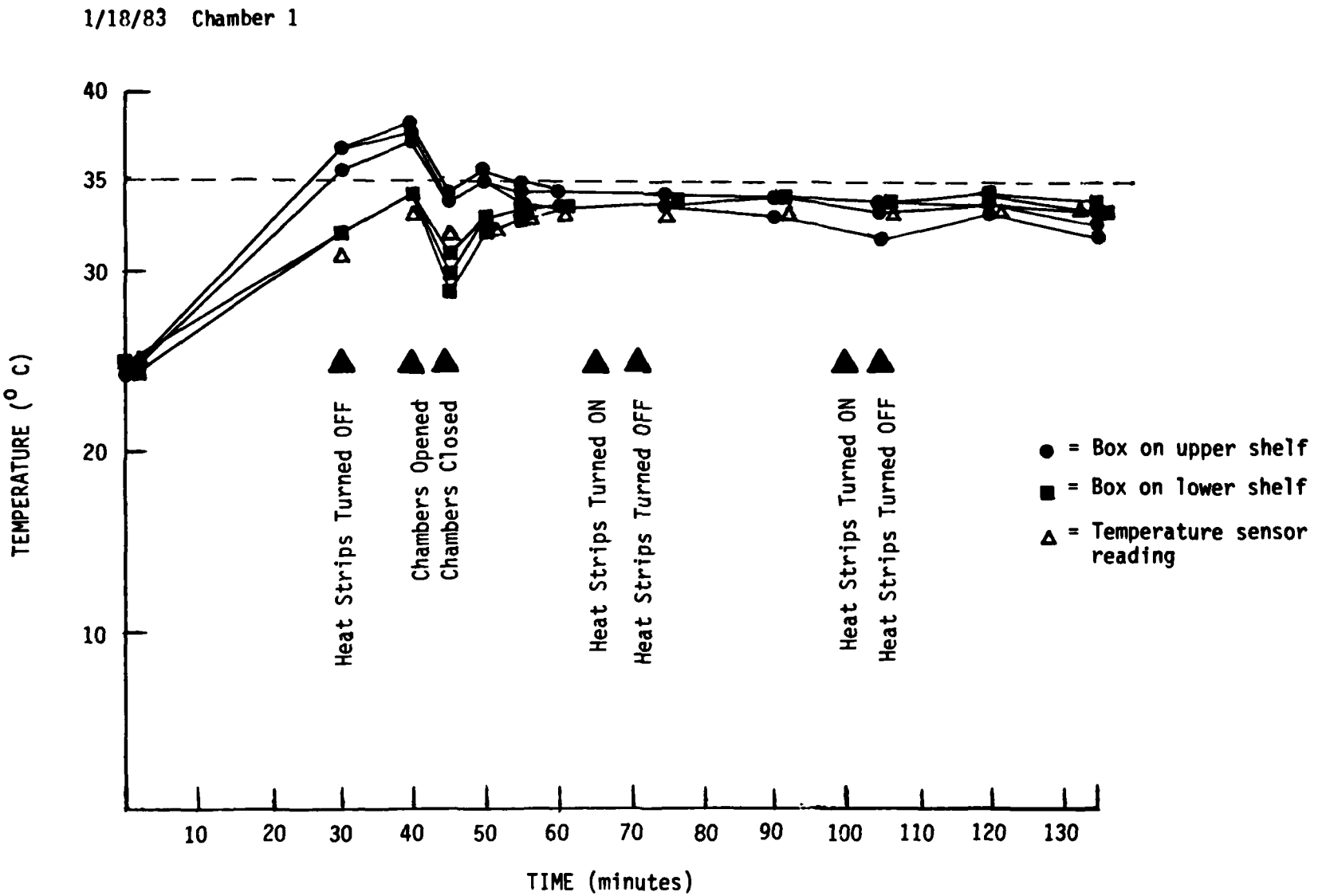


Fig. 10. Temperatures at Seven Locations within Chamber 1 over Time Within the Session. The seven locations were one in each of the six behavioral testing chambers plus one at the site of the permanently mounted sensor.

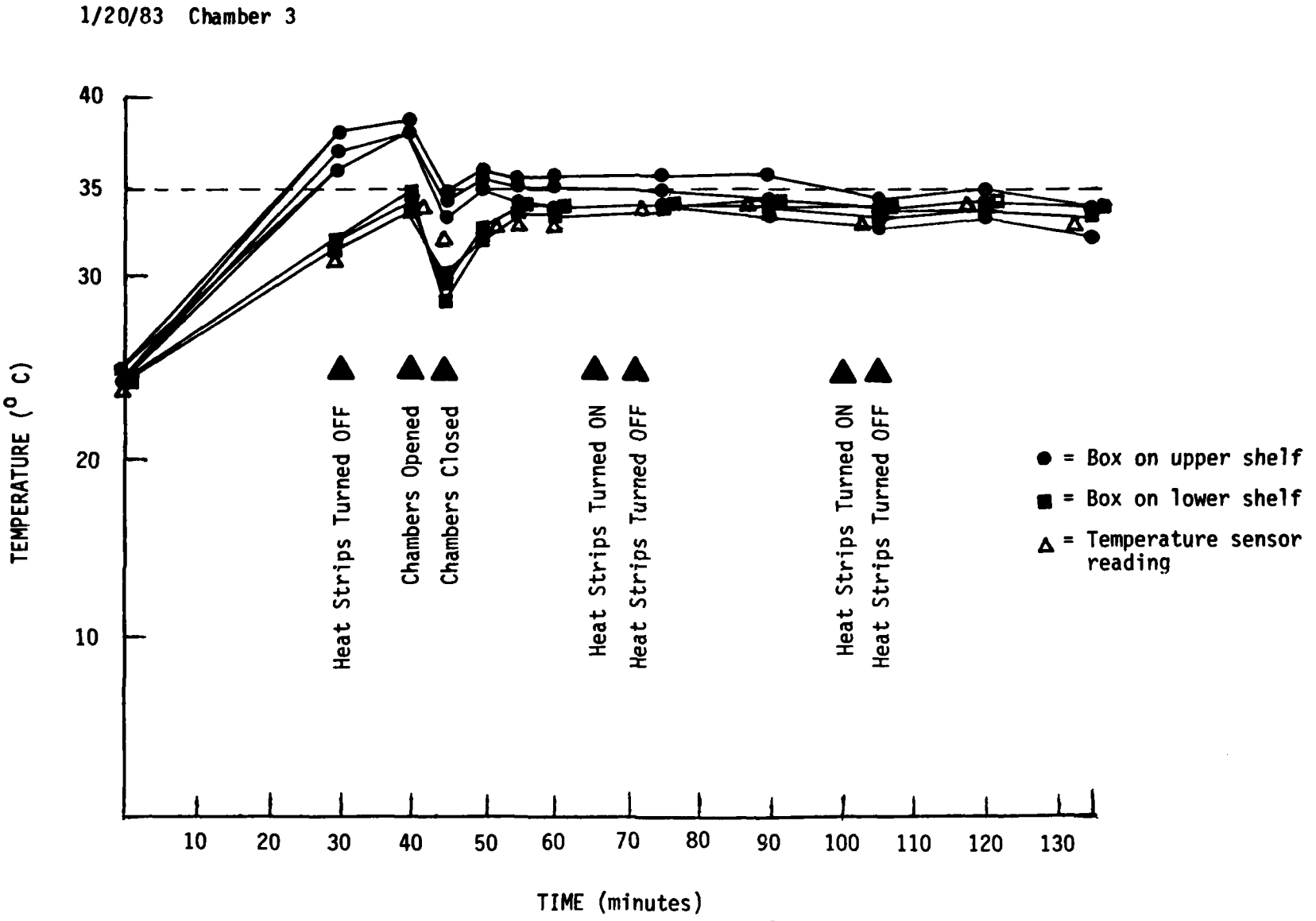


Fig. 11. Temperatures at Seven Locations within Chamber 3 over Time Within the Session. The seven locations were one in each of the six behavioral testing chambers plus one at the site of the permanently mounted sensor.

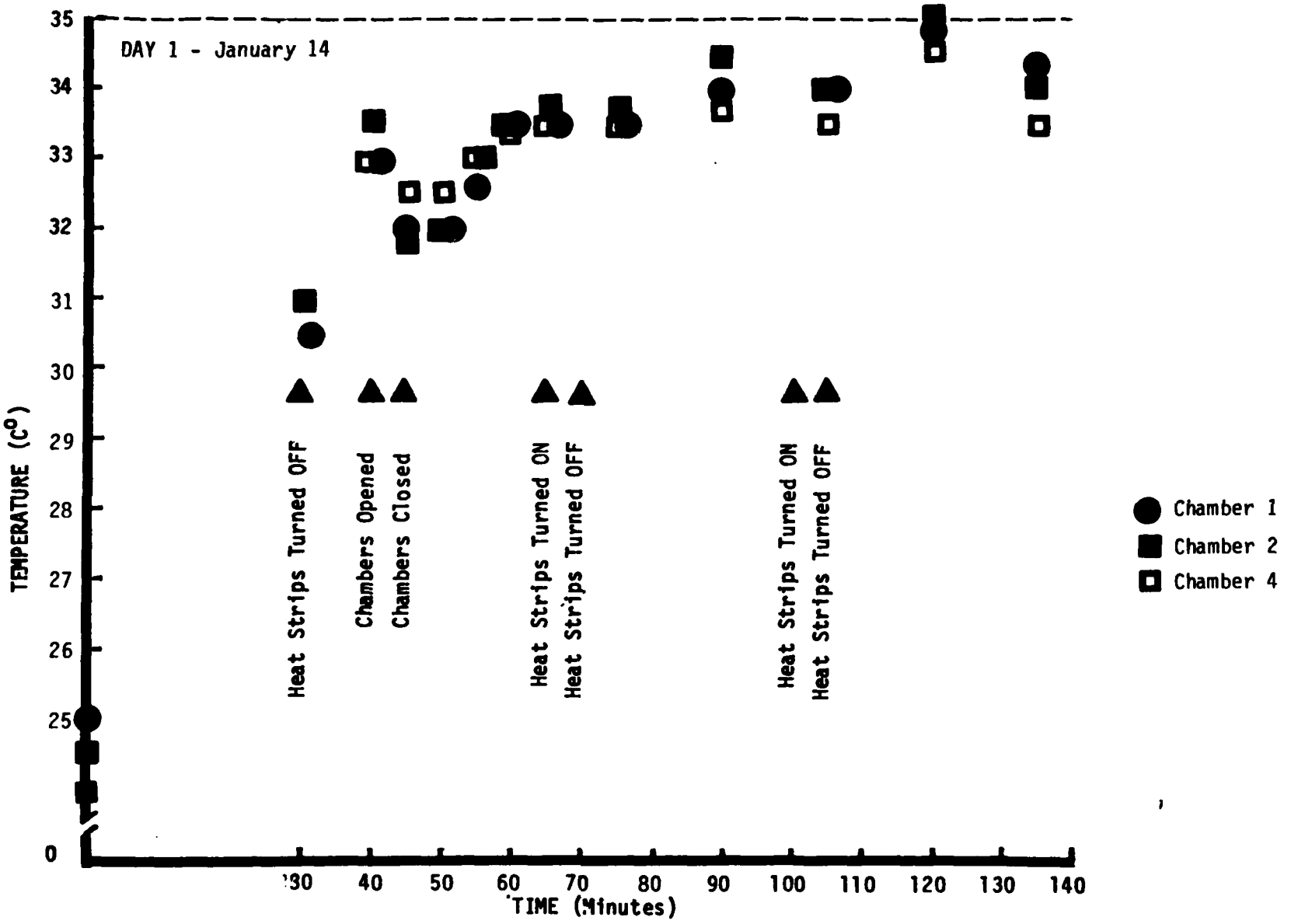


Fig. 12. Temperatures read from permanently mounted sensors in three inhalation chambers (1, 2, and 4) on Day 1 of the experiment. The sensor for chamber 3 was not funtioning.

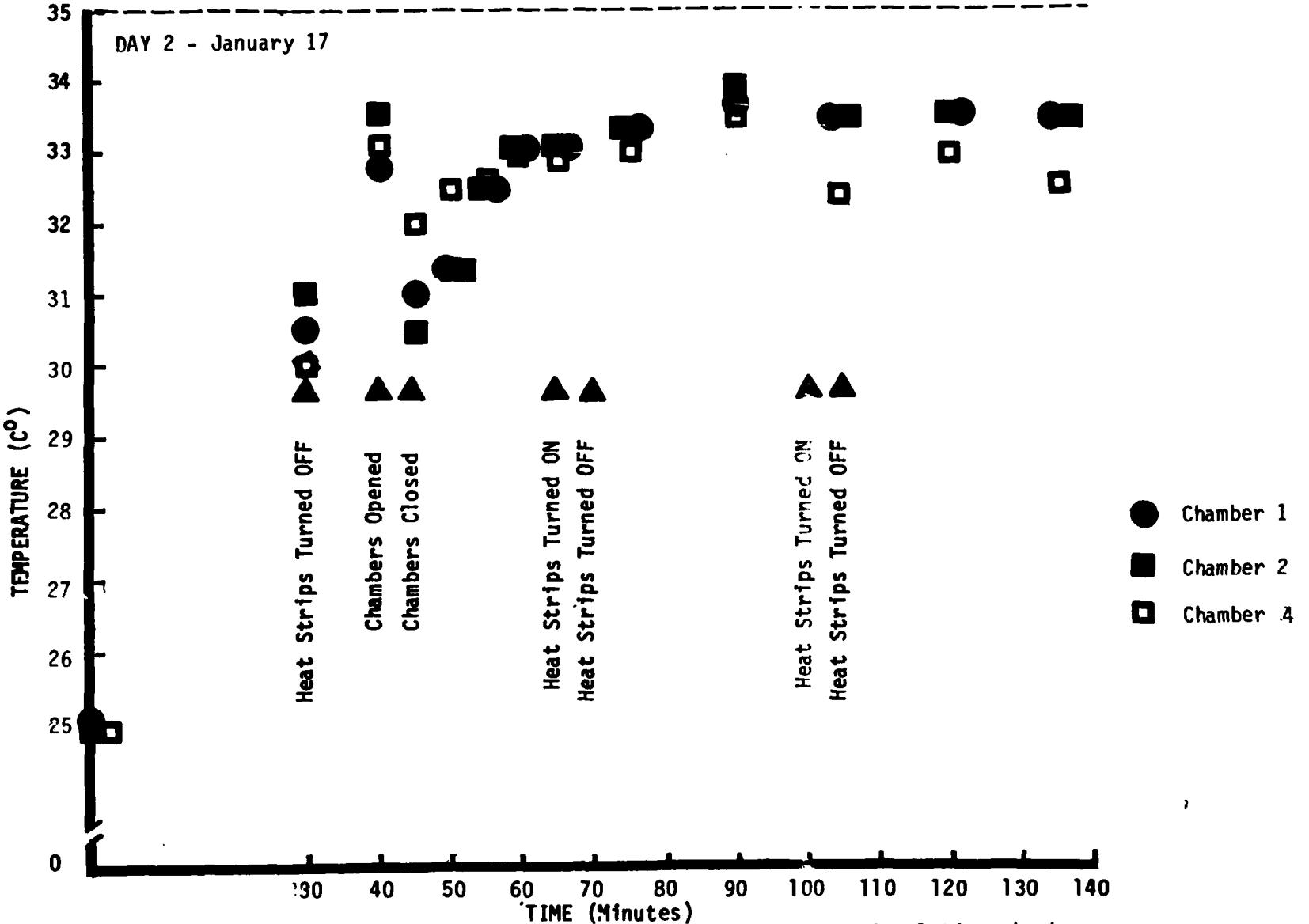


Fig. 13. Temperatures read from permanently mounted sensors in three inhalation chambers (1,2,4) on Day 2 of the experiment. The sensor for chamber 3 was not functioning.

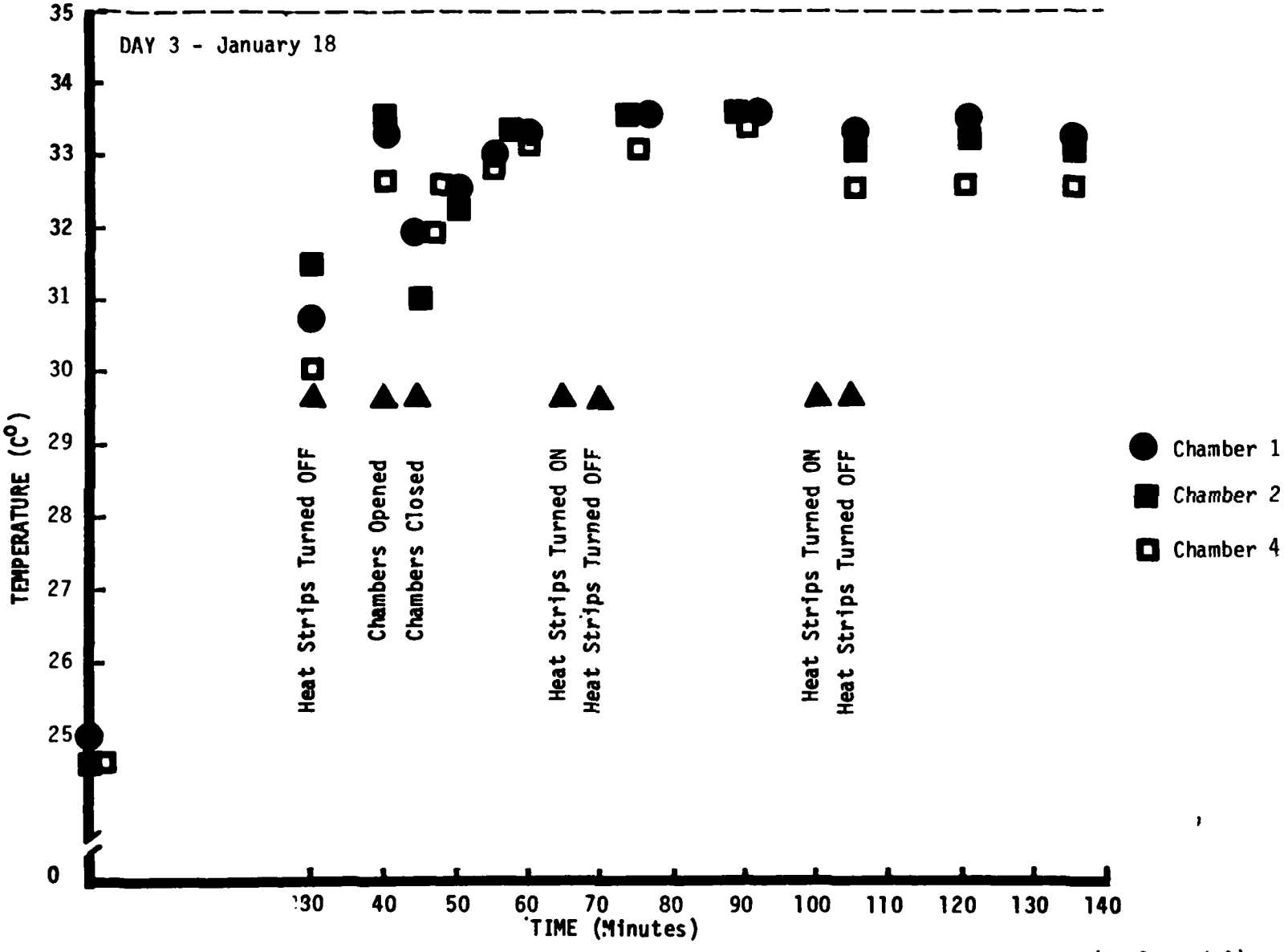


Fig. 14. Temperatures read from permanently mounted sensors in three inhalation chambers (1, 2, and 4) on Day 3 of the experiment. The sensor for chamber 3 was not functioning.

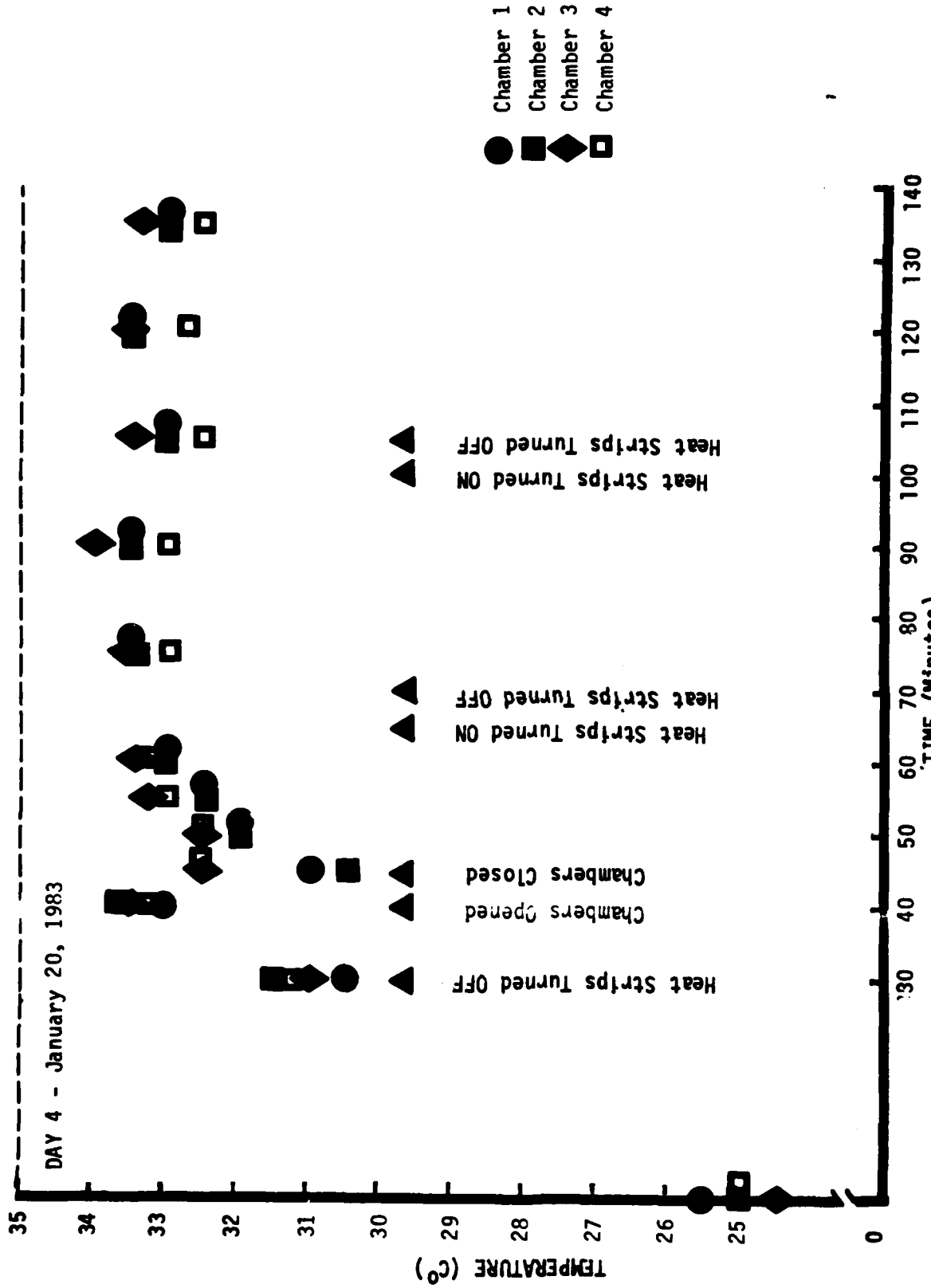


Fig. 15. Temperatures read from permanently mounted sensors in four inhalation chambers on Day 4 of the experiment.

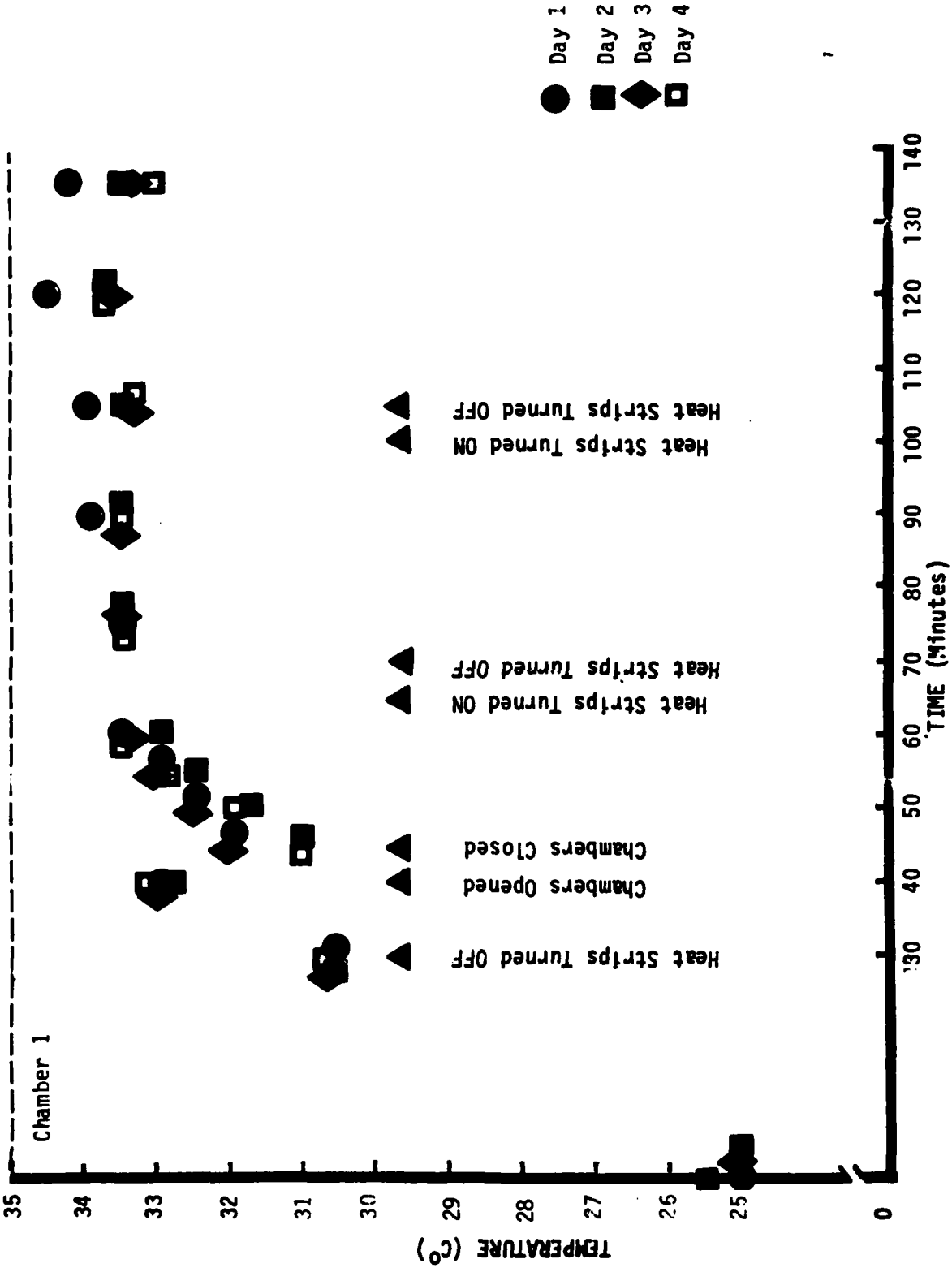


Fig. 16. Temperature readings for Inhalation Chamber 1 from the permanently mounted temperature sensor over 4 days of testing

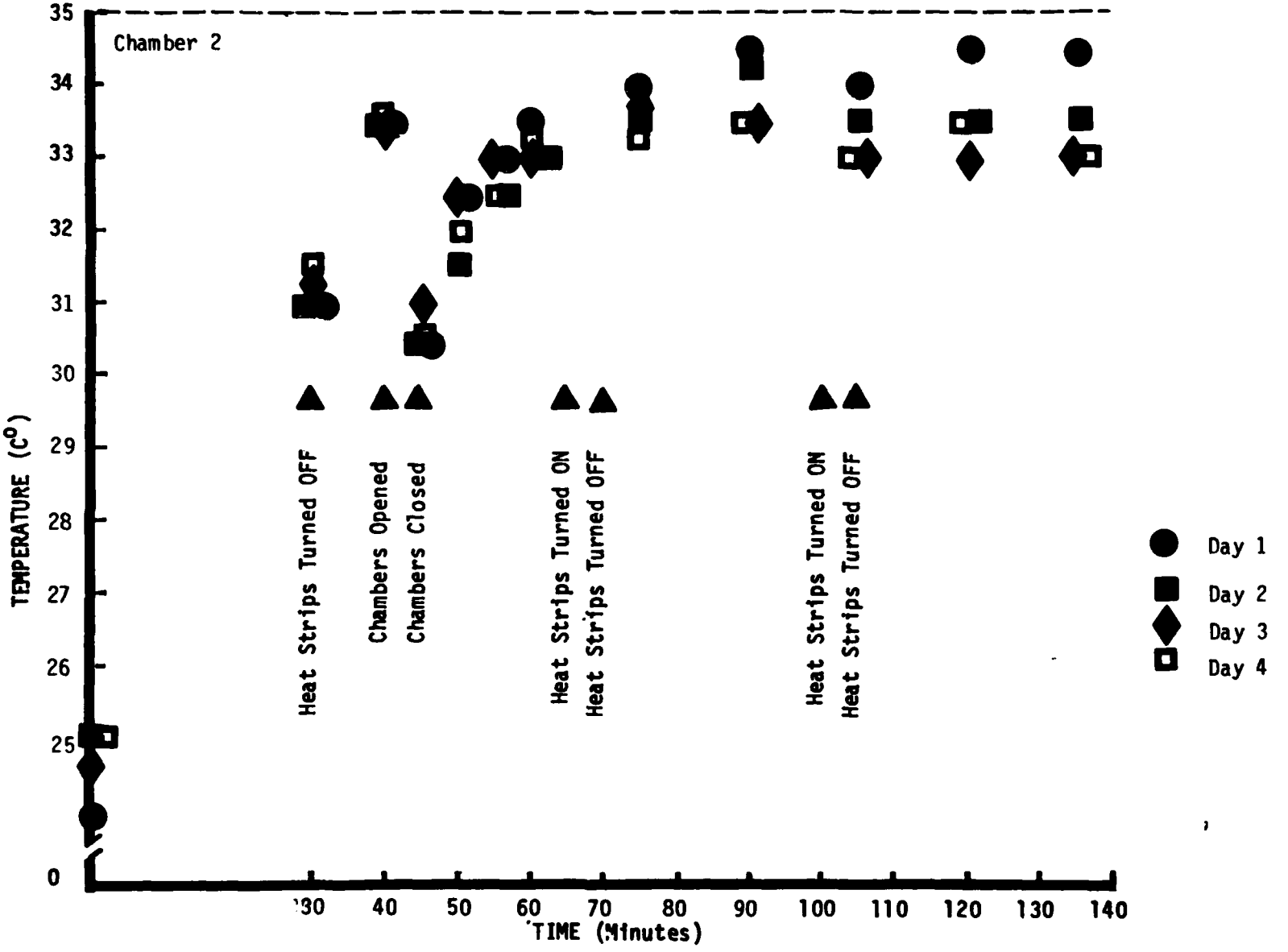


Fig. 17. Temperature readings for Inhalation Chamber 2 from the permanently mounted temperature sensor over 4 days of testing

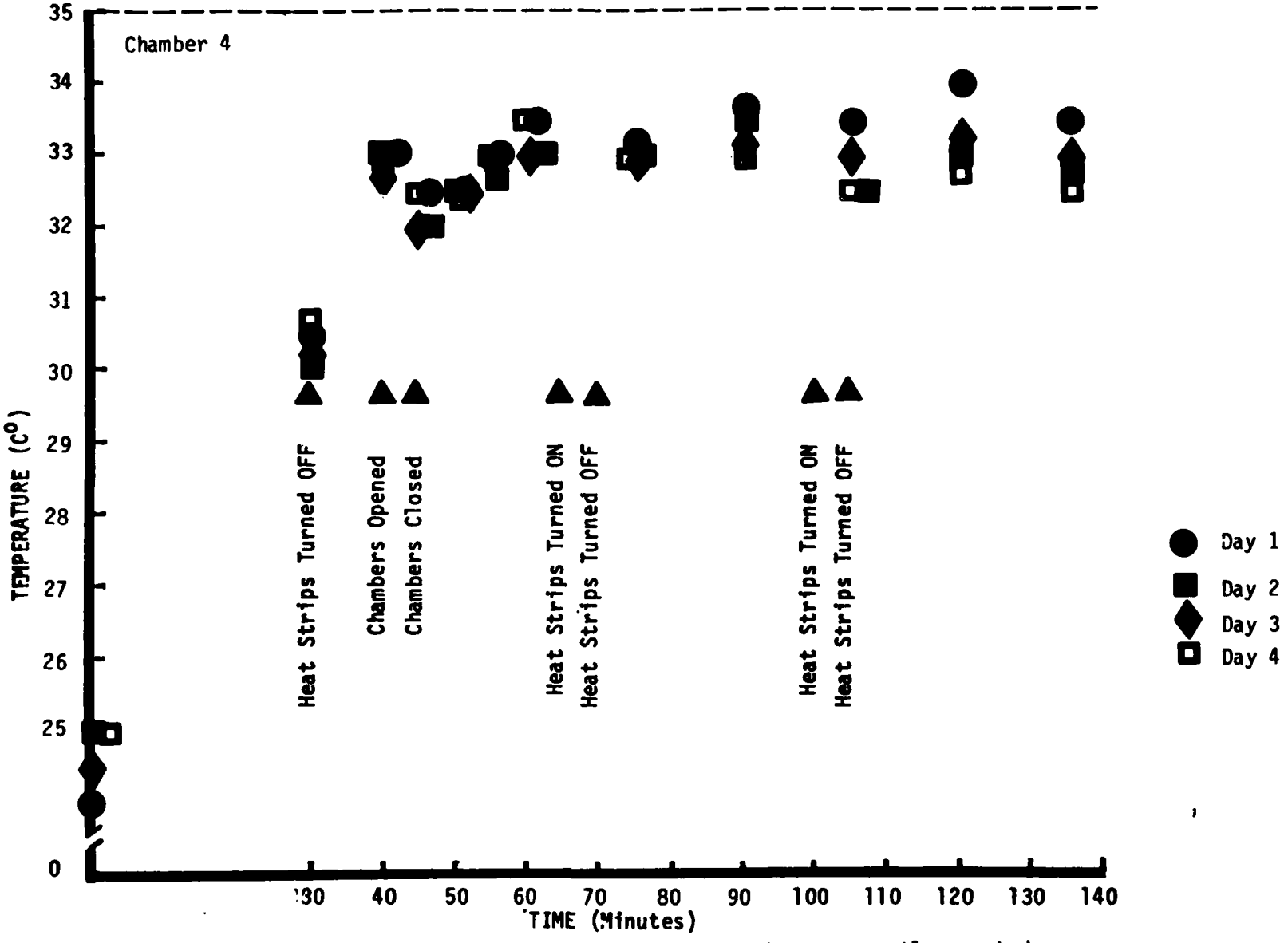


Fig. 18.. Temperature readings for Inhalation Chamber 4 from the permanently mounted temperature sensor over 4 days of testing.

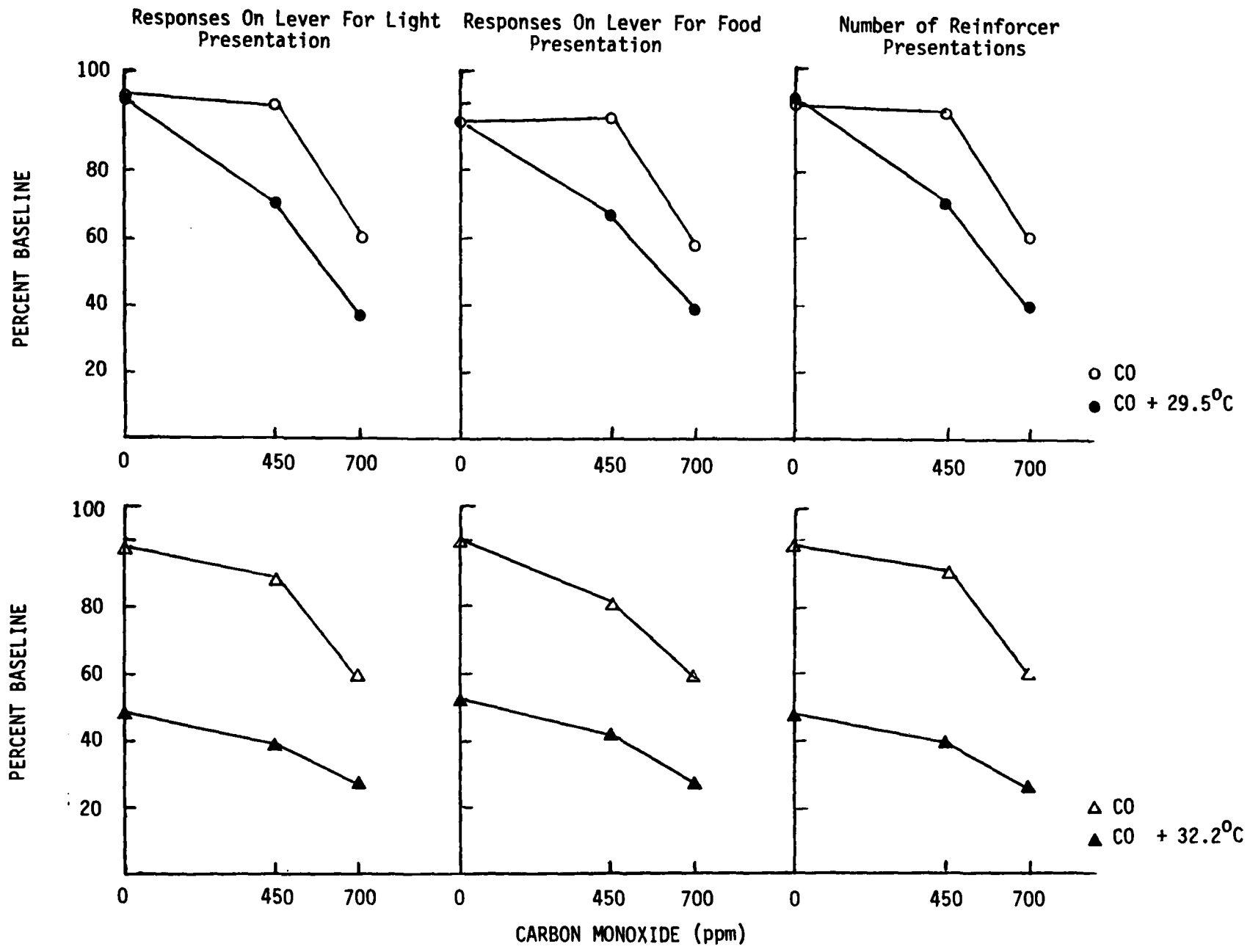


Figure 19. Effects of CO in combination with high environmental temperatures on performance on a chain FR30-FR30 schedule. Baseline performance represents the mean of performance on the three days prior to exposure.

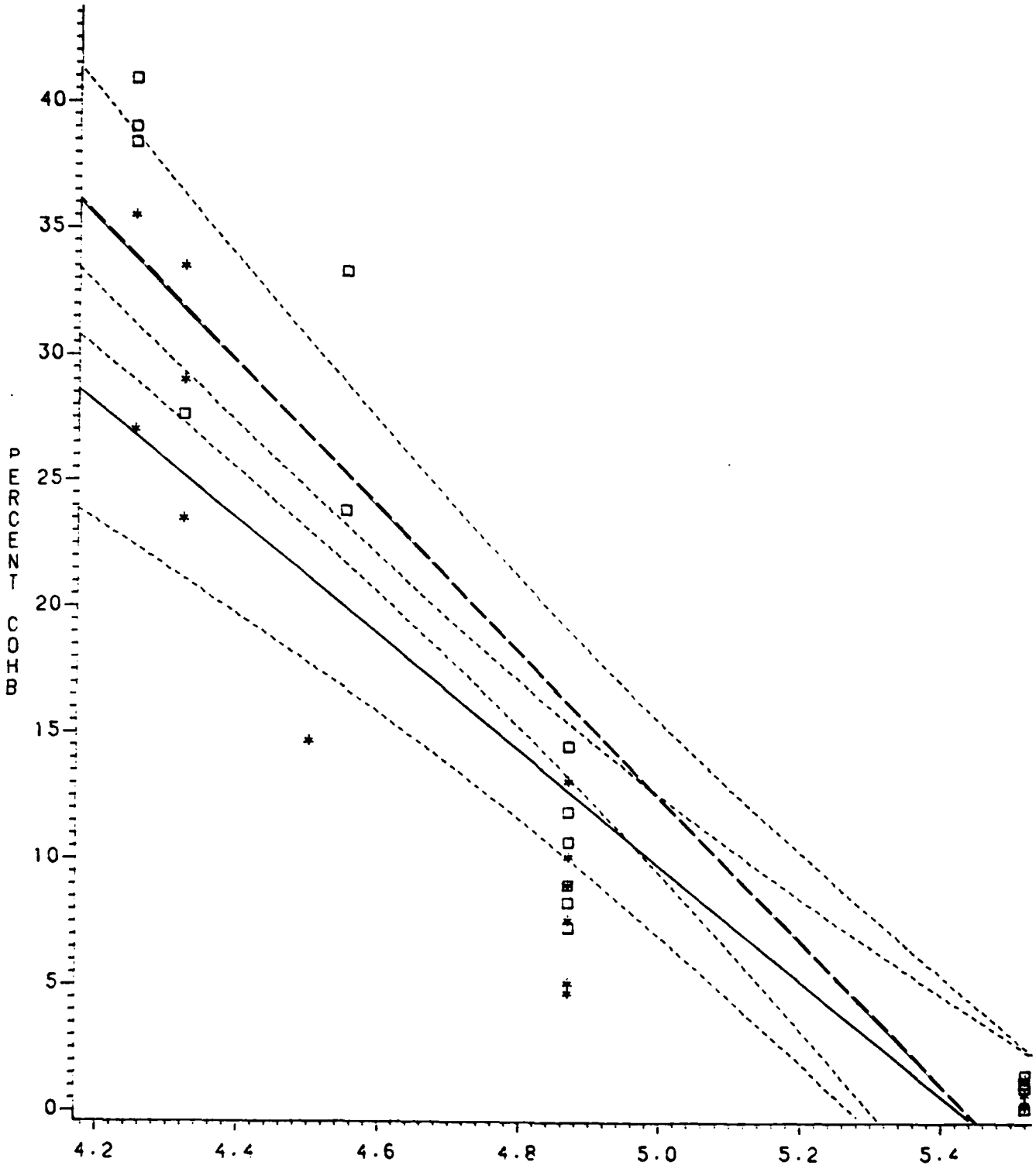


Figure 20. Linear Regression Model for COHb Data with 95% Confidence Intervals.

LEGEND: * = 700 ppm Co; □ = 1250 ppm; --- = 95% Confidence Intervals.

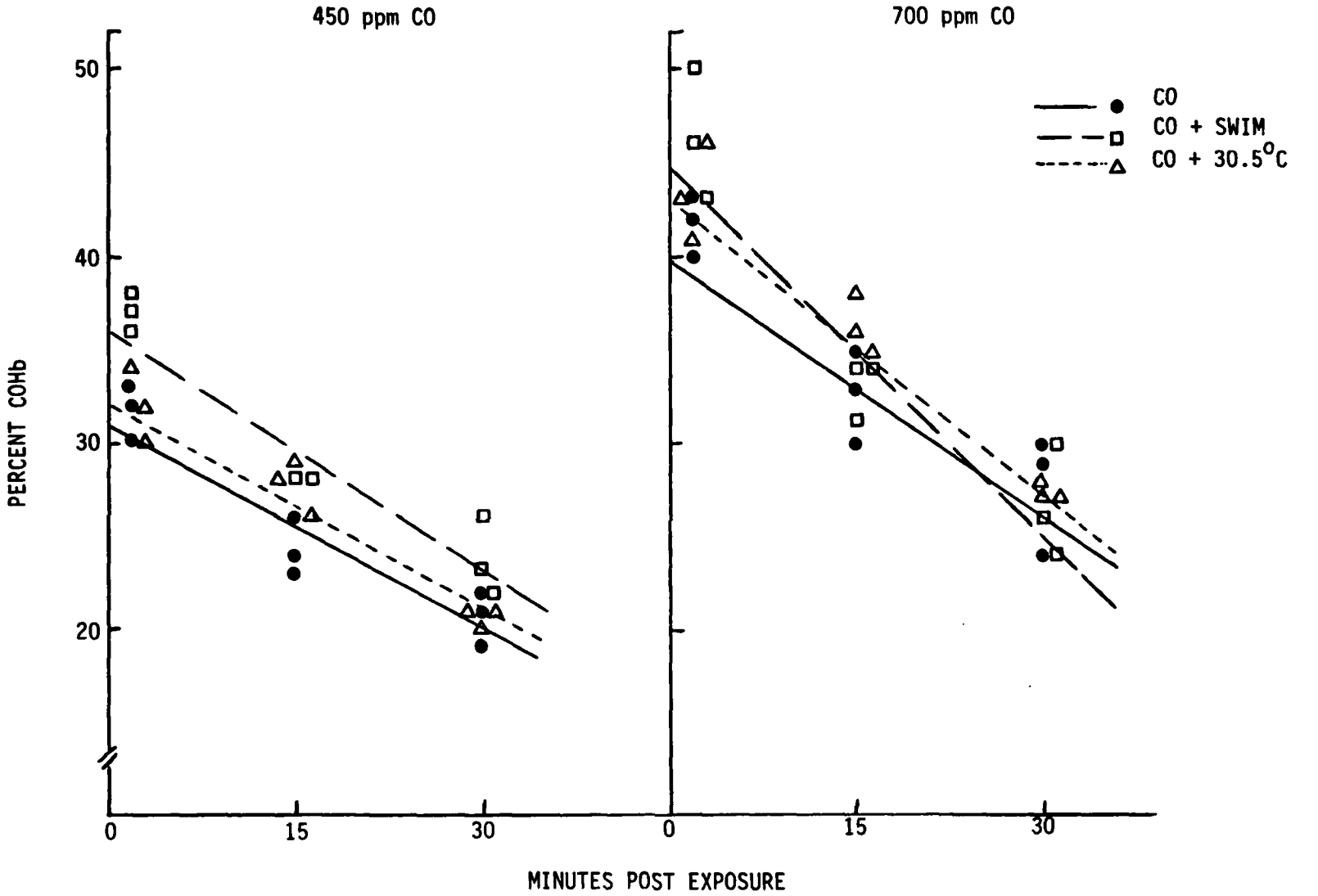


Figure 21. Percent carboxyhemoglobin in blood at different times after exposure to carbon monoxide and/or swim stress or high environmental temperature. The average control value based on six animals that received no CO or stress exposure was 1.2% COHb.

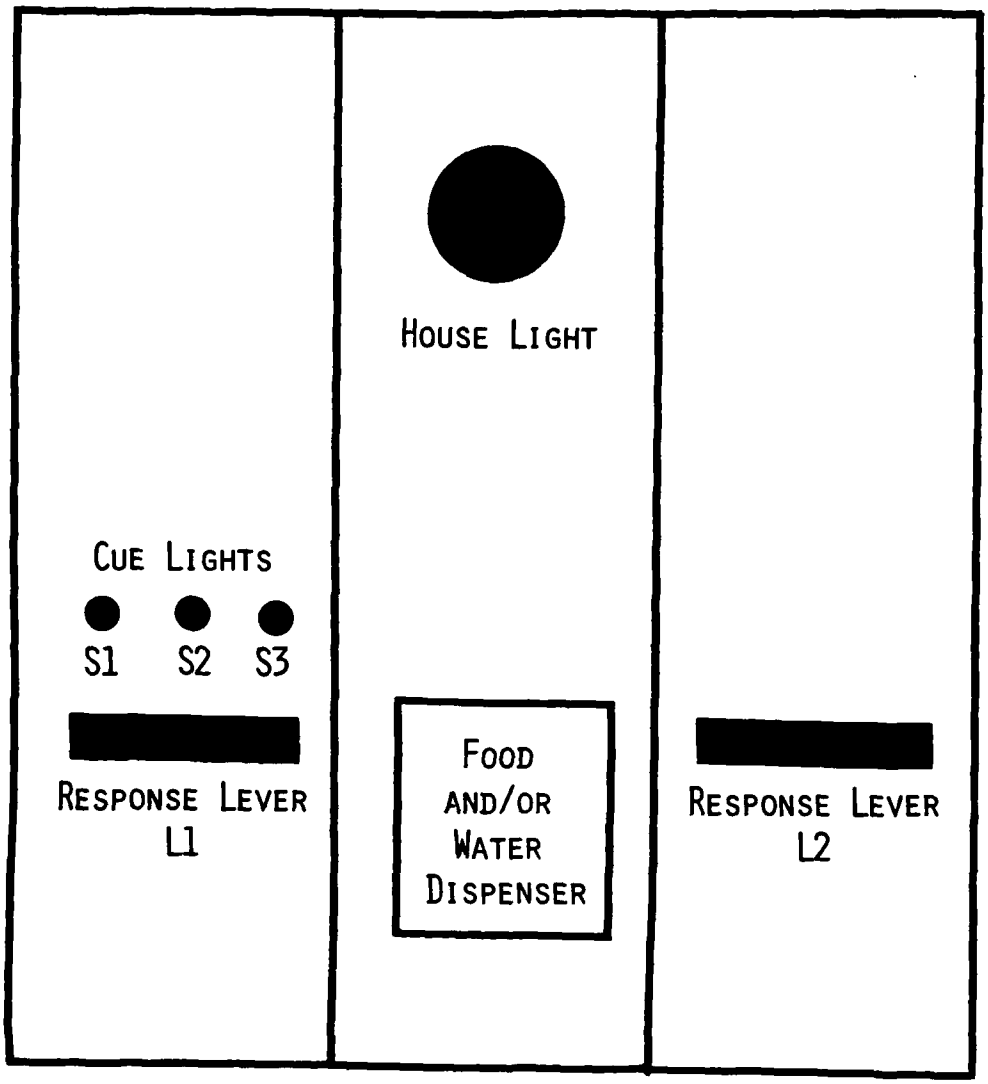


Figure 22. Intelligence Panel: Arrangement for Chain VR5-FR15

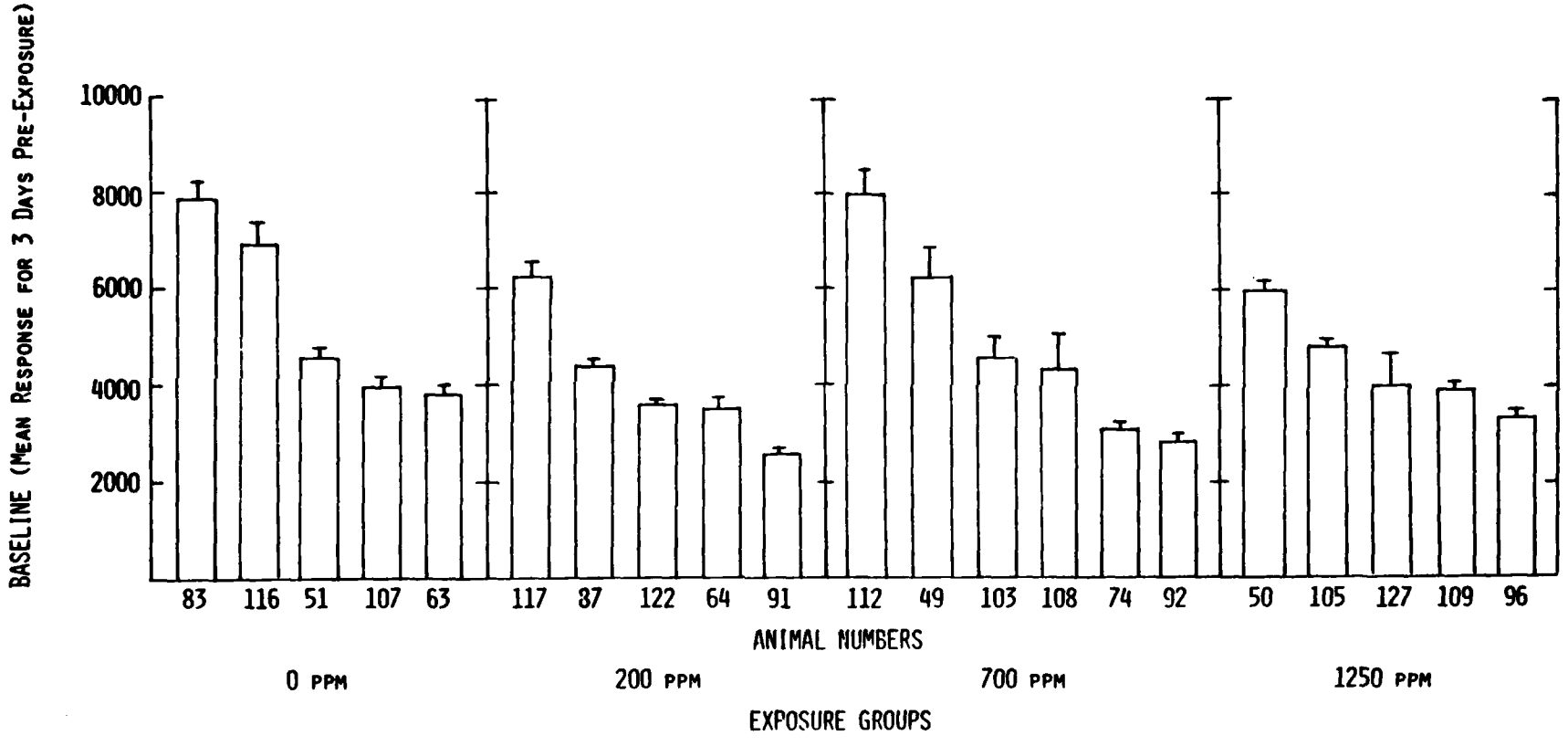


Figure 23. Responses on FR15 Component of Chain VR5-FR15 Schedule

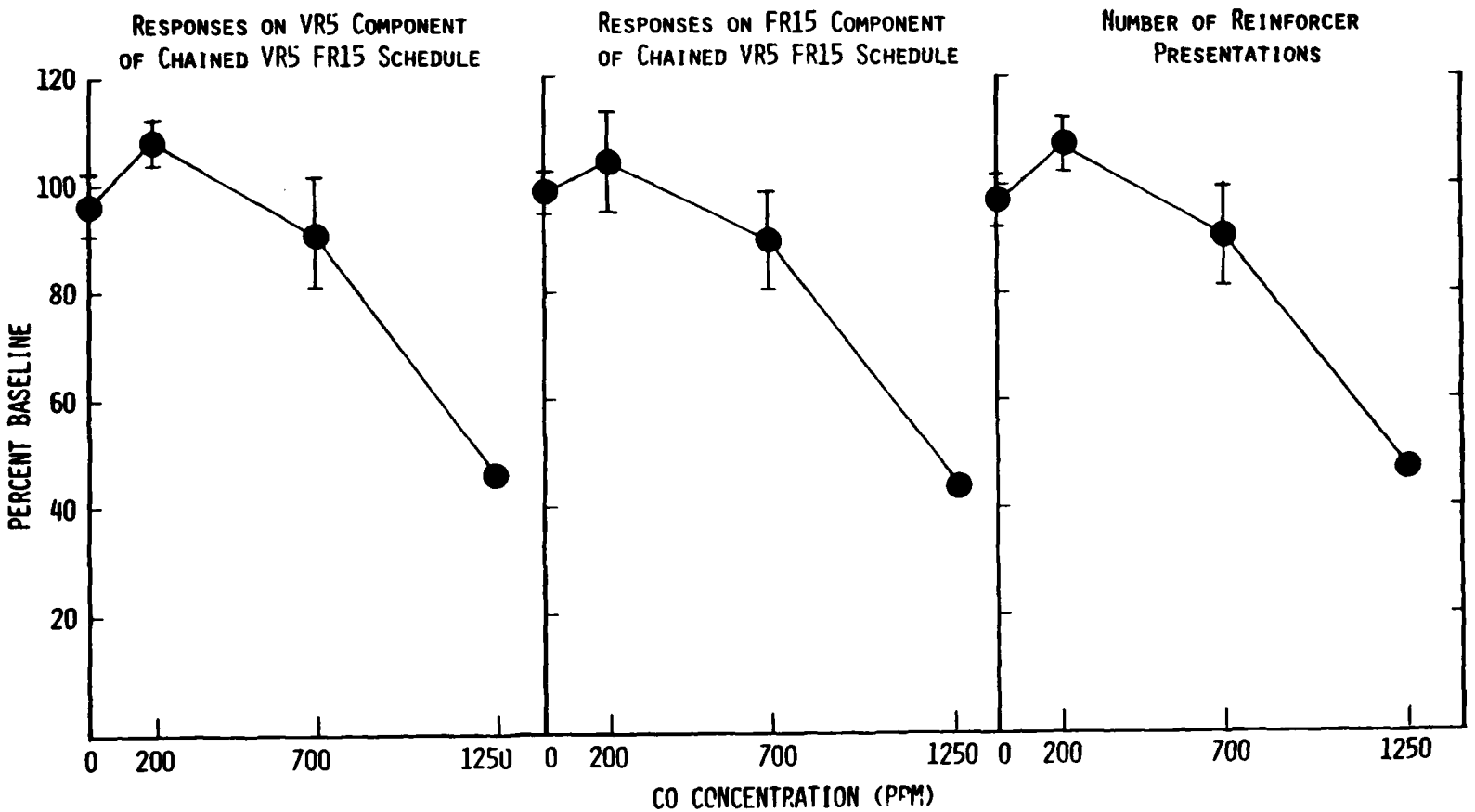


Figure 24. Concentration-Response Curve for VR5-FR15 Performance on the Initial Day of CO Exposure

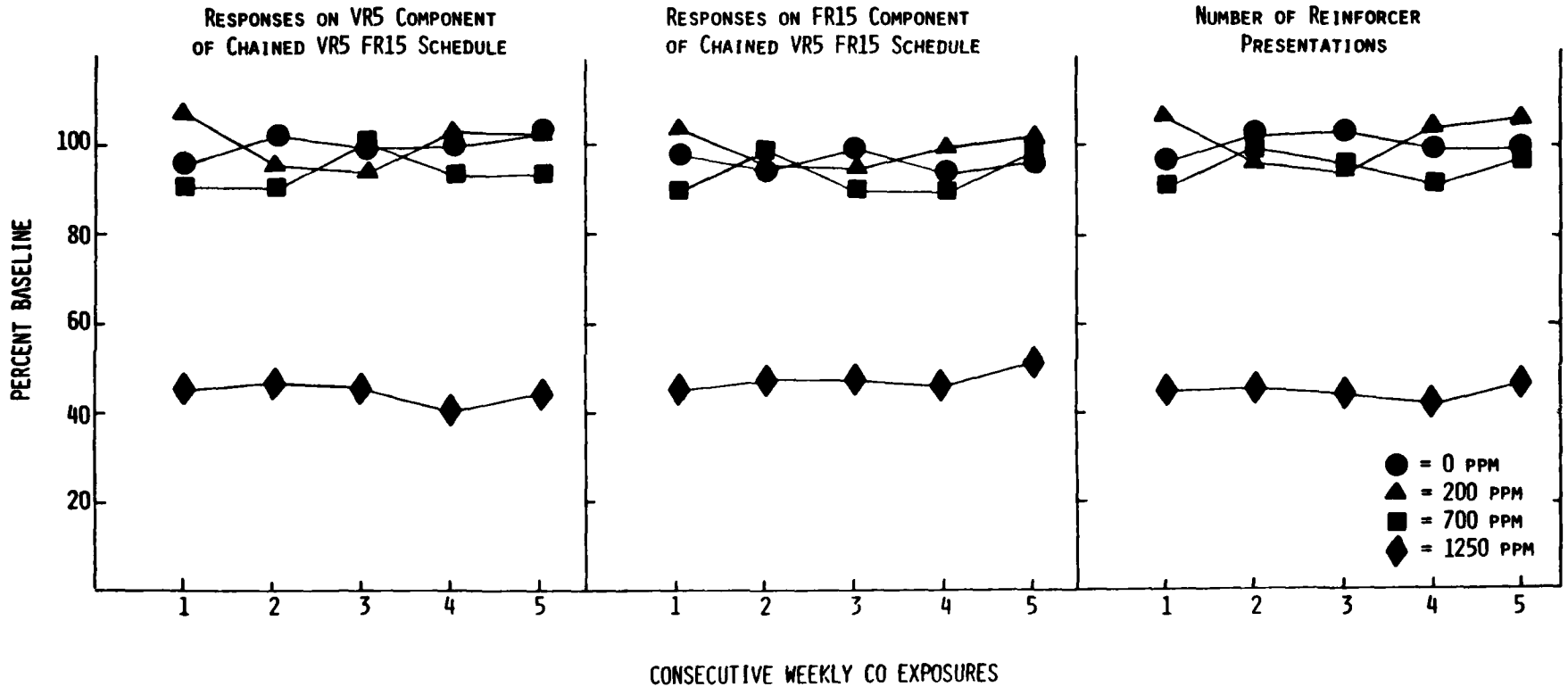


Figure 25. Effects of Five Consecutive Weekly Exposures to CO on VR5-FR15 Performance. Analysis of variance indicated a significant effect of CO ($p < 0.01$) but no differences among the different weeks of exposure.

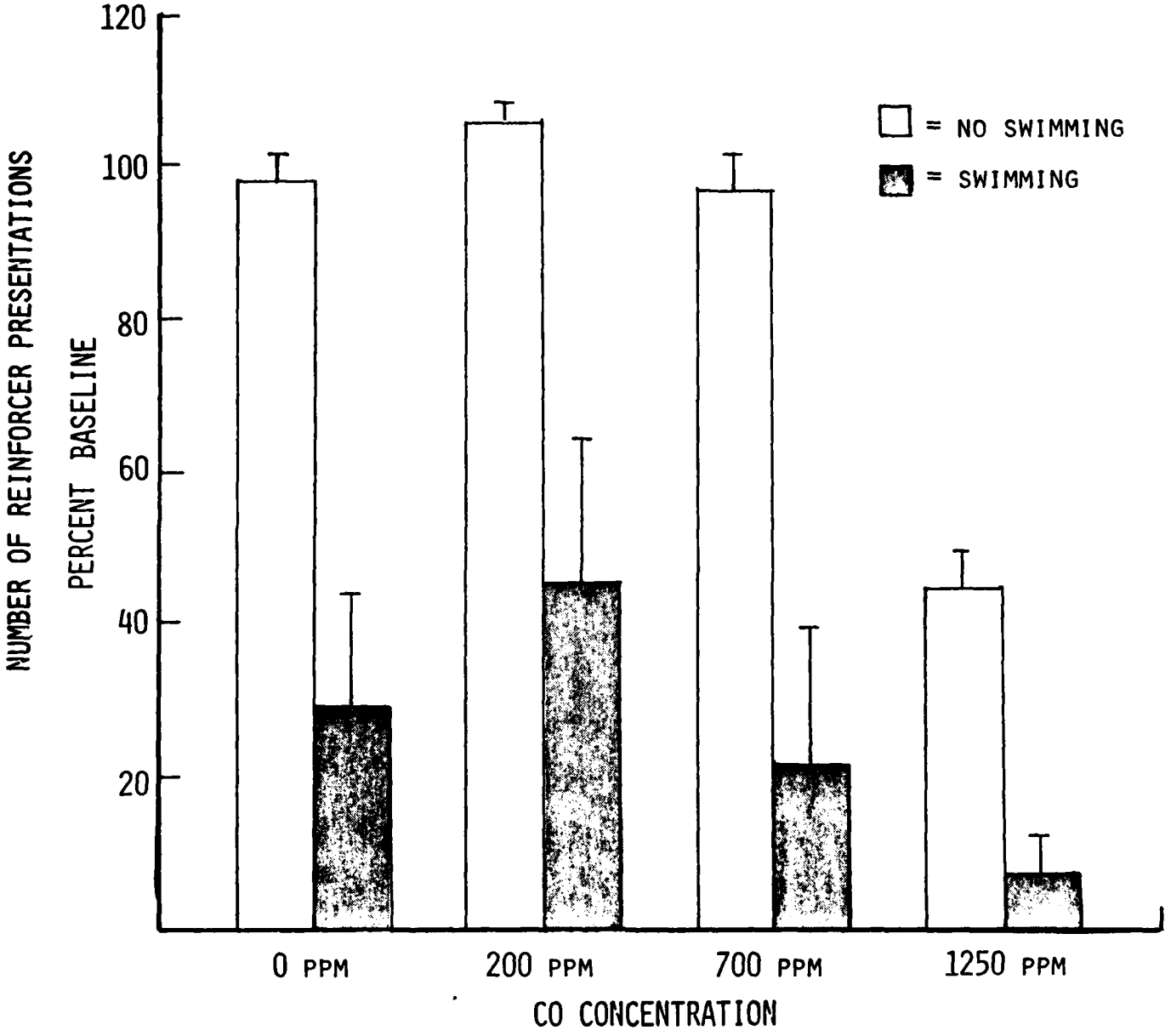


Figure 26. Comparison of the Number of Reinforcer Presentations on a VR5-FR15 Schedule Following CO Alone and CO in Combination with Swim Stress.

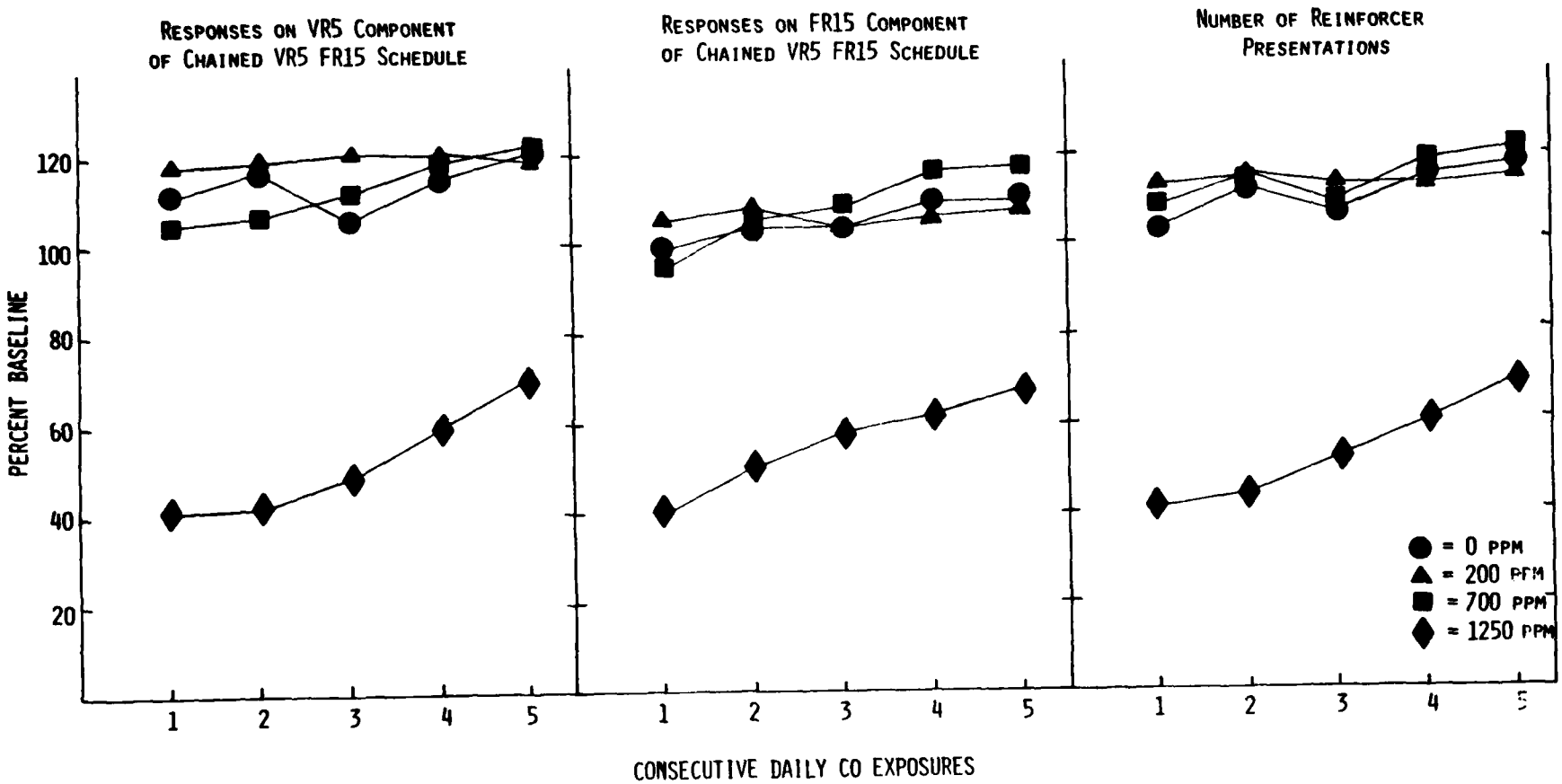


Figure 27. Effects of Five Consecutive Daily Exposures to CO on VR5-FR15 Performance. Analysis of variance indicated a significant effect of CO and a significant difference for days of exposure. ($p < 0.01$)

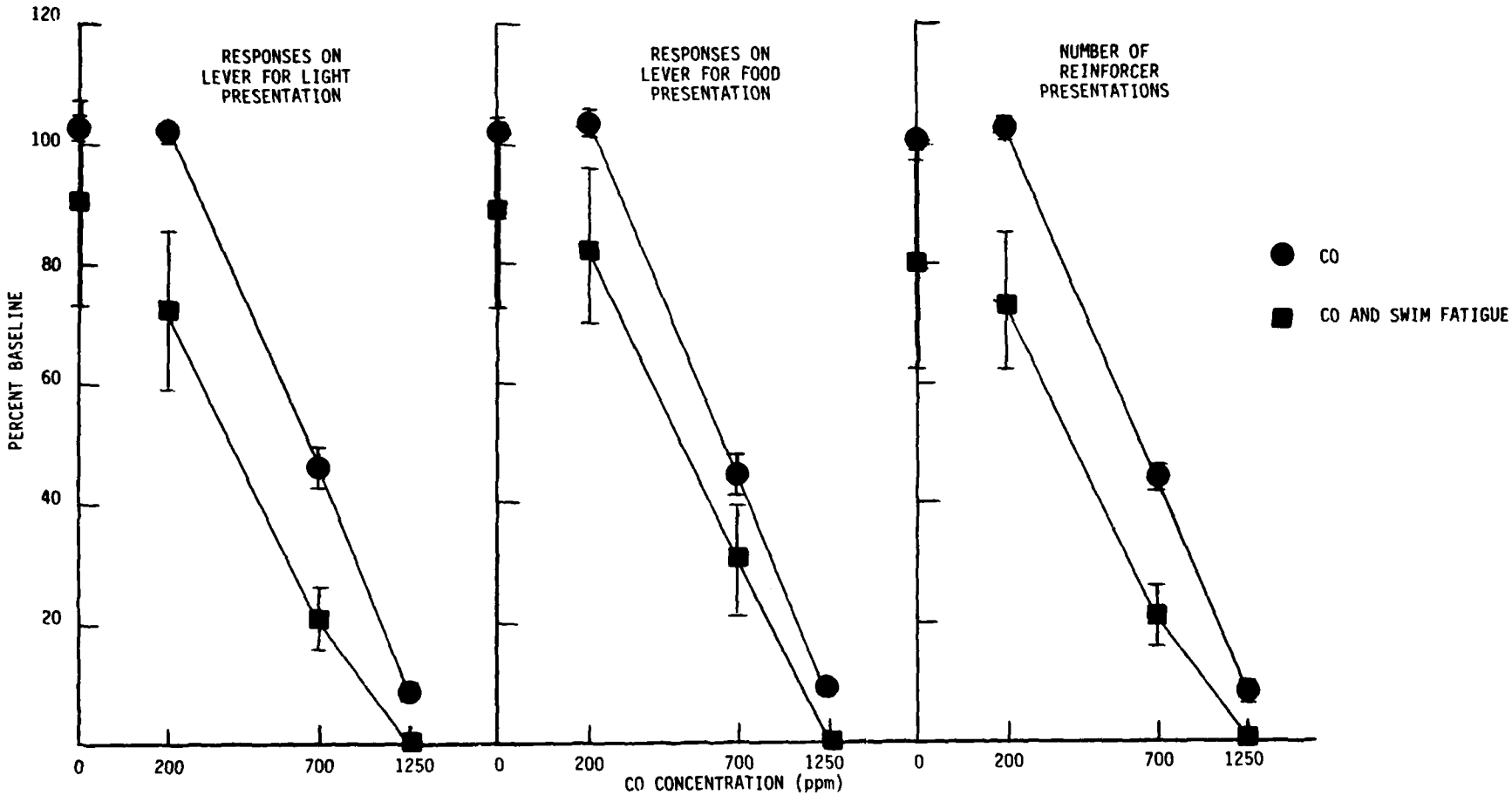


Fig. 28. Effects of CO and Swim Fatigue on Performance of A Chain FR30-FR30 Schedule of Reinforcement. Performance was measured during the last 60 min of a 75 min exposure to CO. Data are presented as percent baseline (Mean + S.E.) for three different measures of performance. The effects of 700 and 1250 ppm CO were significant as was the effect of Swim Fatigue.

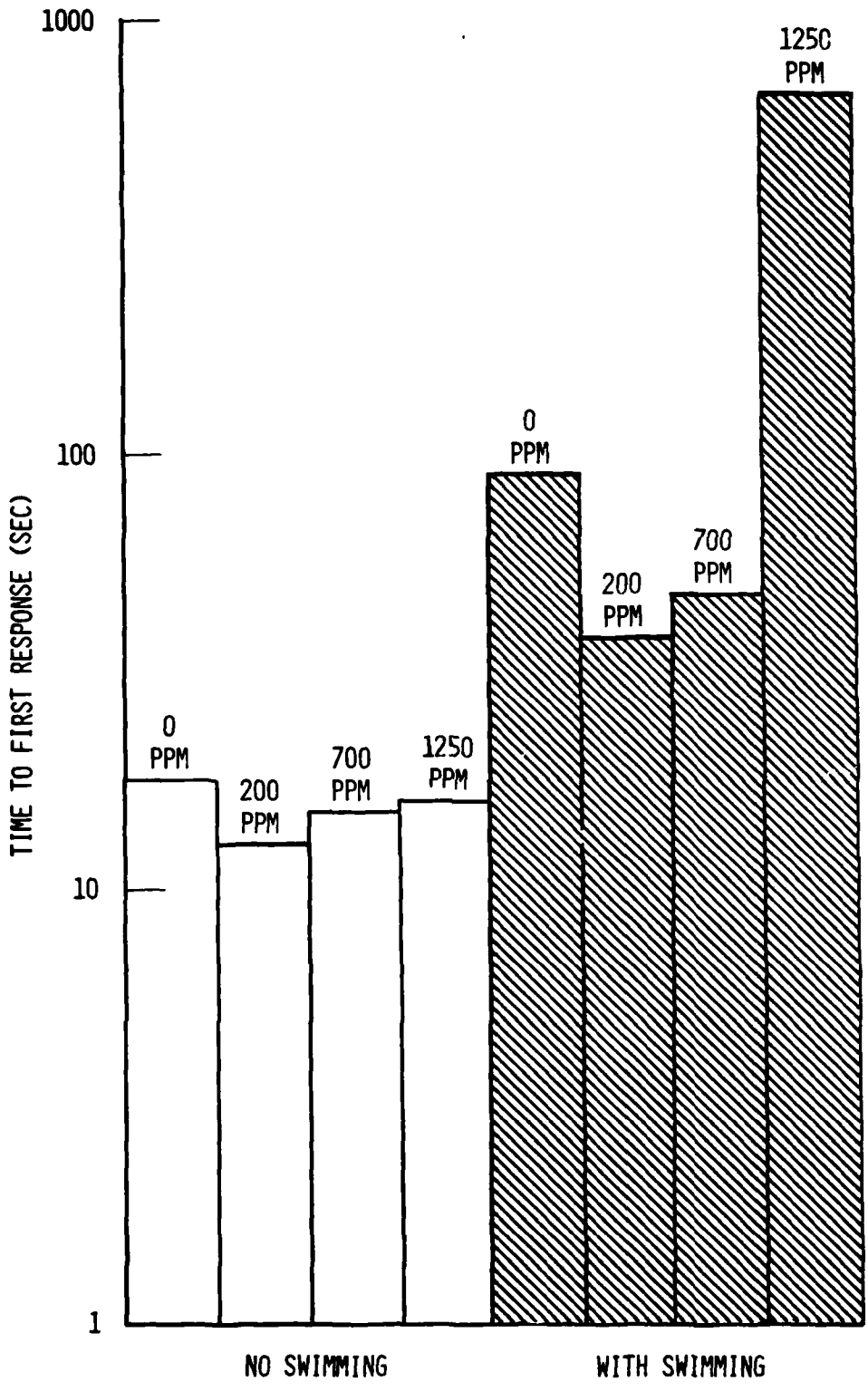


Fig. 29. Time to First Response. The time to first response in performance of a chain FR30FR30 schedule for rats exposed to CO or CO plus forced swimming.

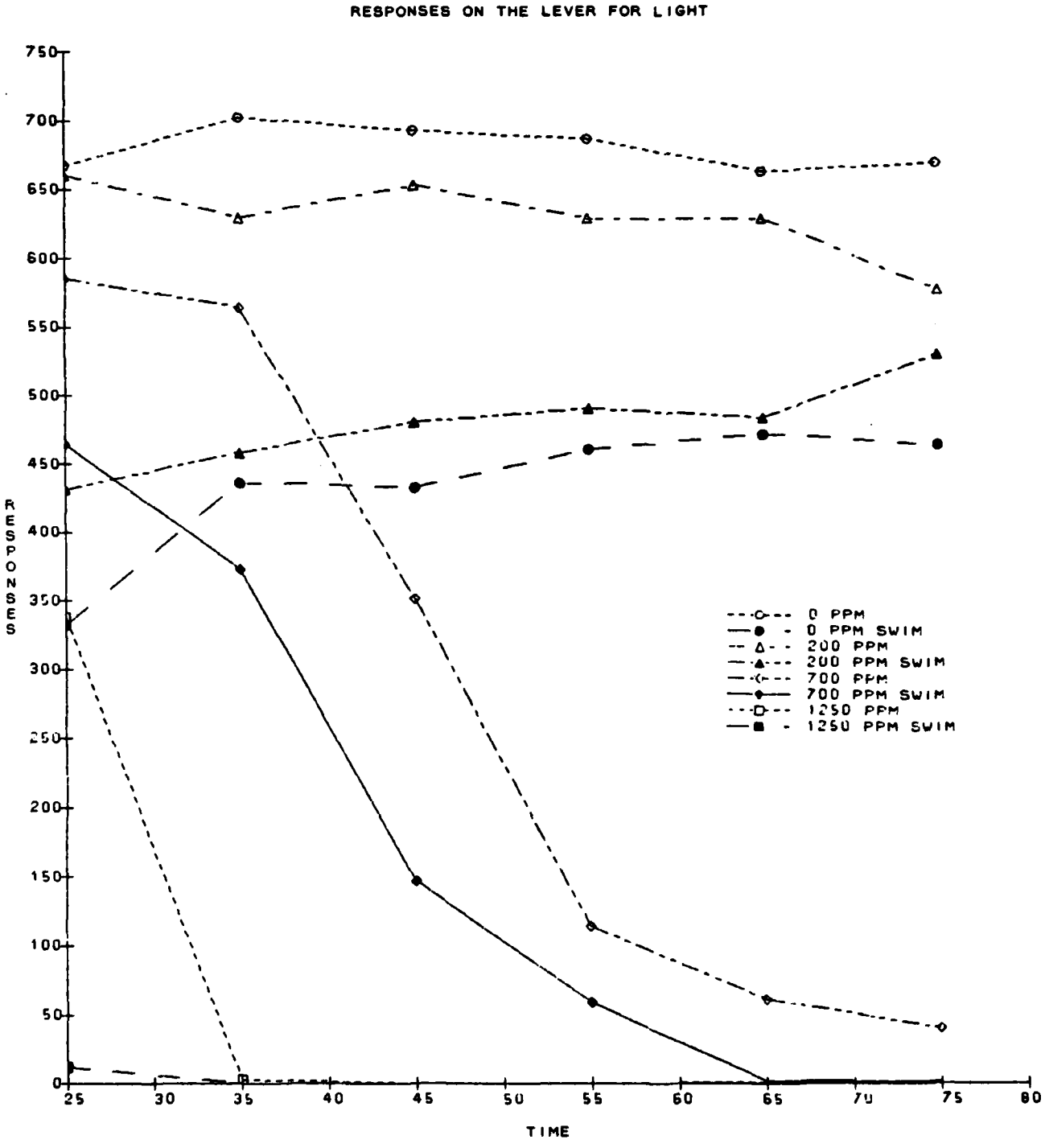


Figure 30. Time Course of Effects following CO and/or Swim Stress: Responses for Light Presentation. Concentration ($p < 0.0001$) and swim stress ($p<0.001$) affected overall performance and the trend over time ($p<0.0001$). The effect began at 25 min for 1250 ppm and at 45 min for 700 ppm. The interaction of CO concentration and swim stress was not significant.

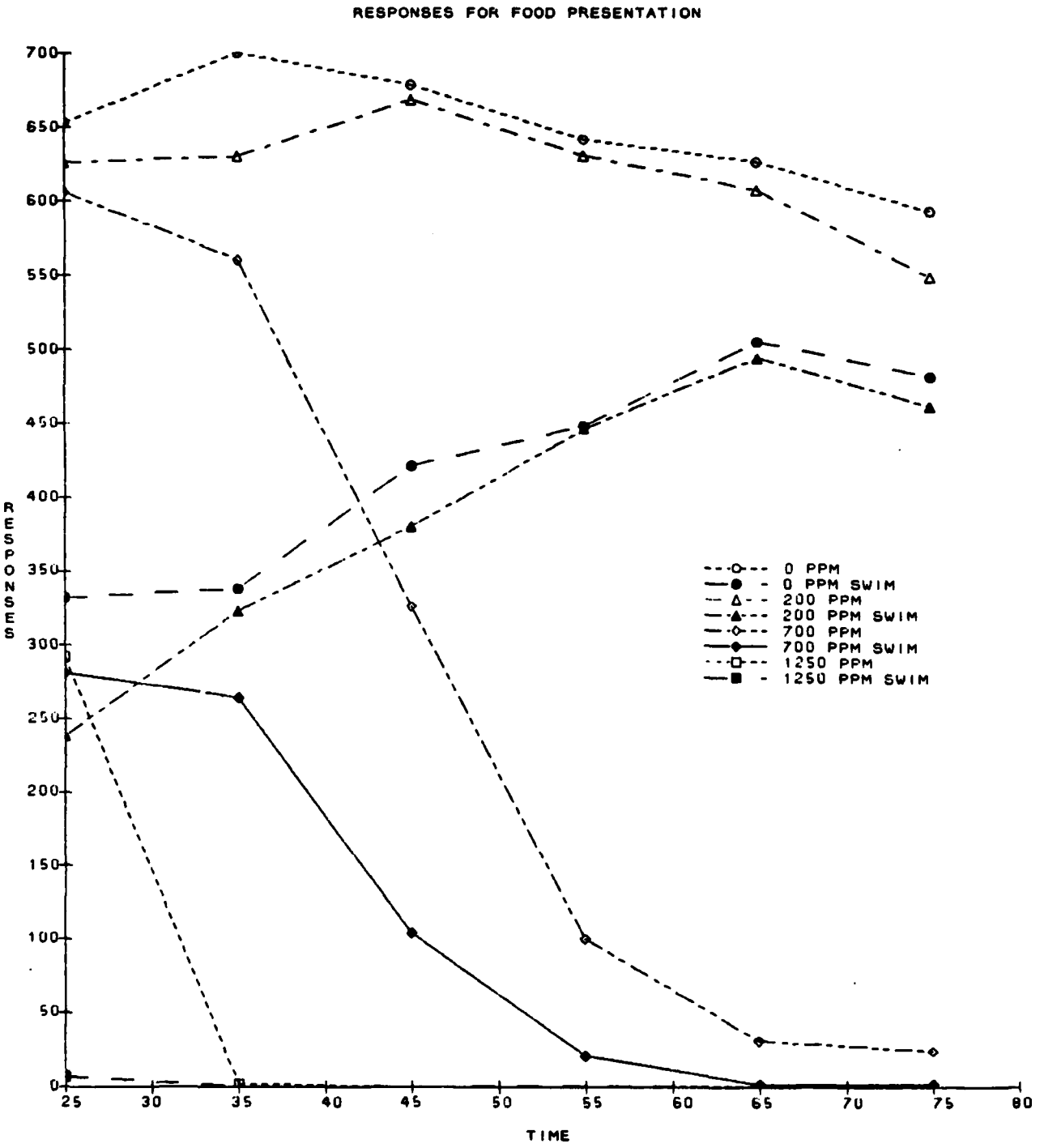


Figure 31. Time Course of Effects following CO and/or Swim Stress: Responses for Food Presentation. Concentration ($p < 0.001$) and swim stress ($p < 0.0001$) affected overall performance and the trend over time ($p < 0.0001$). The effect began at 25 min for 1250 ppm and at 45 min for 700 ppm. The interaction of CO concentration and swim stress was not significant.

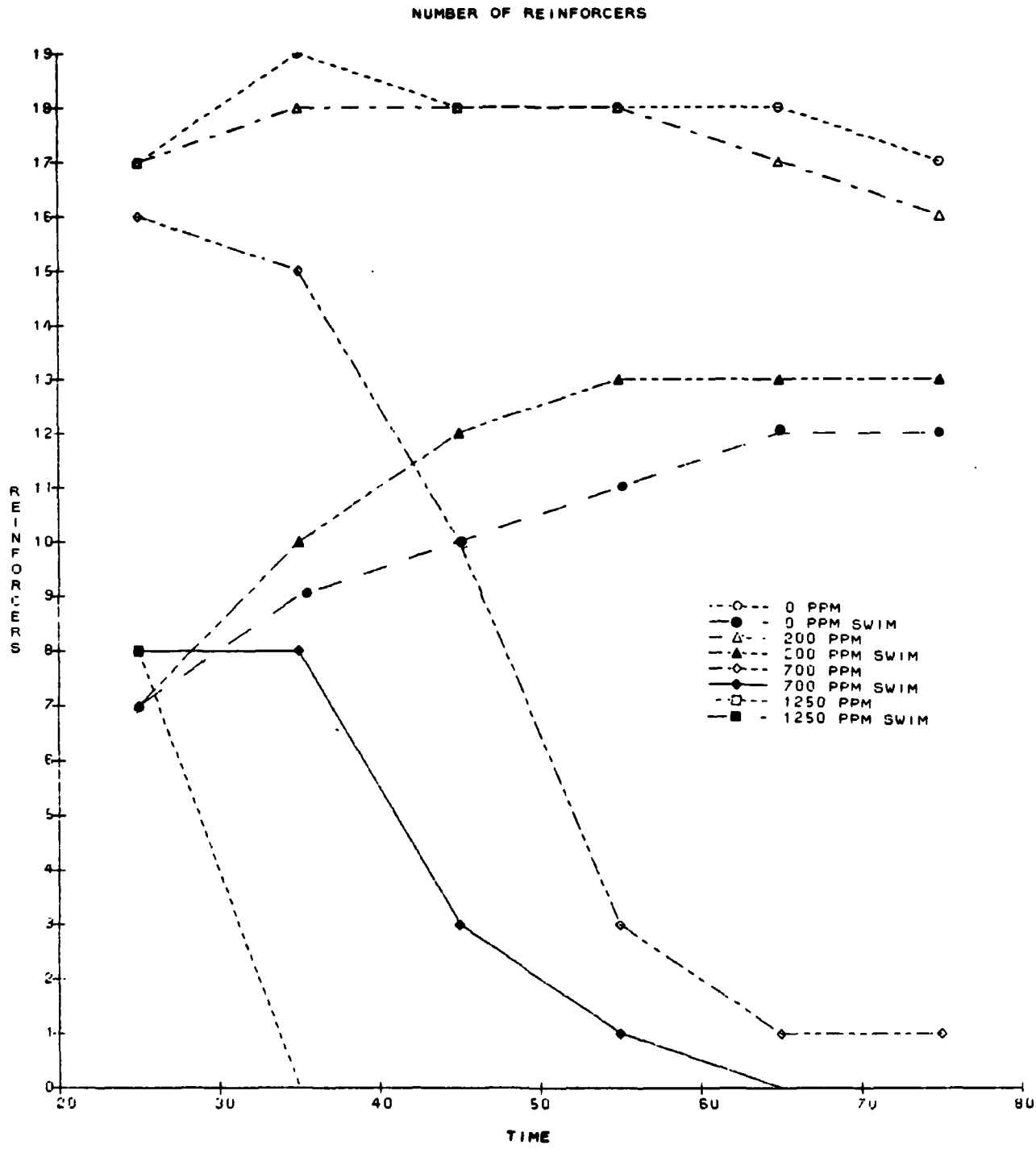


Figure 32. Time Course of Effects following CO and/or Swim Stress: Number of Reinforcers. Concentration ($p < 0.0001$) and swim stress ($p < 0.0001$) affected overall performance and the trend over time ($p < 0.0001$). The effect began at 25 min for 1250 ppm and at 45 min for 700 ppm. The effect of swim stress was present at all time points except 75 min. The interaction of CO concentration and swim stress was not significant.

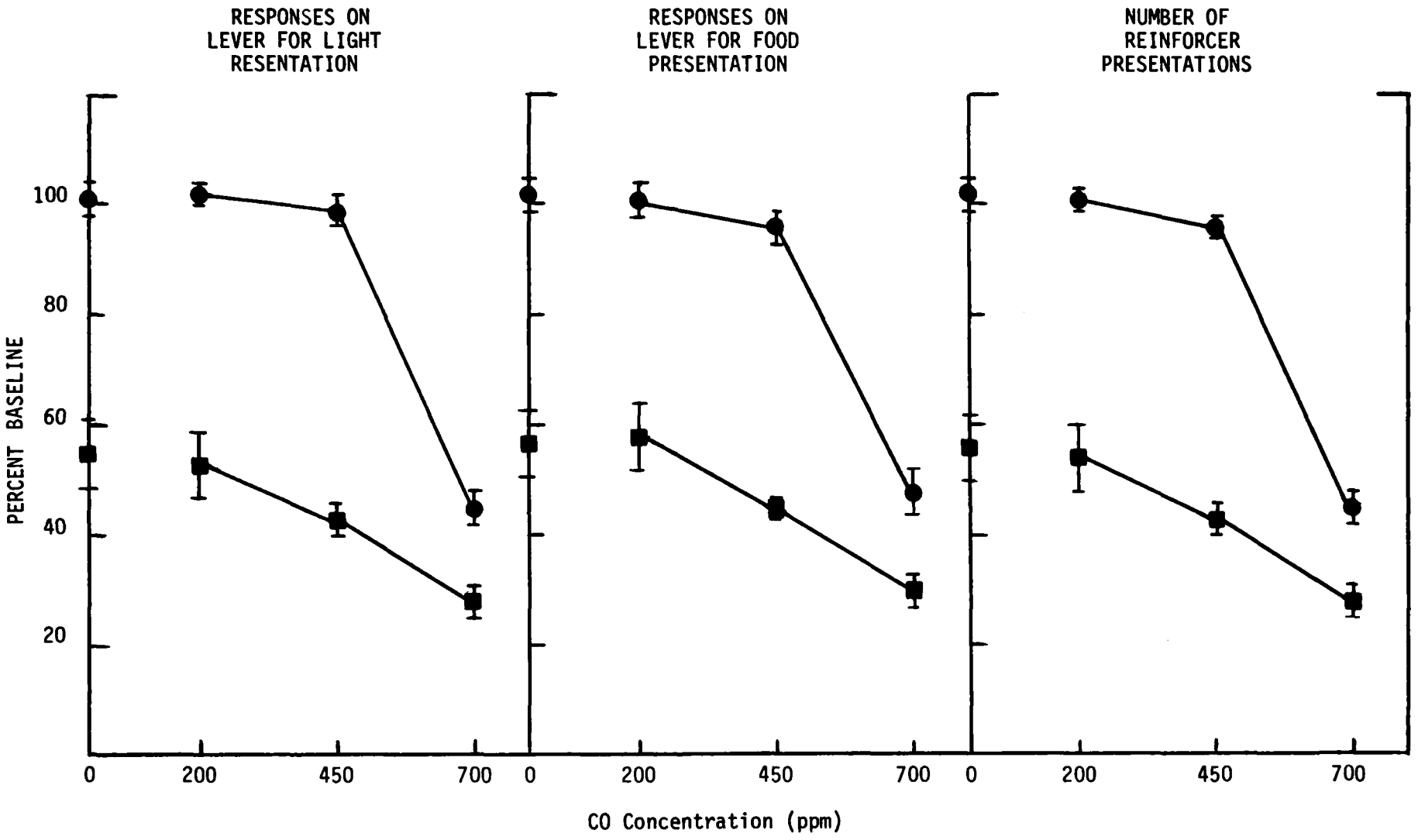


Fig. 33. Effects of CO and heat stress on performance on a chain FR30 FR30 schedule of reinforcement. Performance was measured during the last 60 min of a 75 min exposure session. Data are presented as percent baseline for three different measures of performance (Mean ± S.E.) The effect of heat ($p < 0.0001$) was significant as was the effect of 700 ppm CO ($p<0.0001$).
● are data for CO exposures; ■ are data for CO + 30.5°C

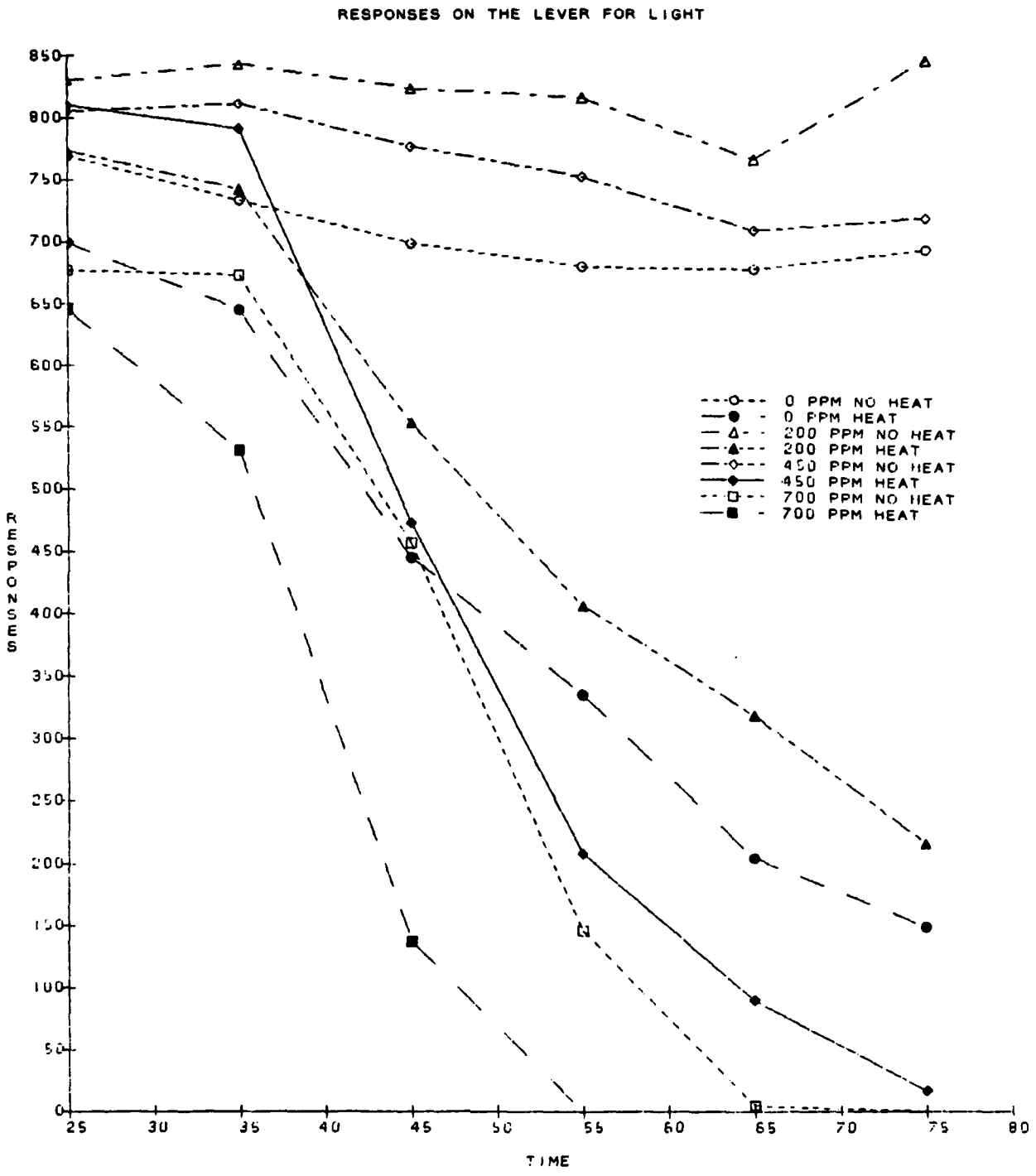


Figure 34. Time Course of Effects following CO and/or Heat Stress: Responses for Light Presentation. A significant concentration x heat interaction was found ($p < 0.0001$). Heat significantly decreased responding lineraly over time ($p < 0.0001$). The 700 ppm group was significantly different from 0 ppm for the last four time points. The 450 ppm group had depressed responding at the last three time points on the day of heat expsoure.

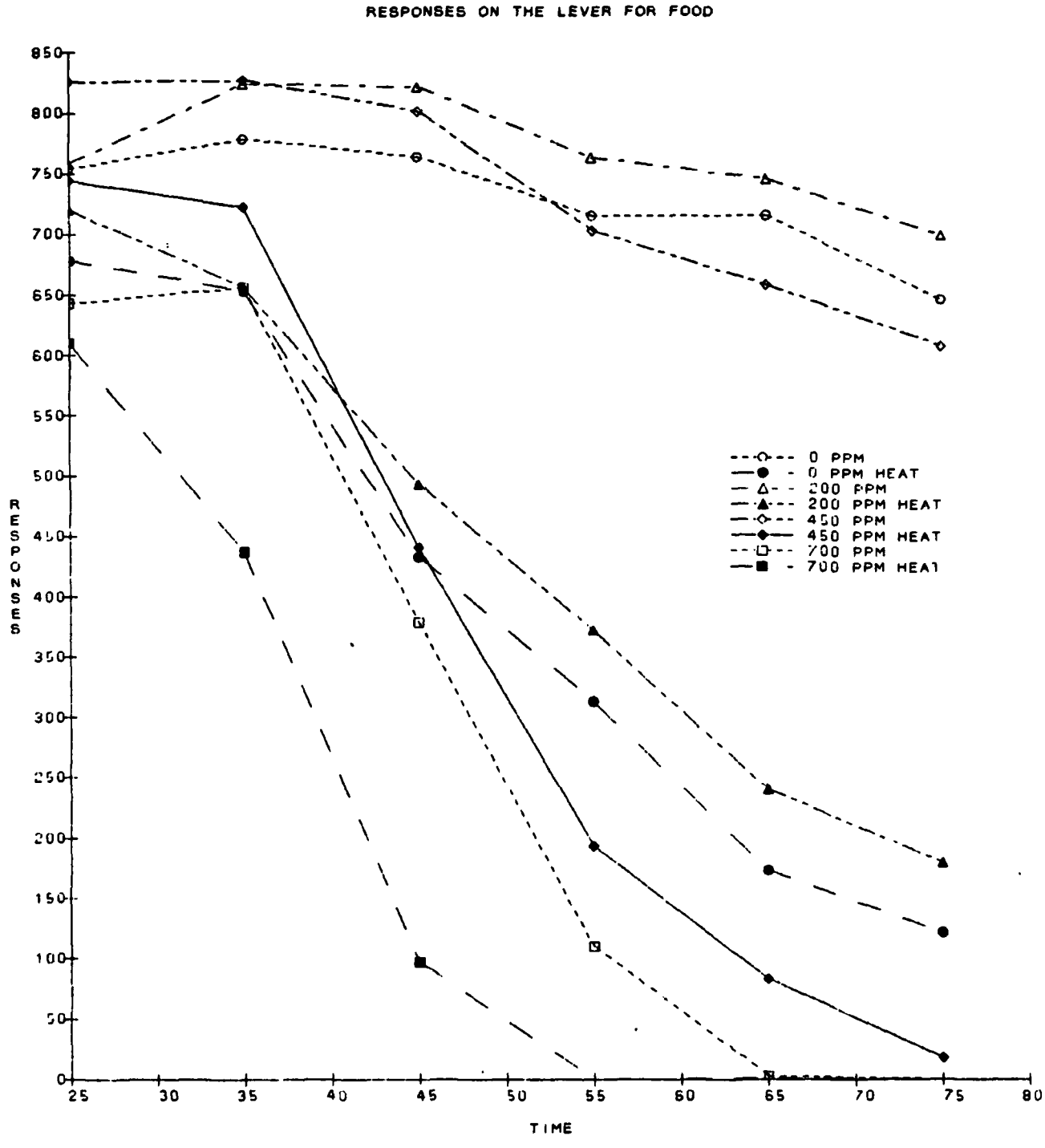


Figure 35. Time Course of Effects following CO and/or Heat Stress: Responses for Food Presentation. A significant concentration x heat stress interaction was found ($p < 0.0001$). This was due to decreases in responding in the 450 ppm heat stress group at the last three time points and both heat and non-heat animals at 700 ppm at the last four time points. The high dose heat stress animals also were lower than controls at the 25 and 35 min time points.

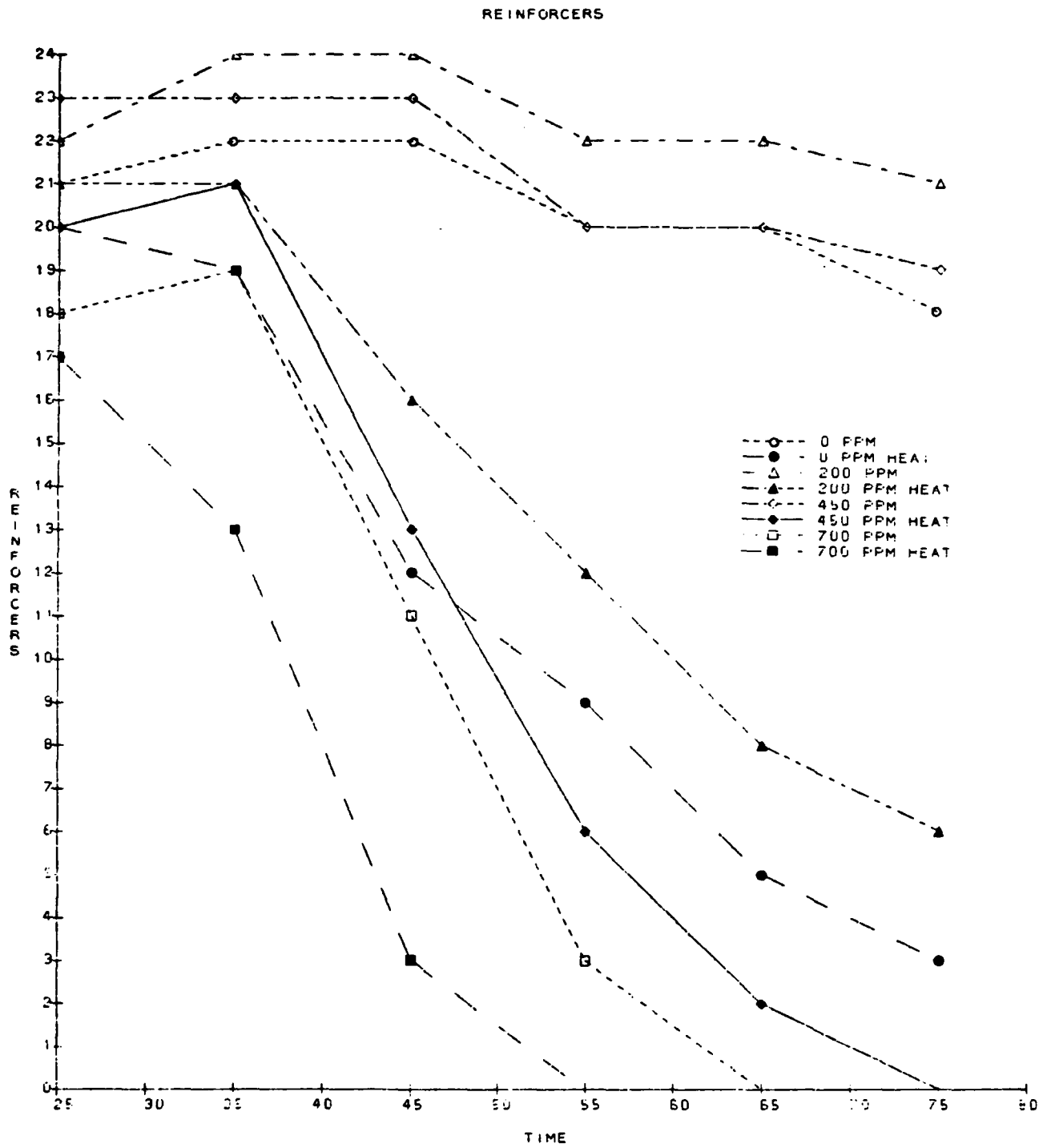


Figure 36. Time Course of Effects following CO and/or Heat Stress: Number of Reinforcers. The concentration x heat stress interaction was signficant at $p < 0.0001$. The interaction was due to decreases in responding in the 450 ppm group exposed to heat for the last three time periods. At 700 ppm, responding was decreased for all but the initial time period.

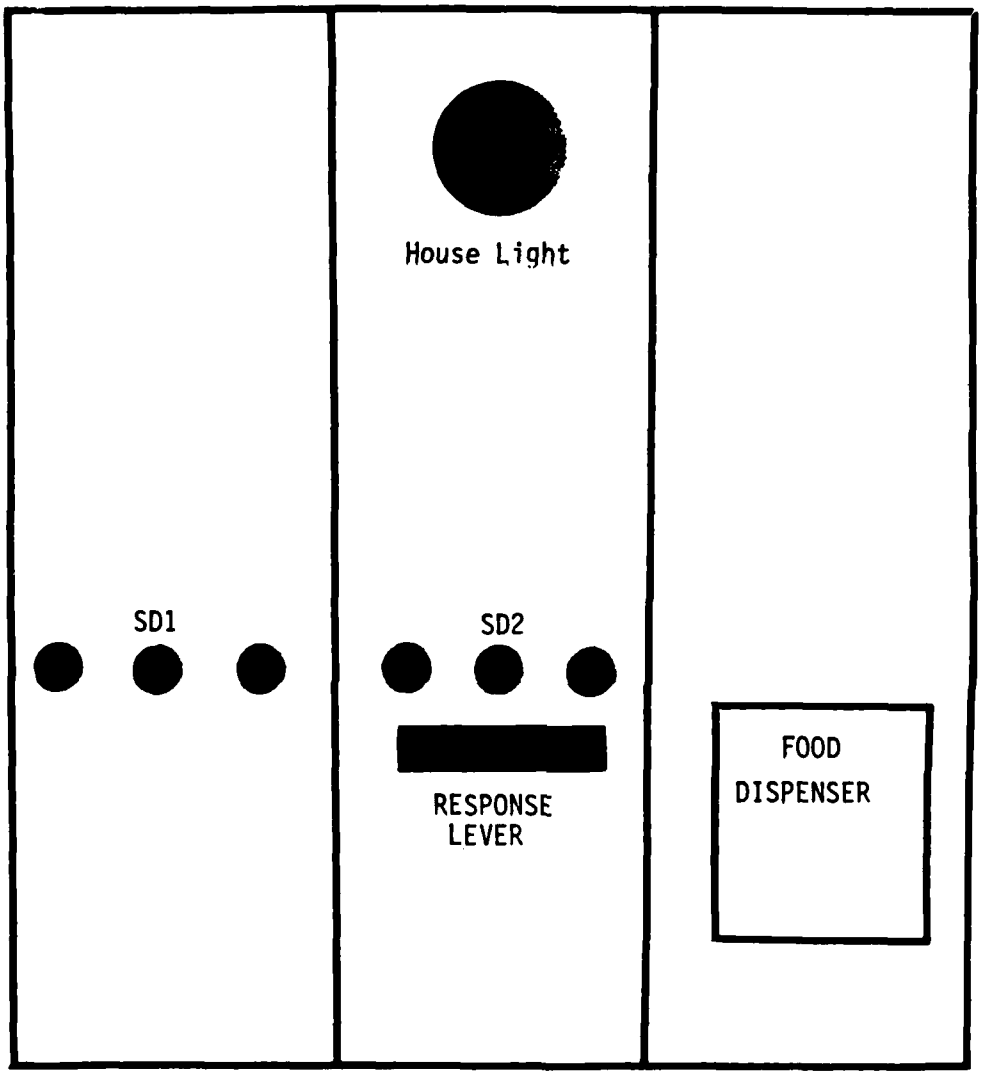


Figure 37: Intelligence Panel: arrangement for reaction time task

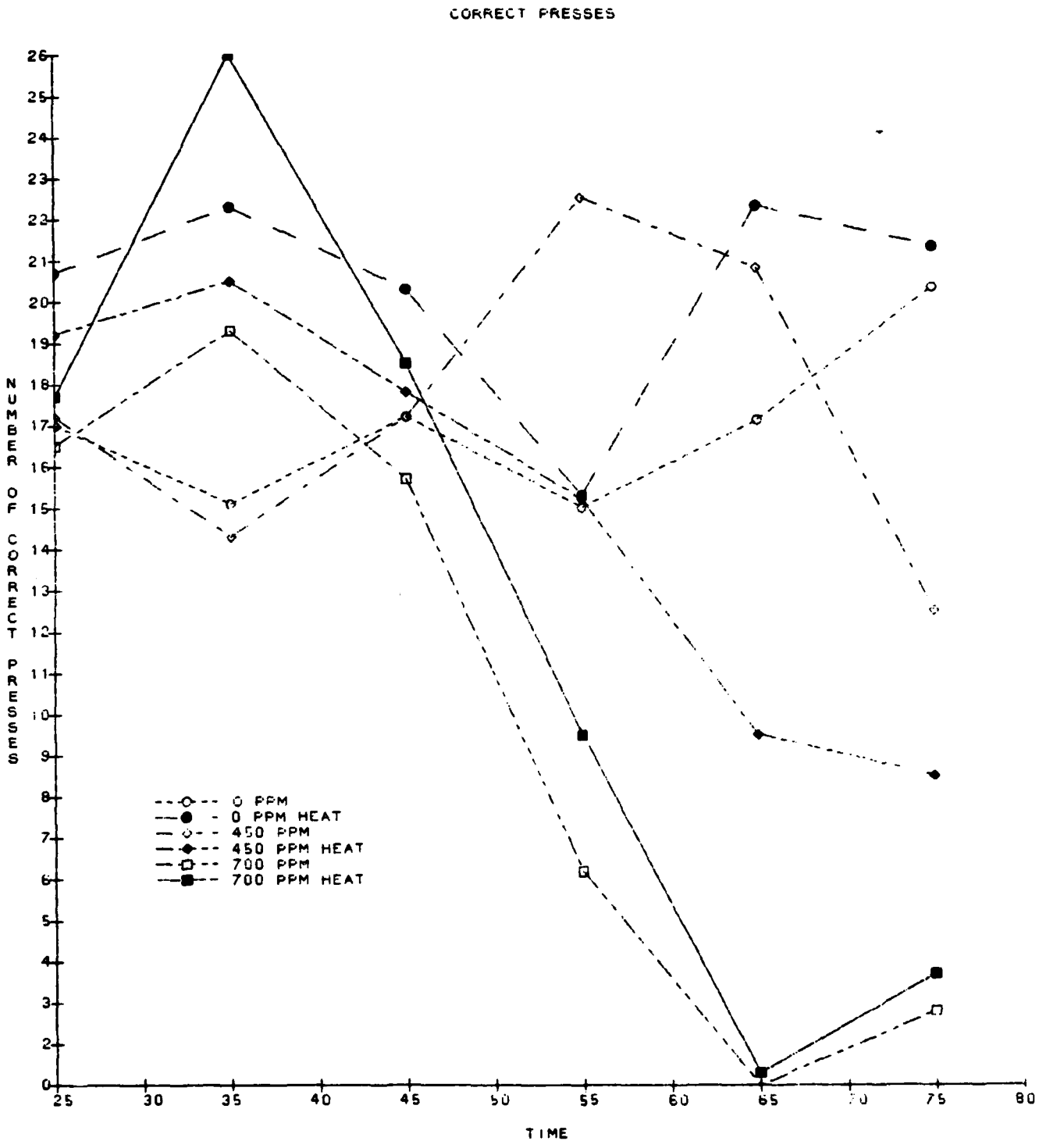


Figure 38. Time Course of Effects for the Reaction Time Task: Correct Lever Presses. There was a significant effect of dose in terms of time trend ($p < 0.0006$). The effect was significant at 75 min for 450 ppm ($p < 0.004$) and at 55 ($p < 0.04$), 65 and 75 min ($p < 0.0001$) for 700 ppm. There were no significant effects of heat.

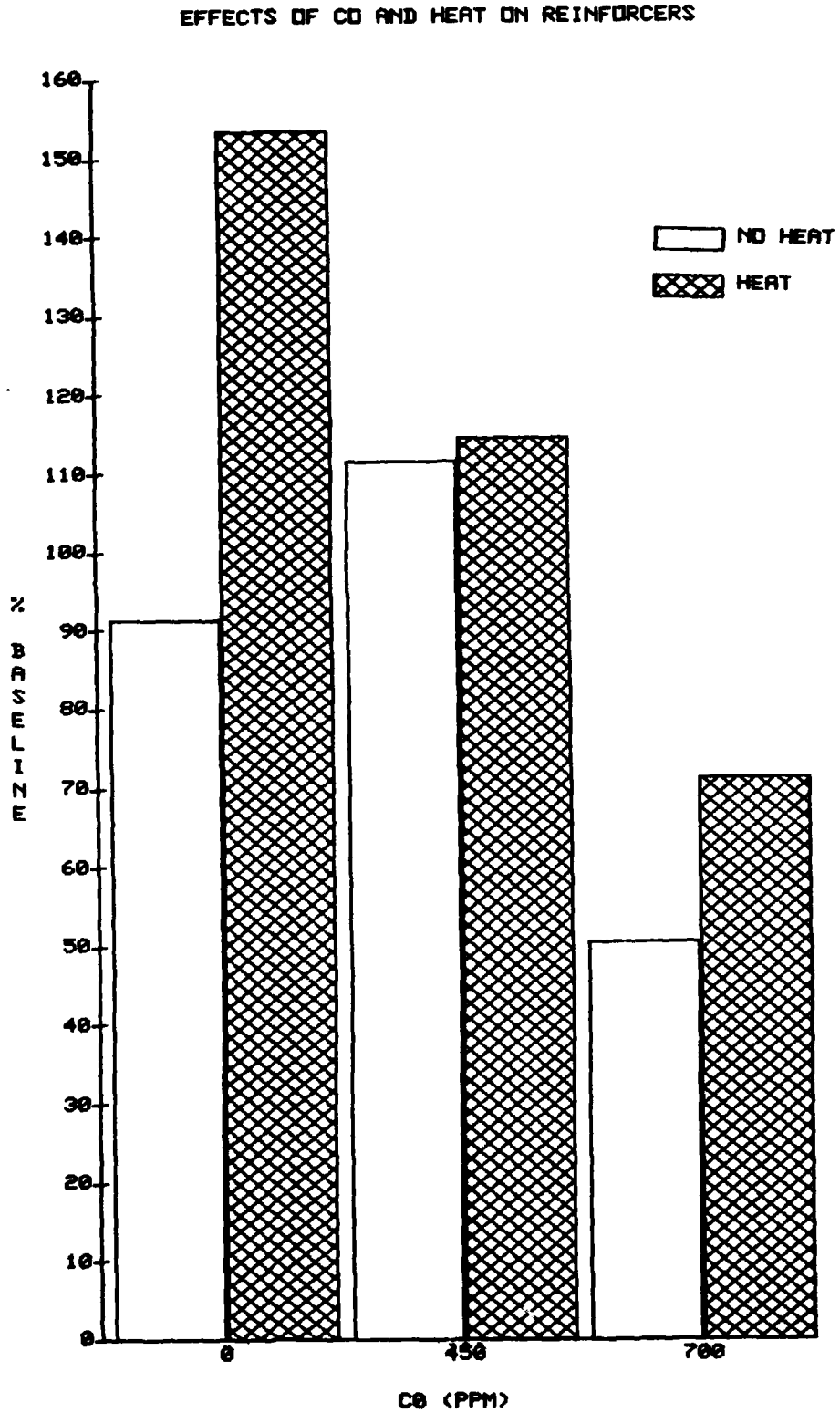


Figure 39. Mean Number of Reinforcers Obtained During 60-Minute Performance Sessions in the Reaction Time Task. Considered as total session performance, the number of reinforcers was not affected by CO or heat stress.

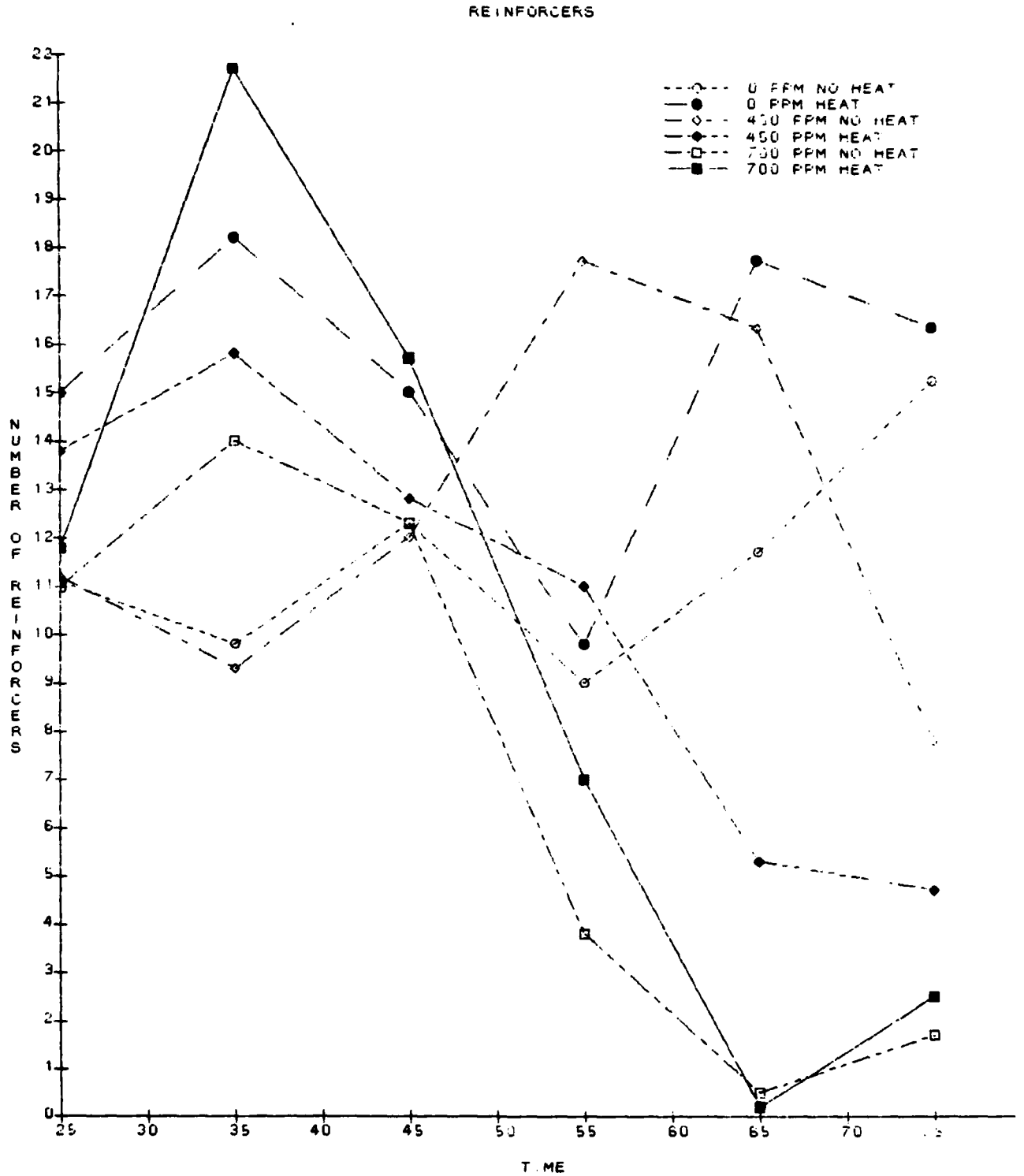


Figure 40. Time Course of Effects for the Reaction Time Task: Number of Reinforcers. A significant effect of dose on time trends was found ($p < 0.006$). The effect of 450 ppm was significant at 75 min ($p < 0.006$) as was the effect of 700 ppm at 65 and 75 min ($p < 0.0001$). There was no effect of heat stress.

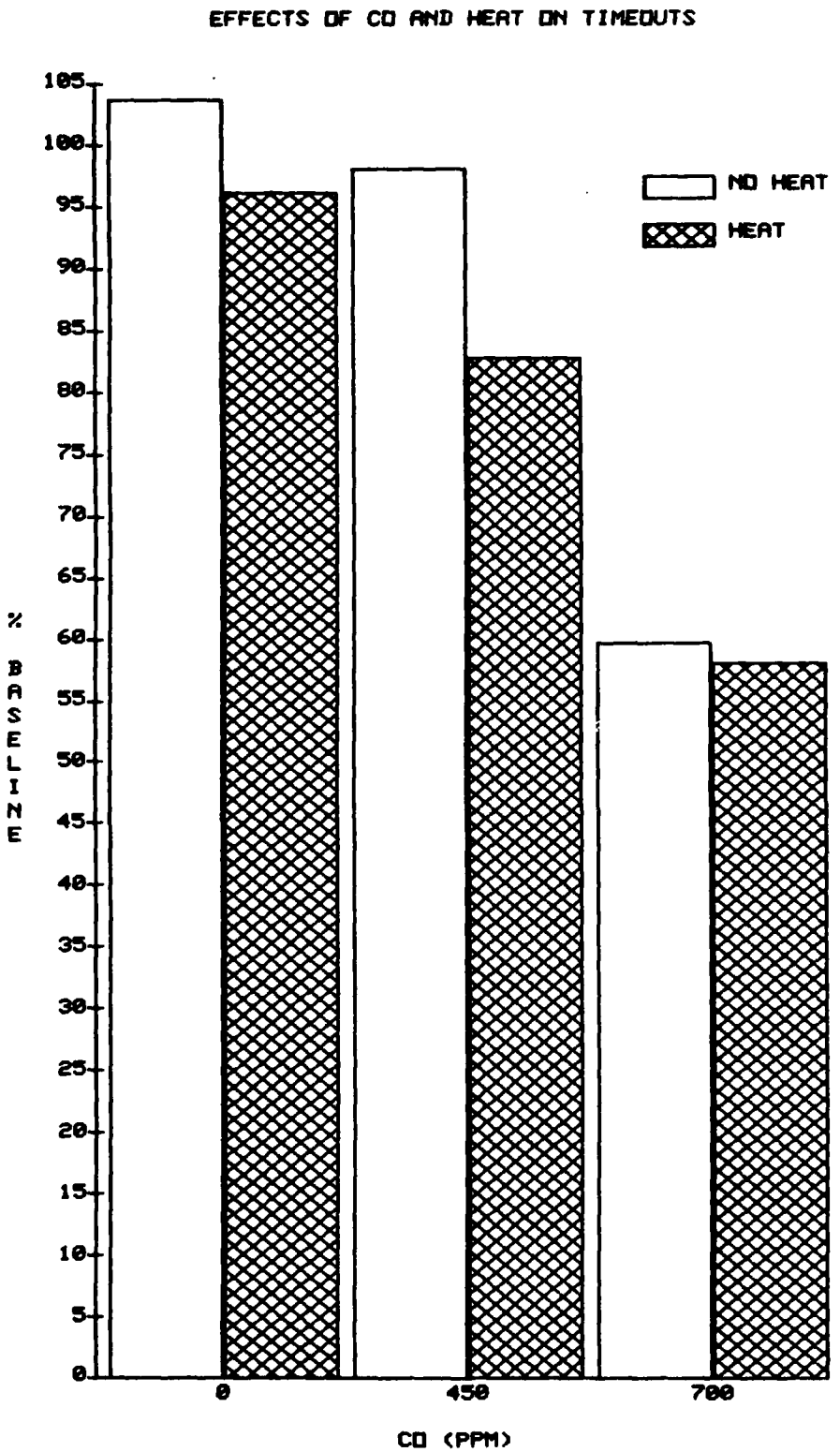


Figure 41. Mean Number of Timeouts Resulting from Premature Releases of the Lever During 60-Minute Performance Sessions in the Reaction Time Task. Timeouts were decreased by 700 ppm CO ($p < 0.0003$) but were not significantly altered by heat stress.

TABLES

AD-A146 576 DEVELOPMENT OF BEHAVIORAL TOXICOLOGY METHODOLOGY FOR INTERACTIVE EXPOSURE REGIMENS(U) IIT RESEARCH INST CHICAGO IL M M PREACHE ET AL. DEC 83 IITRI-L06131-18 2/3

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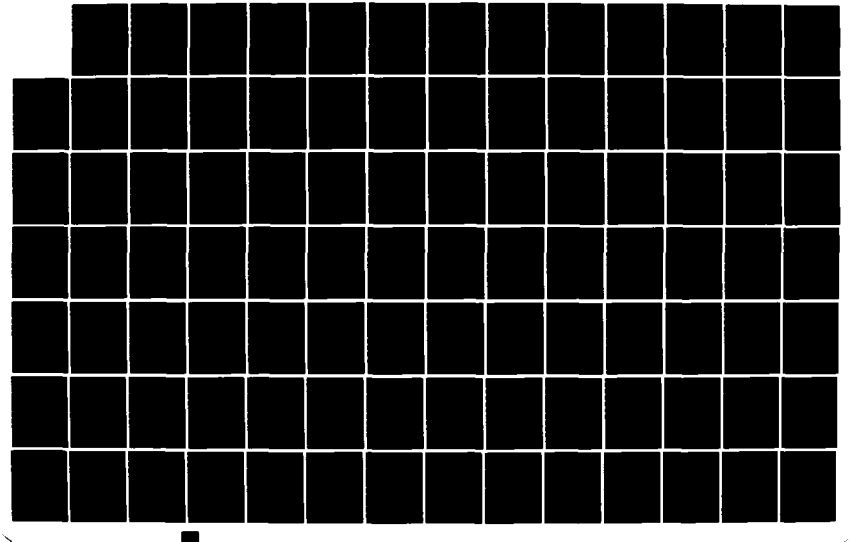

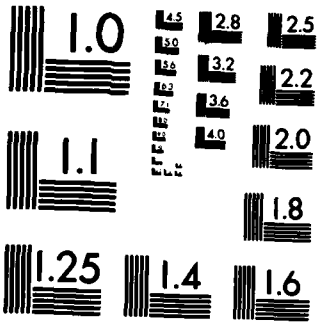

Table 1

CARBON MONOXIDE CONCENTRATION AT DIFFERENT PROBE LOCATIONS[1] WITHIN THE INHALATION CHAMBER

Probe Location	Chamber 1 250 ppm $\bar{X}$ ± S.D.	Range	Chamber 2 250 ppm $\bar{X}$ ± S.D.	Range	Chamber 2 1000 ppm $\bar{X}$ ± S.D.	Range	Chamber 3 1000 ppm $\bar{X}$ ± S.D.	Range
1	265 ± 5.1	261-274	233 ± 0.8	231-233	1010 ± 7.9	1002-1022	1018 ±15.6	1002-1032
2	264 ± 3.2	261-271	233 ± 4.1	231-241	1011 ± 5.7	1002-1022	1011 ±13.6	992-1034
3	264 ± 2.0	261-267	232 ± 1.0	231-233	1013 ± 7.4	1002-1022	1017 ±10.0	1002-1032
4	265 ± 2.4	261-267	233 ± 2.9	231-241	1014 ± 4.2	1012-1022	1012 ± 6.7	1002-1022
5	263 ± 2.5	261-267	233 ± 0.8	231-233	1012 ± 4.7	1002-1012	1008 ± 5.2	1002-1012
6	265 ± 1.3	264-267	232 ± 1.0	231-233	1010 ± 6.3	1002-1022	1020 ± 9.1	1012-1034

[1]All data are summarized as $\bar{X}$ ± S.D. for each location across all sessions. Each data point represents 10 samples. Each probe location was sampled twice during a session for five sessions.

Table 2

CARBON MONOXIDE CONCENTRATION DURING SESSIONS[1]

Session	Chamber 1 250 ppm		Chamber 2 250 ppm		Chamber 2 1000 ppm		Chamber 3 1000 ppm	
	$\bar{X}$ ± S.D.	Range	$\bar{X}$ ± S.D.	Range	$\bar{X}$ ± S.D.	Range	$\bar{X}$ ± S.D.	Range
1	267 ± 0.0	---	233 ± 0.0	---	1004 ± 4.1	1002-1012	1005 ± 5.2	1002-1012
2	267 ± 0.0	---	231 ± 0.0	---	1007 ± 8.4	1002-1022	1034 ± 0.0	---
3	264 ± 0.0	---	233 ± 0.0	---	1015 ±10.3	1002-1022	1012 ± 0.0	---
4	267 ± 5.1	264-274	233 ± 0.0	---	1012 ± 0.0	---	1012 ± 0.0	---
5	264 ± 0.0	---	233 ± 0.0	---	1012 ± 0.0	---	999 ± 5.2	992-1002
6	264 ± 0.0	---	233 ± 0.0	---	1012 ± 0.0	---	1022 ± 4.1	1014-1024
7	264 ± 0.0	---	233 ± 0.0	---	1012 ± 0.0	---	1012 ± 0.0	---
8	261 ± 0.0	---	231 ± 0.0	---	1012 ± 4.1	1002-1012	1029 ± 5.2	---
9	261 ± 0.0	---	236 ± 5.5	231-241	1020 ± 4.1	1012-1022	1014 ± 4.1	1012-1022
10	263 ± 4.1	261-271	231 ± 0.0	---	1012 ± 0.0	---	1005 ± 5.2	1002-1012

[1]Data are summarized as $\bar{X}$ ± S.D. for concentrations during a session regardless of location of the sampling probe.

TABLE 3

FORE- AND HINDLIMB GRIP STRENGTH FOR RATS FOLLOWING FORCED SWIMMING

GROUP	N	AVERAGE GRIP STRENGTH (G) ± SE FORELIMB	CV*	HINDLIMB	CV
0 min swimming	12	1634 ± 56	11.9%	998 ± 41	14.3%
10 min swimming	11	1511 ± 39	8.5%	926 ± 33	12.0%
20 min swimming	12	1430 ± 80	19.4%	875 ± 55	21.6%
40 min swimming	12	1478 ± 77	18.1%	947 ± 45	16.6%

*Coefficient of variation [(SD ÷ Mean) x 100]

TABLE 4

EFFECT OF SWIM STRESS ON HINDLIMB EXTENSOR RESPONSE

0 Min		20 Min	
Rat	Force (g)	Rat	Force (g)
71	817	111	150
57	237	48	533
75	114	110	750
53	323	129	3
119	667	73	433
90	790	114	893
65	307	59	317
30	864	79	0
55	913	68	33
93	933	120	310
81	843	77	223
67	373	44	180
Mean	598		319
S.E.M	88		83

All values are the mean of three scores.

TABLE 5

FORE- AND HINDLIMB GRIP STRENGTH FOR RATS FOLLOWING FORCED SWIMMING WITH 10 G WEIGHTS

GROUP	N	AVERAGE GRIP STRENGTH (G) ± SEM FORELIMB	CV[a]	HINDLIMB	CV
0 min swimming	12	1419 ± 69	16.9%	684 ± 46	23%
10 min swimming	12	1495 ± 34	7.5%	739 ± 36	17%
20 min swimming	12	1394 ± 58	14.5%	727 ± 57	27%
60 mg/kg Phenobarbital	12	925 ±113[b]	42.0%	436 ± 62[b]	49%

[a] Coefficient of variation = [(SD ÷ MEAN) x 100]

[b] $p < 0.05$

Table 6

Effects of Swim Stress Under Varying Conditions on VR10-FR30 Schedule Performance

Animal	Responses on Lever 1 for Light Onset*	Responses on Lever 2 for Reinforcement*	Number of Reinforcer Presentations*
	10 Min Forced Swimming With a 7g Weight		
74	67.7	110.1	68.6
** 49	1.7	0.1	0.0
**122	75.6	58.0	67.0
** 50	0.1	0.0	0.0
	20 Minutes Forced Swimming With a 5g Weight		
92	85.9	89.5	75.7
127	18.0	30.8	18.2
103	0.3	0.0	0.0
** 51	1.6	0.3	0.0
	15 Minutes Forced Swimming With a 5g Weight		
108	14.7	20.8	13.8
87	0.1	0.1	00.0
117	39.0	54.1	37.7
96	0.6	0.1	00.0

* Data are plotted as percent baseline. Baseline is the mean of the three days before the experiment.

** Removed from the water prior to elapse of the specified time.

TABLE 7

EFFECTS OF HEAT STRESS ON RECTAL TEMPERATURES

Exposure to 35.0 degrees C			Exposure to 32.2 degrees C		
Rat	Initial Body Temp	Final Body Temp	Rat	Initial Body Temp	Final Body Temp
311	37.7	38.8	298	37.7	37.7
325	37.9	37.3	327	37.5	37.9
331	37.7	37.8	286	37.7	38.1
316	37.7	38.3	274	37.8	38.8
330	38.8	38.1	290	37.2	38.4
318	37.8	37.9	306	37.4	38.9
Mean	37.9	38.0		37.6	38.3
S.E.M.	.2	.2		.1	.2

All body temperatures given in degrees centrigrade

Table 8

Distribution Of Animals To Exposure Conditions
For Pilot Heat Stress Experiment

Number of Rats - Week 1

CO Concentration	24° Temperature	High Temperature[a]	
		29.5°	32.2°
0 ppm	5	2	2
450 ppm	5	3	2
700 ppm	5	2	3

Number of Rats - Week 2

CO Concentration	24° Temperature	High Temperature[b]	
		29.5°	32.2°
0 ppm	4	3	2
450 ppm	5	2	3
700 ppm	5	3	2

[a]Animals assigned to this group comprised the ambient temperature group for Week 2.

[b]Animals in this group comprised the ambient temperature group for Week 1.

Table 9

EXPERIMENTAL DESIGN SUMMARY -

Allocation of Animals for COHb Determinations

Conditions	Time after Exposure		
	2 min	15 min	30 min
450 ppm CO	3	3	3
700 ppm CO	3	3	3
Swim + 450 ppm	3	3	3
Swim + 700 ppm	3	3	3
Heat + 450 ppm	3	3	3
Heat + 700 ppm	3	3	3

Controls - total of 6, 2/exposure day.

TABLE 10

DURATION OF SWIMMING FOR INDIVIDUAL ANIMALS PRIOR TO TESTING OF VR5 - FR15 PERFORMANCE

	Minutes Swum	Tail Weight as Percent of Body Weight
Control		
51	7.0	3.2
83	9.0	2.1
63	7.5	3.4
116	12.0	2.9
107	7.5	3.1
200 ppm		
91	5.5	3.4
64	5.0	3.0
122	6.0	3.5
117	10.0	2.5
87	8.0	3.3
700 ppm		
112	20.0	2.3
103	20.0	3.0
74	8.0	3.0
92	20.0	2.8
108	20.0	3.2
49	*20.0	2.8
1250 ppm		
105	10.0	2.9
127	10.0	2.7
50	6.0	3.3
96	5.0	3.5
109	6.0	3.6

* Tail weight came off during swimming

Table 11

Distribution of Animals to Swimming and CO Exposure Conditions for a Single Replicate[a]

CO Exposure Group:	Air Control						CO Low Level						CO Middle Level						CO High Level					
Animal No.:	C1	C2	C3	C4	C5	C6	L1	L2	L3	L4	L5	L6	M1	M2	M3	M4	M5	M6	H1	H2	H3	H4	H5	H6
Session No.[b]	Swimming Condition[c]																							
1	S	S	0	0	0	0	S	S	0	0	0	0	S	S	0	0	0	0	S	S	0	0	0	0
2	0	0	S	S	0	0	0	0	S	S	0	0	0	0	S	S	0	0	0	0	S	S	0	0
3	0	0	0	0	S	S	0	0	0	0	S	S	0	0	0	0	S	S	0	0	0	0	S	S

[a] Two replicates of 24 animals were tested

[b] Sessions were spaced at least 1 week apart

[c] S - Animal is given a period of forced swimming prior to the exposure

0 - Animal is not swimming on the day of exposure

TABLE 12

DISTRIBUTION OF ANIMALS TO HEAT STRESS AND CO EXPOSURE CONDITIONS FOR A SINGLE REPLICATE

CO Exposure Group:	Air Control						CO Low Level						CO Middle Level						CO High Level					
Animal No.	C1	C2	C3	C4	C5	C6	L1	L2	L3	L4	L5	L6	M1	M2	M3	M4	M5	M6	H1	H2	H3	H4	H5	H6
Session No.	Heat Stress Condition																							
1	H	H	H	O	O	O	H	H	H	O	O	O	H	H	H	O	O	O	H	H	H	O	O	O
2	O	O	O	H	H	H	O	O	O	H	H	H	O	O	O	H	H	H	O	O	O	H	H	H

Two replicates of 24 animals were tested

Sessions were spaced 1 week apart

H - Animal is exposed to both heat stress (30.5 degrees C) and CO

O - Animal is exposed to CO only

TABLE 13

DISTRIBUTION OF ANIMALS FOR THE REACTION TIME TASK

CO Exposure Group:	Air Control						CO Low Level						CO High Level					
Animal No.:	C1	C2	C3	C4	C5	C6	L1	L2	L3	L4	L5	L6	H1	H2	H3	H4	H5	H6
Session No.	Heat Stress Condition																	
1	H	H	H	O	O	O	H	H	H	O	O	O	H	H	H	O	O	O
2	O	O	O	H	H	H	O	O	O	H	H	H	O	O	O	H	H	H

Sessions were spaced 1 week apart

H - Animal is exposed to both heat stress (30.5 degrees C) and CO

O - Animal is exposed to CO only

TABLE 14

Mean Values for Measures of Performance in the Reaction Time Task

CO (ppm)	Correct Presses		Reinforcers (Correct Releases)		Time Outs		Reaction Time	
	No Heat	Heat	No Heat	Heat	No Heat	Heat	No Heat	Heat
0	102	122	69	92	32	30	40	40
450	104	91	74	64	29	26	66	68
700	62	77	43	59	13	18	44	52

Reaction time is in 1/100 sec.

The concentration analysis indicated significant effects for 700 ppm for correct presses ($p < 0.007$) and time outs ($p < 0.0003$). A significant effect of 450 ppm ($p < 0.03$) was observed for reaction time and a trend towards increased reaction time was present at 700 ppm. There were no significant effects of heat.

APPENDIX A

LITERATURE REVIEW:
BEHAVIORAL TOXICOLOGY METHODOLOGY

TABLE OF CONTENTS

		Page No.
1.	INTRODUCTION	111
2.	LOCOMOTOR ACTIVITY MEASURES IN BEHAVIORAL TOXICOLOGY	113
3.	THE USE OF SCHEDULES MAINTAINED BY POSITIVE REINFORCEMENT IN BEHAVIOR TOXICOLOGY	115
	3.1 Simple Schedules	116
	3.2 Complex Schedules	123
	3.3 The Nature of the Reinforcer	125
4.	THE USE OF NEGATIVE REINFORCEMENT IN BEHAVIORAL TOXICOLOGY	126
5.	EVALUATIONS OF SENSORY FUNCTIONING	127

1. INTRODUCTION

Traditionally, toxicology has been concerned with lethality and morphologic or biochemical changes. Only recently have the effect of toxins on other dimensions of human functioning been recognized and behavioral toxicology emerged as a discipline. It came to the fore in the United States only in the late 1960's although its importance had been recognized and called attention to earlier. (Ruffin, 1963, Magnuson, et al. 1964) Recognition of the potential hazards posed by environmental contaminants came as an aftermath to such incidents as occurred following the thalidamide experience (Lenz, 1962; Taussig, 1962) and the serious sequelae of massive mercury toxicity in Minemata (Matsumoto et al. 1965; Takeuchi 1972). Behavioral toxicology involves the use of behavior as a method of assessing the potential toxicity of compounds. Behavior is the endpoint of the functional integration of the nervous system and thus offers a sensitive method for determining the intact functioning of the central nervous system. Soviet scientists strongly emphasize the importance of the role of the central nervous system in integrating the functions that maintain good health and well being and thus have tended to more frequently incorporate behavioral methods in assessing chemical toxicity or other potential hazards (Ekel & Teichner, 1976).

There are numerous approaches to the study of behavior and comprehensive reviews of the application of behavioral methodology to toxicology have been provided by several authors. Evans and Weiss (1978) have reviewed substances of current interest including carbon monoxide and have examined some of the factors modulating the behavioral effects of toxins mainly in the context of schedule controlled behavior. General reviews of the area of behavioral toxicology have been published by Weiss and Laties (1969), Bignami, (1976), Xinteras and Johnson (1976) and Laties et al. (1977). The intention of this review is to examine some of the more commonly used methods in behavioral toxicology with the primary focus being those methods that are compatible with behavioral evaluations of animals during the actual period of inhalation exposure to a test chemical. The focus will also be on methods more amenable to

detecting subtle but relatively immediate performance decrements as opposed to methods which are more concerned with subtle effects of chronic or multi-generation exposure to the test chemicals. Thus, a number of methods that can be very valuable in behavioral toxicity evaluations will for the most part be ignored. For example, procedures which require continuing experimenter intervention such as assessment of the hindlimb extensor and forelimb grip strength responses (Cabe and Tilson, 1978; Cabe et al., 1978) and sensorimotor screening procedures (Irwin, 1968) are not described because they would be difficult to conduct within inhalation chambers. Similarly, some of the tests of locomotor ability such as rod crossing, treadmill running, or horizontal jumping which are often used in assessing cerebellar defects (Brunner and Altman, 1973; Lynch et al. 1976) have been omitted. Mazes are frequently used in behavioral research with rodents and various types of maze training involving either positional or sensory cues have have been employed in assessing behavioral toxins or teratogens (Brady et al., 1975; Brown, 1975; Brown et al., 1971; Bullock et al., 1966; Snowdon, 1973; Van Gelder et al., 1973); however, the size, nature of construction, and to some extent associated procedures make most mazes incompatible with testing during inhalation exposures. Startle response (Conner et al., 1970) and open field (Hall, 1934) testing are relatively rapid behavioral assessment procedures which have been used in both adult and developmental behavioral toxicology (Ahlenius et al. 1977; Coyle et al. 1976; Reiter et al. 1975; Sobotka et al. 1972). However, with inhalation exposures the time required to establish the test environment for each animal, or set of animals, detracts from the advantage of very short duration testing sessions.

Feasibility was of course not the only factor affecting the selection of behavioral methods for review. The focus also includes a concentration on conditioned responses with little or no attention to unconditioned behaviors such as consummatory or reproductive behaviors or to social behaviors. However, in that locomotor activity is one of the most frequently used measures in behavioral toxicology, because effects on activity levels are important in many performance procedures, and because some of the various methods are amenable to testing in inhalation chambers, the following will give a brief

overview of some of the procedures employed in these measurements.

2. LOCOMOTOR ACTIVITY MEASURES IN BEHAVIORAL TOXICOLOGY

Impaired motor function can be responsible for disruptions in both simple and complex behavioral performance. Increases or decreases in motor activity can interfere with the performance of other behavior through the expression of competing responses.

Activity measurements have been widely used to assess chemically induced alterations in central nervous system function. Various techniques have been used in the assessment of motor activity and these techniques vary in which aspects of motor activity they measure.

A number of devices have been used to measure general activity levels and these have been reviewed and evaluated by (Finger, 1972). The stabilimeter is a cage supported by a central transverse axis that shifts position when the animal inside moves. While the types of movements that will be recorded are dependent on the shape of the cage and the tilt of the axis, the stabilimeter generally does not record movements which occur in only one part of the cage. Using a stabilimeter as the method of assessing activity in rats, Schmidt and Czech (1977) observed increased activity levels in lead treated animals as compared to those of vehicle control animals. Another significant finding in the study was that lead treatment also produced large decrements in food consumption. When the locomotor activity of lead treated and yoked control groups (groups for which the amount of food available was restricted to the amount consumed by lead treated animals) was compared, no differences were observed. This study illustrates the importance of considering the effects of hypophagia induced by the chemical treatment when studying the effects of chemicals on spontaneous motor activity as well as on other categories of behavior.

Photocell apparatuses can be similar in shape to the stabilimeter but differ in that movements are recorded when the light beams are interrupted. The sensitivity of devices that are based photocell detection is a function of the number and arrangement of the photocells throughout the box.

Residential mazes are used to measure the activity of rats over long periods. As described by Norton et al. (1975), the residential maze consists of a series of interconnecting alleys equipped with photocell detecting devices. Locomotor activity is measured as photocell beam interruptions. The residential maze has received much use in the evaluation of toxic agents including lead (Reiter et al., 1975) carbon monoxide, and x-irradiation (Norton et al., 1976). It offers several advantages over traditional activity measuring devices. During the period of assessment the animal resides in the maze thus allowing measurement of activity over time. Exploratory activity appears to habituate fairly quickly and control rats follow a normal pattern of circadian rhythmic activity to which the effects of a treatment can be compared. Thus, disruption in the rhythmicity of activity as well as alterations in total activity can be considered simultaneously. As rhythmic changes in activity may influence the sensitivity of organisms to an agent (Reinberg and Halberg, 1971), this is an important advantage.

The running wheel (or activity wheel) is a cylinder which rotates around its axle when an animal walks or runs in it. Some of the commercially available apparatus are designed with a live-in cage adjacent to the wheel so that the animal can be given free or limited access to the wheel. In the simplest usage of this apparatus, wheel rotations are accumulated on mechanical counters and the number of rotations per unit time is used as the measure of activity. Activity wheels have received only limited application in behavioral toxicology. This may in part be due to the fact that they require physical exertion by the animal. Interpretation of the results is thus complicated by the relative contributions of exercise effects and gross motor activity effects. Some investigators have modified the running wheel so that the wheel turns only when the animal makes a lever-press response to free the wheel. In one such modification Collier and Hirsch (1971) showed that the wheel running could be used as a reinforcer for lever pressing. By using lever pressing as an additional dependent variable, some of the objections noted for the activity wheel may be diminished.

The activity device used in evaluating the effects of an agent on motor activity can be a critical variable and must be considered in the analysis of the results. For example, the finding of lead-induced hyperactivity has been reported by Silbergeld and Goldberg (1973, 1974), Sauerhoff and Michaelson (1973), Golter and Michaelson (1975), Overmann (1977) and Dubas and Hrdina (1978). Hyperactivity has not been observed following lead treatment by Sobotka et al. (1975) Krehbiel et al. (1976) or Modak et al. (1975). While a number of important variables have differed among these studies, the different measures of activity used can only have complicated the interpretation of the data further.

Activity measures, while they undoubtedly reflect important aspects of central functioning, are basically measures of unconditioned behavior. It is important to be aware of the effects of any toxin on measures of general activity because, as was pointed out previously, changes in activity can influence the performance of other behavior. However, where the concern is subtle effects on the central nervous system which may result in performance decrement other behavioral methods may be more valuable. Such measures can frequently include some assessment of activity effects.

3. THE USE OF SCHEDULES MAINTAINED BY POSITIVE REINFORCEMENT IN BEHAVIORAL TOXICOLOGY

The assessment of higher nervous system function through the use of animal models presents a challenging problem. Drawing from the methods of the behavioral sciences and applying these to toxicology, behavioral toxicology was equipped at its onset with a relatively advanced technology which had been successfully applied to pharmacology. The technology used in both behavioral toxicology and behavioral pharmacology was drawn from work in experimental psychology, mainly the techniques originally developed and pioneered by B.F. Skinner (1938) and frequently referred to as operant psychology.

The fundamental premise of operant psychology is that behavior is determined by its consequences. Stated simply, how an organism behaves is the result of rewards and punishments. In the experimental situation, the environment is manipulated so that what an organism does is either rewarded

or punished. By arranging behavioral consequences in an orderly fashion, the organism comes to respond in a well defined and predictable manner. This arrangement of reinforcement contingent on the completion of specified response requirements defines a schedule of reinforcement. For ease of reference brief definitions of some of the more commonly used schedules are given in Table A1. Comprehensive discussion of standard schedules of reinforcement can be found in Ferster and Skinner (1957) and Reynolds (1968). Additional sources for detailed analysis and interpretations of schedules include Morse (1966) and Zeiler (1977).

3.1 Simple Schedules

Schedules produce characteristic patterns of responding which are stable and replicable. They provide ongoing performance against which various insults can be evaluated in an objective manner. In addition to their usefulness in studying the determinants of behavior they may be useful in determining underlying biochemical mechanisms.

Schedules of reinforcement are most often defined in terms of time or responses (number). On time based schedules, reinforcement is contingent upon response requirements following the passage of specified periods of time. The standard time based schedules are fixed interval and variable interval schedules. Fixed interval schedules require that a response be made following a specified time; variable interval schedules program reinforcement at intermittent time periods.

When responding is reinforced on a fixed interval schedule, a pattern of responding which is characteristic of the schedule emerges and appears to be independent of species, response mode, or the nature (quality) of the reinforcer (Kelleher and Morse, 1968). During the early part of the interval responding is virtually absent, but shows some increases as time within the interval progresses. As the time at which reinforcement is available becomes more immediate, responding rapidly accelerates to a high terminal rate. This response pattern has been interpreted by some as indicative of time discrimination. However, Morse (1966) offered an alternative interpretation based on the principle that responses occurring just prior to delivery of the reinforcing stimulus would be more strongly reinforced. The strength of

TABLE A1

DEFINITIONS OF STANDARD SCHEDULES OF REINFORCEMENT

Continuous Reinforcement or Fixed Ratio 1 (CRF, FR 1):	Each response is followed by reinforcement.
Fixed Interval (FI):	Reinforcement is contingent upon a response being made after a specified period of time has passed. Responses during the interval have no consequences.
Variable Interval (VI):	Responses are intermittantly reinforced over time.
Fixed Ratio (FI):	Reinforcment is contingent upon a specified number of responses being made after the last reinforcement.
Variable Ratio (VR):	The number of responses required for reinforcement varies from reinforcement to reinforcement in an irregular manner.
Differential Reinforcement of Low Rates of Responding (DRL):	A response is reinforced only after a specified period of time has elapsed; responses earlier in the interval reset a timer and reinitiate the interval.
Differential Reinforcment of High Rates of Responding (DRH):	A response is reinforced provided that a specified number of responses occurs before a specified time elapses
Concurrent:	Two or more responses are reinforced according to two or more schedules at the same time.
Multiple:	Two are more independent schedules are presented successively, each in the presence of a discriminable ext oceptive stimulus.
Mixed:	Two or more independent schedules are presented successively without any external stimulus to indicate which schedule is in effect.
Fixed Consecutive Number (FCN):	A specified minimum number of responses must be made on one response device before a response on a second device will be reinforced.
Progressive Ratio:	The response requirement for each successive reinforcement is progressively increased.

reinforcement would be progressively weaker for responses more distant in time from the delivery of the reinforcer. Although not the method of choice if the intent is to study disruption of time estimation, fixed interval schedules have been widely used in pharmacology and have been shown to be sensitive to a number of pharmacological agents (Kelleher and Morse, 1968).

On variable interval schedules, some average interval value is typically selected and the interval between successive reinforcer deliveries is varied around that average value. A moderately high sustained rate of responding is generated by reinforcement on a variable interval schedule and is considered to be due the uncertainty of reinforcement (Iversen and Iversen, 1975) this schedule has not been widely used in behavioral toxicology, although both increased and decreased responding have been reported in studies of the effects carbon monoxide on variable interval performance.

Two standard response-number based schedules are the fixed ratio and variable ratio schedules. Fixed ratio schedules set a fixed number of responses as the criterion for reinforcement. Fixed ratio schedules generate a pattern of behavior which includes a steady high rate of responding from the first response after delivery of a reinforcer up to the response that fulfills the ratio requirement for the next reinforcer (Reynolds, 1968). Where the magnitude of the ratio is large these runs of steady responding are typically separated by a period of nonresponding or pausing after reinforcement, whereas with small ratios postreinforcement pausing is not typical of the response pattern. There are species differences in what represents a large or small ratio. For example, for lever pressing responses with rats, a ratio of 15 may be small. With pigeons performing a key pecking response, 50 is a small ratio. These differences in relative values of the ratios are most likely in part dependent on the topography of the criterion response and how well it fits within the response repertoire of the species being tested.

Where the value of the ratio in a fixed ratio schedule is set at one (FR 1), i.e., one response required for each delivery of the reinforcer, the schedule is more frequently denoted as a continuous reinforcement schedule

(CRF). Shaping of responding often begins with reinforcement on a CRF schedule. Although CRF schedules are used in various areas of behavioral research including behavioral toxicology, satiation can be a problem with some reinforcers if the experimental sessions are long. Another characteristic of the CRF schedule that is important when considering its use is that compared to responding maintained by intermittent schedules of reinforcement, responding associated with a CRF schedule of reinforcement tends to extinguish rapidly when delivery of reinforcement is discontinued.

When each delivery of the reinforcer is contingent upon completion of some specified number of responses but that number fluctuates over successive reinforcements, the schedule is called a variable ratio schedule. A single number is typically used to denote the magnitude of the ratio in such schedules (e.g., VR 15) but the number actually indicates the average of ratio requirements. Once established variable ratio performance is characterized by a very high, nearly constant rate of responding at almost all ratio values. However, pausing may occur if the average ratio exceeds certain values or if not enough small or medium ratios are included in the schedule. Strain is the term used to denote abrupt pauses in a constant, rapid rate of responding on a variable ratio schedule and is often due to overly rapid advancement of the ratio requirements. Performance on variable ratio schedules tends to be slower to extinguish then performances on a comparable fixed ratio schedule.

These four schedules - fixed interval, variable interval, fixed ratio, and variable ratio - are some times designated as "simple" schedules of reinforcement. A number of other schedules can be derived from these by imposing more specific criteria for reinforcement delivery or by combining simple schedule components into more complex schedules.

The DRL (differential reinforcement of low rates of responding) schedule is a variant of the interval schedule in that one of the contingencies for reinforcement is the passage of a specified amount of time since the last reinforced response. The added criterion is that the cumulative response during the criterion interval must not exceed some specified number. If this number is exceeded before the interval elapses, the opportunity for

reinforcement is lost and timing of the criterion interval and counting of responses begins at the time the response total was exceeded. DRL schedules tend to generate low rates of responding. One method of examining DRL performance is to examine the distribution of interresponse times (IRT). If a relative frequency distribution is plotted for IRT durations, the curve characteristically shows a pronounced peak at about the IRT value that equals the criterion interval. IRT durations slightly longer or slightly shorter than the criterion interval occur somewhat less frequently and very short IRT's even less frequently (Reynolds, 1968). Where the number of responses that cannot be exceeding during the criterion interval is set at one, the schedule is a pure example of differential reinforcement of interresponse time.

An even more stringent variation of differential reinforcement of interresponse times is represented by a schedule in which both minimum and maximum limits on the IRT are made criteria for reinforcement. In such a schedule only those responses which terminate an IRT that is longer than a specified duration and shorter than a slightly longer duration are reinforced. For example, the contingency might be that the response that produces the reinforcer be made no sooner than 10 sec and no later than 12 sec after the last reinforced response. A response too early in the interval would result in restart of the interval as would the lapsing of the criterion interval without a response. In either case an opportunity for reinforcement is lost. Performance on schedules which involve differential reinforcement of interresponse times are considered indicative of time discrimination (Reynolds, 1968) and thus should be considered where disruption of time estimation is of interest.

There are also variations of the ratio schedules. For example, a progressive ratio schedule is one in which the ratio is advanced by some specified number after each delivery of reinforcement, that is, the animal is required to emit a progressively increasing number of responses in order to receive each successive reinforcement. The pattern of responding on progressive ratio schedules is characterized by periods of high rates of consistent responding or "runs" and periods of pausing.

The DRH schedule involves the differential reinforcement of high rates of responding. This is basically a variant of fixed ratio schedul? with the added criterion that the ratio must be met within a specified time interval. If the interval elapses without the ratio being met, any responses made during the interval do not count towards meeting the ratio criterion for reinforcement. Although this schedule generates high rates of responding it has not been used extensively in behavioral toxicology.

Simple schedules of reinforcement have been widely used in behavioral pharmacology to investigate the effects of drugs on behavior. Their usefulness has been pointed out repeatedly (e.g. see Kelleher and Morse, 1968; Thompson and Boren, 1977). A comprehensive summary of the effects of drugs on schedule controlled behavior can be found for specific agents and individual schedules in Seiden and Dykstra (1977). The importance of ongoing behavior in determining a drug's effect was illustrated in an early experiment by Dews (1955). Pigeons were trained to peck a response key under two different intermittent schedules of food presentation , either a fixed interval or a fixed ratio schedule. Administration of pentobarbital (1 - 4 mg/bird) markedly reduced fixed interval responding. In contrast, these doses of pentobarbital increased fixed ratio responding. Lower doses produced increases in rates on both schedules. These data illustrate the importance of drug dose, as well as the schedule of reinforcement maintaining behavior in determining the drugs effect.

Certain schedules have also been shown to be particularly sensitive to specific classes of drugs. For example, McGuire and Seiden (1980) have shown that the effects of tricyclic antidepressants differ from other classes of drugs on DRL schedules. The tricyclic antidepressants at certain doses produce a decrease in response rate which is reflected in a decrease in responses having very short interresponse times. In contrast, cholinergic blocking agents increase response rate and decrease reinforcement rate (McGuire and Seiden, 1980). Psychomotor stimulants increase response rate,

decrease reinforcement rate and shift the IRT distribution to the left (e.g. Schuster and Zimmerman, 1961; Campbell and Seiden, 1973; Seiden et al. 1979). Ethanol generally decreases response and reinforcement rate at all doses that have effects (e.g. Sidman, 1955; Sanger et al. 1974).

The extent of the effects seen with different tricyclic antidepressants also appears to be related to the underlying neurotransmitter systems involved. The tricyclic antidepressants have been shown to block the reuptake of norepinephrine (NE) into nerve terminals (Axelrod et al. 1961; Waldmeier et al., 1976) with a potency relationship for NE uptake blocking properties of desmethylimipramine>imipraine>chlorimipramine. This relationship paralleled the potency relationship of the effects of these drugs on the DRL schedule, suggesting that the underlying biochemical changes may be related to the behavioral effects of these drugs. Thus, schedules of reinforcement have the potential for assisting in elucidating the underlying mechanisms of drug action. The selective disruption of schedules by certain classes of agents and the correlation of these disruptions with biochemical changes offer a potential advantage to behavioral toxicology.

Although the use of schedule-controlled behavior in the assessment of toxins is still a relatively new area, selective sensitivity of schedules to different agents has been shown. Dietz and McMillan (1979) have shown greater sensitivity to DRL than FR schedules following administration of the pesticides mirex and kepone. Although their effects on DRL differed (mirex increased very long IRTs, while kepone increased very short IRTs) these effects were apparent before the disruption occured on FR responding. The disruption in FR responding did not occur until overt signs of toxicity were also apparent.

Cory-Slechta and Thompson (1979) used a fixed interval schedule to assess the effects of chronic postweaning lead exposure in rats. They found disruptions in fixed interval performance including increased response rates and decreased time to initiation of responding in the interval. This was the first study to show that lead could produce effects on performance on operant schedules when administered to adult animals.

A number of simple reinforcement schedules were considered in the carbon monoxide literature (Appendix B). One of the earliest investigations was the work of Beard and Wertheim (1967) in which VI, FI, VR, FR, and DRL schedules were employed. This study revealed no indications of differential sensitivity of the schedules as effects were seen at the same levels on all schedules. However, as discussed in Appendix B methodological questions could be raised concerning the studies and these might account for the lack of apparent differential sensitivity. Other studies in which simple schedules were used in the study of the behavioral effects of carbon monoxide are described in Appendix B. In general, these studies would seem to indicate that schedules which generate high rates of responding would be more readily affected by carbon monoxide but this of course would not be expected for all agents.

3.2 Complex Schedules

Evaluation of more than one schedule during an experimental session can be accomplished through the use of concurrent, multiple, or mixed schedules. With concurrent scheduling of reinforcement, two or more responses are reinforced according to two or more schedules with both schedules in operation at the same time. For example, lever pressing may be maintained on one lever by reinforcement on an FR schedule at the same time lever pressing on a second bar or some other response if reinforced on an FI schedule. Although the contingencies for reinforcement on the two component schedules of a concurrent schedule are independent, interactions may result in patterns of behavior that are different than would be obtained if one or the other simple schedules were used.

In a multiple schedule two or more schedules alternate, each schedule having a different discriminative stimulus associated with it. A mixed schedule also has two or more alternating schedules but there are no discriminative stimuli associated with the different schedules.

The interaction of schedule controlled behavior and chemical agents was recognized early in behavioral pharmacology (Dew, 1956; Morse and Herrnstein, 1956) and led to widespread use of the multiple fixed interval/fixed ratio schedule in both behavioral pharmacology and behavioral toxicology. This schedule allows the simultaneous analysis of performance on a fixed interval schedule which temporally defines contingencies and on a fixed ratio schedule which assesses high rate responding.

One of the earliest studies in behavioral toxicology made use of the multiple FI FR schedule to assess the effects of mercury vapor (Armstrong et al. 1963). Exposure to mercury vapor caused a decrease in the average rate of responding in both the FI and FR components. Upon discontinuation of exposure response rates returned to normal.

Exposing animals performing on a multiple fixed interval, fixed ratio schedule to carbon disulfide produced a selective decline in FI response rate while FR responding remained intact under exposures that eliminated FI responding (Levine 1976).

The contrasting effects of these toxins on the same schedule illustrates the differential sensitivity of this schedule. Examination of the nature and extent of the disruption can provide not only an indicator of the potential toxicity of an agent but also some insight into the underlying mechanism responsible for the disruption.

Considering a different type of complex schedule, methylmercury exposed pigeons were evaluated on a fixed consecutive number schedule (Evans et al., 1975). Evans and coworkers found that a single large dose of methylmercury disrupted performance on the fixed consecutive number schedule 72 hours after treatment.

Operantly controlled behavior comes under the control of stimuli of different types. Control can be either external or internal. As with certain other schedules, there is evidence that performance on a fixed consecutive number schedule is more easily disrupted if there is no external cue to signal completion of the number requirement in the first schedule component (Laties, 1972). When a signal light was added

to indicate the completion of the response requirement on the first key, the disruptive effects of d-amphetamine previously observed disappeared.

The area of internal versus external control has not been extensively dealt with in behavioral toxicology, however, data using drugs suggests that external stimulus control is less disrupted by drugs than is behavior maintained by internal stimulus control (Laties, 1972; Laties and Weiss, 1966). The same may prove to be the situation with toxins and this area deserves investigation.

3.3 The Nature of the Reinforcer

In using schedules of reinforcement to assess any environmental challenge, reinforcers are manipulated. Consideration of the nature of the reinforcing stimulus is critical. Skinner (1953) has defined a reinforcer as "any stimulus that increases the probability of a response that it follows". In common terms "reinforcers" are rewards (e.g. food, water, sex, or brain stimulation) for which an organism will work or in the case of negative reinforcers, aversive or painful stimuli (e.g. electric shock) which the organism will work to avoid.

Food or water are the most frequently used reinforcers and their reliability as reinforcing stimuli is with few exceptions unquestioned. The use of food or water, however, requires manipulation of the deprivation level of the organism and this cannot be ignored especially when using an agent which is known or suspected of affecting central appetite control centers.

Electrical brain stimulation has been shown to function as a reinforcer when stimulating electrodes are placed in certain brain regions (Hall et al., 1977). Annau (1975) has used this model to assess the behavioral effects of carbon monoxide and hypoxic hypoxia. Both decrease self-stimulation with very short exposures. Acute exposure to trichloroethylene also decreases self-stimulation (Baetjer et al. 1970). The high rates typically maintained by brain stimulation and differential effects as a function of electrode placement may restrict its usefulness as a tool in assessing performance. Both time to prepare the animals and the equipment needed for delivery of brain stimulation make this reinforcer less attractive as a candidate for

general use.

In addition to these commonly used reinforcers, other stimuli, including access to running wheels for rats, observation of another animal for monkeys, drugs in several species, and access to heat in a cold environment can function as reinforcers. While the nature of the specific reinforcer needs to be considered, it is reassuring to find similar patterns of responding maintained regardless of the reinforcers.

4. THE USE OF NEGATIVE REINFORCEMENT IN BEHAVIORAL TOXICOLOGY

An increase in responding following the termination of some event is defined as negative reinforcement. The use of schedules of negative reinforcement offers the advantage of not involving deprivation as is the situation with schedules using some positive reinforcers. The most commonly used negative reinforcer is electric shock. It has been used in several different paradigms, which have been used in behavioral toxicology.

Shuttle box avoidance requires that an animal jump from one compartment to another to avoid or escape electric shock. A stimulus is presented prior to the shock and a response of jumping to the other compartment in the presence of the stimulus constitutes an avoidance response. If the animal does not respond to the stimulus but waits until the shock presentation to make a response, his response constitutes an escape response. Shuttle box avoidance is often used to assess learning and memory following *in utero* exposure to toxins. Animals are trained to avoid shock and trials to criterion or acquisition are used as an index of learning. The response is then extinguished. Following extinction, the animal is retrained and this period of reacquisition is taken as a measure of memory. Hughes and Annau (1976) trained mice exposed *in utero* to 3.0 or 5.0 mg Hg/kg methylmercury on a shuttle box avoidance task. They found a significant increase in the number of trials to criterion in the treated mice. Mactutus et al. (1980) found differences in both learning and retention following prenatal carbon monoxide exposure.

Using a lever press response, Sidman (1953) described an avoidance schedule which requires the animal to make a response in order to avoid or postpone a shock. This procedure is referred to as Sidman or non-discriminated or continuous avoidance. The procedure has been widely used in behavioral pharmacology (see Seiden and Dykstra, 1977 for review of drug effects on avoidance). Its application to behavioral toxicology has been somewhat limited. The effects of CO alone or in combination with alcohol were investigated using a continuous avoidance schedule (McGuire, unpublished observation). CO had little effect on avoidance responding during a 30 minute exposure period until very high concentrations (1500 ppm). In combination with 4 gm/kg alcohol the CO-induced disruption of avoidance responding occurred at lower concentrations and persisted into the post exposure period.

Unsignalled avoidance was one of the schedules used by Dietz and McMillan (1979) to assess the behavioral effects of mirex and kepone. While they found decreases in response rate on the avoidance schedule with the administration of both of these insecticides, they found that avoidance responding was less sensitive to disruption than either DRL or FR schedule performance. In addition, the effects on avoidance occurred only at the time overt signs of toxicity were apparent.

5. EVALUATIONS OF SENSORY FUNCTIONING

The integration and execution of behavior requires function in one or more of the primary sensory modalities. Sensory systems are often the targets of environmental contaminants. For example, methylmercury causes visual disturbances (Evans et al. 1975) and noise produces hearing impairments (Stebbins, 1970).

The sensitivity of behavioral methods can be utilized in the determination of functional deficits in sensory systems before morphological indices may show damage (Evans and Weiss, 1978). Both the visual and auditory systems have had refined techniques developed which allow for the detection of subtle deficits following toxic insult. Stebbins (1970) has used auditory discrimination tasks with monkeys to assess hearing impairments following administration of aminoglycosides.

The procedure provides a method for determining changes in auditory thresholds and frequency difference thresholds. To assess disturbances in peripheral vision following exposure to methylmercury Evans et al. (1975) used monkeys trained to detect form differences at luminance intensities to which only rods (peripheral retinal sensing elements) would be sensitive.

These examples have involved sophisiticated techniques using complex tasks with primates. The assessment of the functioning of any sensory modality must take into consideration the species and the biological constraints on that species. The capabilities of the animal will restrict the modality and determine the extent of generalization across species. For behavioral investigations of the visual system, the pigeon has most frequently been used because of it's well developed visual system which includes color vision. The problem when one attempts to use behavioral responses based on visual acuity with pharmacological or toxicological challenges is the difference in responsiveness of the avian and other species. Underlying physiological and biochemical differences in the transformation of the chemical agent often result in effects different from those seen in other species. For example, morphine and other opiates increase response rates in pigeons in contrast to their rate decreasing effect in other species including humans, non-human primates and rodents. Thus, extrapolations from avians of the effects of chemicals on behavioral measures must be made with caution and knowledge of the biotransformation of the agents involved.

The visual system of the rat is unfortunately poorly developed and lacking in color discrimination. Rats, however, are capable of visual tasks and sensitive tests need to be developed utilizing this species.

The species of choice in visual testing and with other sensory dimensions is the non-human primate. While much behavioral work has been done with this species, their use in toxicology studies is often prohibited because of their expense.

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APPENDIX B

LITERATURE REVIEW:
CARBON MONOXIDE EFFECTS ON BEHAVIOR

TABLE OF CONTENTS

		Page No.
1.0	INTRODUCTION	137
2.0	EFFECTS OF CARBON MONOXIDE ON ANIMAL BEHAVIOR	143
	a. Unconditioned Behaviors in Rodents	143
	b. Operant Conditioning Procedures in Rodents	145
	c. Aversive Control of Behavior in Rodents	150
	d. Studies in Non-Human Species Other than Rodents	150
3.0	EFFECTS OF CARBON MONOXIDE ON HUMAN PERFORMANCE	151
4.0	SUMMARY	153

LIST OF TABLES

B1	PERCENT CARBOXYHEMOGLOBIN IN RATS DURING AND FOLLOWING CO EXPOSURE	139
B2	PERCENT CARBOXYHEMOGLOBIN IN RATS AFTER CO EXPOSURE	141
B3	TABULAR PRESENTATION OF EFFECTS OF CARBON MONOXIDE ON BEHAVIORAL RESPONSES IN ANIMALS	157
B4	LIST OF STUDIES OF CARBON MONOXIDE EFFECTS ON HUMAN PERFORMANCE BY TYPE OR PERFORMANCE TASK	161

1.0 INTRODUCTION

Although the behavioral effects of carbon monoxide (CO) were first reported in 1895 (Haldane, 1895) and some work on this chemical commenced during World War II (e.g. McFarland et al. 1944) extensive investigation of CO did not begin until the 1960's. Concern with the potential toxicity of CO in aircrafts, spaceships and nuclear submarines (Malorny, 1972; Theodore et al., 1971; Schulte, 1973) led to a resurgence of investigation of CO. Awareness of the general environmental exposures to CO as a result of cigarette smoke and automobile exhaust, led to further concern with the possibility of deleterious effects as a consequence of CO.

Several reviews are available dealing with various aspects of carbon monoxide. In the area of behavioral effects, Laties and Merigan (1979) have reviewed extensively both the animal and human data. Reviews by the National Academy of Sciences (1969, 1977) critique the human data and offer recommendations for further research. The biological effects of CO were discussed in the New York Academy of Sciences Proceedings (1970). discussions of the physiological effects of CO can be found in standard reference books including Goodman and Gilman (1975) and Doull et at. (1980).

CO is a colorless, odorless gas found in the environment as a result of the incomplete combustion of organic matter. It is released from both natural and anthropogenic sources. The major natural source of CO results from the oxidation of methane. Other contributors to atmospheric CO include forest fires, terpene oxidation, and the oceans (National Academy of Science, 1977). The principal anthropogenic source is the incomplete combustion of carbonaceous fuels.

In the body CO combines with hemoglobin to form carboxyhemoglobin (COHb). The affinity of hemoglobin for CO is approximately 240 times greater than the affinity of hemoglobin for oxygen, consequently inhalation of CO results in rapid combination of CO with hemoglobin. The toxicity which follows exposure to CO is mainly the result of the tissue hypoxia caused by the inability of the blood to carry sufficient oxygen. Blood

COHb levels are the most frequently used indicator of CO toxicity and consequently attempts have been made to relate COHb and the effects of CO. The physiological signs and symptoms of CO poisoning have been correlated with COHb levels. At 0-10% of blood saturation, there are no physiological symptoms in humans. The first symptoms of CO toxicity occur at blood COHb levels of 16-30% and are in the form of headaches and throbbing in the temples. From 30-50% COHb, severe headaches, weakness, dizziness, dimness of vision, nausea and vomiting occur. Higher COHb levels result in coma with intermittent convulsions and death when COHb levels reach 70% or greater (Swinyard, 1975). Attempts to relate behavioral disruption following CO exposure and COHb levels has been less successful than the elucidation of the relationship between COHb levels and physiological symptoms.

Assuming a positive correlation between behavioral disruption and COHb saturation, it might be predicted that behavioral changes would parallel the changes in COHb. In behavioral studies using human subjects, the relationship between COHb levels and behavioral responses remains a controversial area. Studies which have shown a positive correlation between these variables (e.g. Beard and Werthiem, 1967) have not been replicable. Many studies with humans have only estimated COHb levels which raises the question of the reliability of the estimates. Because of the concern for human safety, only low level exposures can be conducted and effects at higher concentrations must either be extrapolated or based on cases of serious intoxication. Thus, it is impossible to directly determine the behavioral effects of CO in human subjects over a range of saturations. These problems make it necessary to employ animal models in an attempt to determine the effects of CO and the relationship to COHb levels.

The time course of blood saturation with CO in rats has been determined empirically by Montgomery and Rubin (1971). Exposing rats to 150, 250, 500 and 1000 ppm CO for 240 min they reported a half-time of saturation for CO of 25-35 minutes and 95% equilibrium saturation by 90 minutes. The half-time for desaturation was 32-35 minutes. COHb levels at the various CO concentrations are shown in Table B1.

TABLE B1

PERCENT CARBOXYHEMOGLOBIN IN RATS DURING AND FOLLOWING CO EXPOSURE

	Minutes During Exposure				Minutes Following Exposure		
CO ppm	60	120	180	240	60	120	180
150	10	10	12	12	5	2.5	-
250	15	20	20	20	5	2.5	-
500	32	40	40	40	10	5	-
1000	60	60	60	60	20	5	2

All values are approximates from curves shown by Montgomery and Rubin (1971).

Unfortunately, in the animal literature, studies employing behavioral measures do not routinely report COHb levels. Ator et al. (1976) reported COHb levels at 15, 30, 50, 120 and 240 minutes during exposure to 240, 500, 750 and 1000 ppm. Their results showed a dose and time related increase in COHb. No behavioral effects were reported until exposure to 750 ppm. Although blood COHb levels were 39.6% after 2 hours of exposure to 500 ppm and 22.1% after 2 hours of exposure to 250 ppm performance was not affected at these levels (Table B2).

Plevova and Frantik (1974) reported COHb levels of 22.8% following exposure to 200 ppm CO for 24 hours and 19.6% after exposure to 700 ppm CO for 30 minutes. Under both exposure conditions, they report decreased endurance on a treadmill. Geller et al. (1979) reported that exposure of rats to 50 ppm CO for 2 hours produced blood COHb levels of 13.9 - 19.6%. Performance of FI 2-min, FR 60 and VI 2-min schedules all showed slight response rate increases at this exposure level. Annau (1975) exposed rats trained to respond for electrical brain stimulation to 1000 ppm CO for 192 min. As shown Figure 1, increases in COHb paralleled decreases in response rate.

Thus, attempts to correlate COHb levels and behavioral responses in rats have been limited and findings have been inconsistent. While COHb levels are consistent across studies, with the exception of Annau's findings using self-stimulation, behavioral disruptions do not appear to be directly related to changes in COHb levels.

Such findings have led to the speculation that COHb levels may, in fact, not be the best predictor of behavioral toxicity (e.g. Lilienthal, 1950; Plevova and Frantik, 1974; and Sokal, 1975). CO exposure concentration and duration have been proposed as better indices and unquestionably these variables need to be considered. It is also possible that venous COHb levels do not adequately reflect momentary fluctuations in cerebral levels of COHb which may be directly correlated with the behavioral disruptions produced by CO exposure.

TABLE B2

PERCENT CARBOXYHEMOGLOBIN IN RATS AFTER CO EXPOSURE

	Minutes of Exposure				
CO (ppm)	15	30	60	120	240
250	7.7	12.4	16.8	19.3	22.1
500	16.2	23.9	31.9	38.2	39.6
750	25.7	38.0	48.9	50.9	52.4
1000	-	52.0	55.7	55.1	56.9

Adapted from Ator et al. (1976)

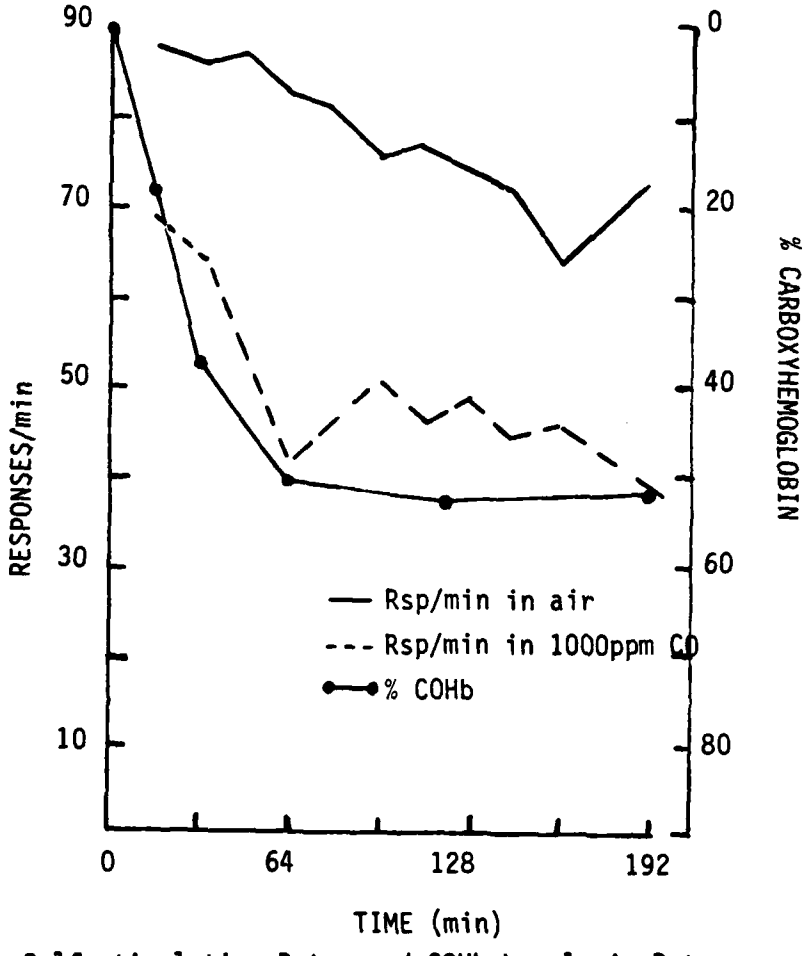

Fig. B1. Self-stimulation Rates and COHb Levels in Rats During Exposure to 1000 ppm CO or Air

(Adapted from Annau, 1975).

2.0 EFFECTS OF CARBON MONOXIDE ON ANIMAL BEHAVIORS (TABLE B3).

a. Unconditioned Behaviors in Rodents

Typically, the first consideration in determining the behavioral effects of any agent is its effects on unconditioned behaviors. Dependent variables in this category include measures of food and water intake and motor activity.

Exposure to hypoxic hypoxia has been reported to produce immediate weight loss and suppression in growth in animals. Since the major effects of carbon monoxide have been attributed to tissue hypoxia, Koob et al. (1974) were interested in comparing the effects of hypoxic hypoxia and carbon monoxide hypoxia on food intake, water intake and body weights. These dependent variables were monitored in Long-Evans and Sprague Dawley rats following a single 24-hour exposure to 250, 500 or 1000 ppm CO. In the Long-Evans rats, they found significant reductions in food and water intake following all exposure levels. Exposure to 500 ppm CO and higher concentrations also produced significant decreases in body weight. The Sprague-Dawley strain exhibited similar decreases with significant alterations first observed at 250-500 ppm CO. Similar effects were found in both strains during exposure to 16% oxygen, 14% oxygen and 10% oxygen. Unfortunately, no follow-up data were provided and the transitory nature of this phenomenon may be questioned.

Two other studies have also investigated weight changes following long-term, continuous exposures to CO. Stupfel and Bouley (1970) exposed rats to 150 ppm CO for 95 consecutive hours per week for 3 months or lifetime. They found decreases in water consumption and weight gain during exposures but weight decrements were made up on weekends. Theodore et al. (1971) exposed rats to 460 mg/m^3 (400 ppm) CO for 71 days and then to 575 mg/m^3 (500 ppm) CO for an additional 97 days. While the weights of CO treated animals were slightly below the weights of the control animals during the exposure period, by the end of the treatment both groups were equal in weight.

No studies using single short (<6 hours) exposures have reported weight data. Repeated short-duration exposures may produce some weight changes but the available data suggest that weight decrement in adult animals as a consequence of low or moderate level CO exposure is not a permanent effect.

Data on the effects of CO on activity measures are also very limited. Activity levels of CO exposed rats were measured by Culver and Norton (1976) using a residential maze (Norton et al. 1975). The animals lived in the maze thus allowing continuous recording of changes in activity. Recordings were made on counters when photocell beams were interrupted by the animals' movement through the maze. Male and female rats were exposed to 0.6% CO until respiratory arrest occurred. The exposed groups included females 3, 5, 10, and 18 months old and males 10 and 18 months old. Culver and Norton observed general recovery of motor function, including eating, drinking and walking in all animals on the day after exposure. No hyperactivity occurred for 3 weeks after exposure to CO. However, when the animals were retested 6 weeks post-exposure persistent hyperactivity was observed in all groups except the 18 month old males when tested during the nocturnal period. It should be noted that the number of 18 month old males tested was only three as six animals died during or shortly after CO exposure and one other animal died 6 weeks after the exposure. In all other CO-exposed groups, mortalities were comparable with a combined mortality of 38%.

The exposure conditions used by Culver and Norton were quite severe and their finding of a persistent change in activity levels is not unexpected. While these data suggest an interesting direction for further research, the lack of parametric work makes it impossible to extrapolate to implications for behavioral changes under less extreme exposure conditions.

In an early study of the effects of CO on various physiological parameters, Musselman et al. (1959) investigated the effects on activity in rats following exposure to 50 ppm CO continuously for 3 months. They reported no effect on activity measured by means of "squirrel type" activity cages but the lack of information on testing apparatus and on time and duration of testing makes it difficult to compare these data with other findings.

The effects of CO on activity measured as running behavior has been investigated using treadmills and running wheels. Plevova and Frantik (1974) measured running behavior using a treadmill and found significant decreases in performance following exposure to 200 ppm CO for 24 hours or 700 ppm CO for 30 minutes.

Spontaneous wheel running in the white mouse was investigated by Malorny (1972) following 14 hours of exposure to 55, 85 and 160 ppm CO. During 3 hours of additional exposures, running performance was significantly reduced in a concentration related manner. Under control conditions, running performance in 3 hours averaged about 1500 m. The distance covered decreased to about 1000 m with exposure to 55 ppm, 740 m with exposure to 84 ppm and 400 m after 160 ppm. While these data suggest low CO concentrations decrease activity, the use of running wheel performance raises the question of whether this is a behavioral disruption resulting from central nervous system impairment or due to other physical or physiological effects.

Malorny (1972) also examined swimming performance following one hour exposures to 300 or 500 ppm CO. Both exposure conditions resulted in decreased swim time suggesting that physical capacity is reduced by exposure to CO.

b. Operant Conditioning Procedures in Rodents

The use of behavioral methodology, especially operant conditioning techniques has recently been applied to the assessment of toxic agents. These techniques allow objective measures of behavior and have come to be more widely used when interest is in the functioning of the intact central nervous system. See behavioral methodology review for a more complete discussion of operant techniques.

Several investigators have used a continuous reinforcement schedule (CRF) to investigate the behavioral effects of CO. The continuous reinforcement schedule provides reinforcer presentation following each response the animal makes.

Goldberg and Chappell (1967) exposed rats performing on a continuous reinforcement schedule for food to 250 or 500 ppm CO for 55 minutes on 3 consecutive days. At the lower concentration rats showed a slight increase in responding on the first day of exposure; this was followed by a progressive decrease in responding on the two consecutive days of exposure. At 500 ppm CO produced progressive decreases in responding on all three days of exposure. On the day following the third exposure, the animals were run in normal atmosphere. Responding in the 500 ppm group increased to above control values. Responding in the 250 ppm group returned to near control levels. Goldberg and Chappell also investigated the effects of 2 hour exposures to 200 ppm CO on CRF and extinction following CRF training. Responding on CRF in animals exposed to CO was decreased below levels seen in air-exposed animals. Although the methods are somewhat unclear, the procedure in this study differed from the experiment described above in that stable baseline performance was not established prior to exposures. From the data it appears that transitional behavior was being measured with CO exposed animals showing an increase in CRF responding but the magnitude of this increase was below that seen in CRF performance in untreated controls.

In extinction a response which was formerly reinforced is no longer followed by reinforcement. The typical pattern of responding in extinction is an initial increase in response rate (called an extinction burst) followed by a gradual decrease in responding until the subject responds at pre-conditioning levels (called the operant level). Rats with no prior history of CO, were trained on a CRF schedule and were then exposed to CO (200 ppm for 2 hours). Exposure to CO produced fewer extinction responses than were emitted by the control group.

Goldberg and Chappel also investigated the effects of CO exposure on performance of rats on a VR schedule. The exposures (200 ppm) occurred 1 hour prior to and during the 1 hour experimental sessions. In their initial study of effects VR performance, responding was decreased in comparison with air-exposed control rats; however, in a repetition of the study in which older animals with a different training history were used no effects on VR performances were detected.

Teichner (1967) used a CRF schedule to assess the effects of CO following 5 hours exposure to 500 ppm CO for 5 consecutive days. Although his data were highly variable, CO exposure did produce a significant decrease in response rate. This decrease persisted for all 5 days of exposure.

Comparing the effects of hypoxic hypoxia and carbon monoxide hypoxia on self-stimulation behavior Annau (1975) also used a CRF schedule. Rats were exposed to 250, 500 or 1000 ppm CO for 16 minutes. There was a dose related decrease in self-stimulation. In subsequent experiments using the same paradigm, rats were allowed to self-stimulate for 2 hours in the presence of 500 or 1000 ppm CO. Both exposures produced decreases in self-stimulation rates. At 1000 ppm the time course of these decreases paralleled the increases in COHb levels.

From Annau's data it would appear that exposures to 250 ppm CO as brief as 16 minutes can produce behavioral effects. As the author points out, the novelty and stress caused by the changes in the animals' environment may be the cause of the disruption rather than direct central nervous system (CNS) effects of CO. A second aspect of this work which must be considered is the nature of the reinforcing stimulus. Self stimulation differs from food or water reinforcement in several respects. It is more immediate and there is no consummatory response involved. Comparisons between studies must be made cautiously. However, in general the data do suggest that performance on CRF schedules is disrupted by exposure to 250 ppm CO for short periods (i.e. less than 60 minutes).

A number of simple schedules were investigated by Beard and Werthiem (1967). These included FI 3, FR 25, VI 25, VR 25, VR 16 and DRL's with values of 2-30 sec. CO exposure concentration were 250, 500, 750, 1000 ppm for 96 minutes. On all schedules effects were reported at 250 ppm with the onset of the effects being very rapid. The effects on DRL were first apparent at exposure to 100 ppm after 11 minutes. Unfortunately, few details of the methods were provided and data presentation was limited. Thus, comparisons with other findings and generalizations must be made cautiously.

Two additional studies examined the effects of CO on FR responding. Following exposure to 1000 ppm CO for 1 hour, Carter et al. (1973) reported a 95% decrease in response rates in rats performing on a FR 15 schedule for 30 minutes in the presence of CO. CO decreased mean response rate from 90.44 rsp/min to 3.89 rsp/min.

Geller et al. (1979) exposed rats to 25, 50, 100, 200 or 500 ppm for 2 hours. During the second hour of exposure, the animals performed on FI 2-min, FR 60, or VI 2-min schedules. On the VI schedule, although averaged data showed no effect until responding was decreased at 500 ppm individual animals showed increases in response rate in the concentration range of 25 to 200 ppm CO. These increases were small and the lack of any statistical analysis makes this effect at best suggestive. Both FR and FI schedules showed some decrease in response rates at 200 ppm CO, with the FR schedule being slightly more affected. Again, these increases were small and probably not statistically significant. Exposure to 500 ppm produced reliable response rate decreases.

Response rate decreases on FI, FR, VI and VR schedules occur at CO concentrations of 500 ppm or greater for exposure durations of 1 hour or longer. At lower concentrations, the effects of CO are somewhat questionable. Beard and Werthiem (1967) observed rate decreases at 250 ppm on all schedules as did Goldberg and Chappel in one study on FR performance. Geller and coworkers saw increases but investigated only interval schedules. None of the studies had adequate statistical analysis; the number of animals used was small; and from the data presented the effects at levels below 500 ppm appeared to be minimal.

DRL schedule performance was used by Ator et al. (1976) investigating the effects of CO. Rats performing on a DRL 21-SEC schedule were exposed to CO for 30 minutes prior to the session and for the duration of the one-hour session. CO concentrations of 100, 250, 500 and 600 ppm had no effect on DRL performance. Only exposure to 750 and 1000 ppm produced decreased response rates. This effect was not due to a disruption in the IRT distributions but rather was related to extended pausing.

The differences in Ator's results and those of Beard and Werthiem on DRL performance are difficult to reconcile with the available information. Ator's study was well controlled and the animals baseline performance was stable before CO exposures were initiated. However, the number of animals used was small (N=3). Beard and Wertheim provided little pre-exposure performance data. Based on these limitations, at this time, it appears DRL performance is resistant to disruption by low concentrations of CO. However, replication of these findings is needed before definitive statements on the effects of CO on DRL schedules will be warranted.

Examining the effects of CO on performance on a more complicated schedule of reinforcement Smith et al. (1976) exposed rats performing on a fixed-consecutive-number (FCN) schedule to 200, 400 and 600 ppm CO for 30 or 60 minutes before and during a 45 minute session. This schedule required that the rats make 20 or more consecutive responses on one lever followed by 1 response on a second lever to obtain food reinforcement. Consistent decreases in response rate due to decreased local rates[a] and extended pauses were observed at 600 ppm. At the lower exposure concentrations occasionally decreased response rates and lowered percentage of reinforcements were also observed.

On progressive ratio schedules the response requirement is increased arithmetically for each successive reinforcement and terminated when the animal does not respond for a specified period. Using progressive ratio schedules with increments of 5 or 7 responses, Merigan and McIntire (1976) examined the effects of 155, 330, 520 and 700 ppm CO. Exposures were conducted for 30 minutes pre-session and for the duration of the session which varied. They found a decreased breaking point[b] at 520 and 700 ppm. At 700 ppm local rates were decreased and pause lengths increased. Lower concentrations had no effect on performance.

[a]The number of consecutive lever responses on the first lever preceding a response on the second lever is defined as a run. Response rate during a run is defined as the local rate of response.

[b]The magnitude of the ratio at which the progression is terminated due to failure to respond is called the breaking point.

c. Aversive Control of Behavior in Rodents

Aversive control has also been used in the analysis of CO's effects. Most experiments involving aversive control use the presentation of electric shock as the aversive stimulus.

Zorn (1972) exposed rats to 150 ppm CO for 8 hours each night, 5 nights/week for 2, 4 and 10 weeks. In addition, they were exposed to CO for 12 hours on the 6th and 7th days of every 4th week. When tested on a conditioned escape response CO-exposed animals showed a significant reduction in performance reflected in longer learning time. From subjective observations there appeared increases in emotional behavior.

Stupfel and Bouley (1970) exposed rats to 50 ppm CO, 95 hrs/week for 3 months and included in their experiment both air and unmanipulated control groups. When tested on shuttle box avoidance the groups differed in the percentage of avoidances with the unmanipulated control avoiding most frequently and the air control less frequently. The investigators reported this as an effect of CO but recognized that due to the overall low rate of avoidance (12%) clear interpretations were difficult.

d. Studies of Non-Human Species Other Than Rodents

Most investigations of CO have used rodents, predominantly rats, as the subjects. A few studies, however, have employed other species, either pigeons or non-human primates.

McMillan and Miller (1974) examined the effects of CO exposure on pigeons performing on a multiple fixed-ratio 30 fixed-interval 5-min schedule. The animals were exposed to 380, 490, 1000, 1410 and 1720 ppm CO for one hour prior to the experimental session and for the duration of a one hour experimental session. They found decreases in responding at 490 ppm CO, little responding occurred at 1410 ppm and responding was completely abolished at 1720 ppm. Both components of the multiple schedule were similarly affected.

A complicated program utilizing continuous and discrete avoidance performance in monkeys was used by Back and coworkers (Theodore et al. 1971) to evaluate the effects of continuous long-term exposures to CO. Exposure

conditions examined were 55 mg/m^3 for 100 or 105 days, 110, 220, 440 mg/m^3 for 7 days, 220 mg/m^3 for 100 days and 440 mg/m^3 for 99 days. None of the exposure conditions produced any significant changes in operant performance.

Geller et al. (1979) used baboons on a match-to-sample discrimination task to investigate the effects of CO. Exposures to 25, 50 and 75 ppm for 6 hours/day, for 5 days at each exposure condition produced no significant effects although an "occasional mistake" occurred at each dosage level.

3.0 EFFECTS OF CO ON HUMAN PERFORMANCES (TABLE B4)

The effects of carbon monoxide on various aspects of human performance have been reviewed by Stewart (1975, 1976) and Laties and Merigan (1979). In order to put the animal data in perspective and make rational choices of an appropriate behavioral model, the major findings of the human experimental work will be briefly summarized. Psychological testing of humans under experimental exposure to CO has involved the areas of vision and audition, time discrimination, motor behavior, vigilance and driving.

Dimness of vision occurs in humans with CO exposures that result in 30-40% blood reduction (Swinyard, 1975) and with severe poisoning there may be permanent visual and auditory impairment (Laties and Merigan, 1979). The effects of exposure to lower concentrations of CO remains questionable.

In 1970, Beard and Grandstaff reported on research by Wertheim which examined the effects of CO on four aspects of vision: the absolute threshold for detecting light, brightness difference thresholds, critical flicker fusion and visual acuity. Following the first set of tests, subjects were exposed to 50, 150 or 250 ppm CO for 1 hour during which time they continued to perform on the visual tests. Wertheim reported consistent impairments in brightness difference thresholds, critical flicker fusion and visual acuity. Subsequent attempts to replicate these findings have been unsuccessful. Stewart et al. (1970) found no effect on visual acuity, depth perception or color vision following 8 hour exposures to 100 ppm CO. Wright et al. (1973) exposed subjects to CO until COHb levels increased to 7% in smokers and 4.4% in non-smokers. They reported no effects on night vision, glare recovery or depth perception as a consequence of the CO exposure. Exposure to 300, 650 or 950 ppm CO for 45 minutes had no effects on brightness

discrimination or depth perception (Ramsey, 1972).

In contrast to Wertheim's findings with critical flicker fusion, no effects of CO on this measure were observed by Fodor and Winneke (1972), Guest et al. (1970), Lilienthal and Fugitt (1946), O'Donnell et al. (1971), Ramsey (1972), Vollmer (1946) von Post-Lingen (1964), and Winneke (1974. Seppanen et al. (1977) however, did report decreased critical flicker fusions in smokers when compared to non-smokers.

Vigilance tasks involve the detection and reporting of small environmental changes ("signals") occurring at infrequent intervals. Signals are usually visual or auditory stimuli.

Auditory vigilance tasks have been used by Groll-Knapp et al. (1972), Fodor and Winneke (1972), and Haider et al. (1975) in the examination of the effects of CO. Groll-Knapp et al. (1972) exposed subjects performing on an auditory vigilance task to 0, 57, 115 and 172 mg/m^3 CO for 2 hours. They reported a dose-related decrease in the number of missed signals following CO exposure. Attempts to replicate these results have been unsuccessful (Haider et al. 1975, Winneke 1974). Subjects were exposed to CO (57 mg/m^3, 50 ppm) for 80 minutes prior to and during three 45 minutes vigilance testing sessions (Fodor and Winneke, 1972). Performance during these periods was compared to that during air control sessions. The CO exposure significantly impaired performance during the first of the three sessions, had less effect in the second, and by the third session performance was not different from that in the comparable control period.

Subjects performing a visual vigilance task were exposed to 0, 57, 200 or 286 mg/m^3 CO (0, 50, 175 or 250 ppm) for 1.5 hours (Beard and Grandstaff, 1970). Performance was reportedly decreased at 50 and 175 ppm but not at 250 ppm CO.

Horvath et al. (1971) exposed subjects for 1 hour to 0, 29 and 126 mg/m^3 (0, 25 and 110 ppm) CO and for the duration of a one hour session while performing on a visual vigilance task. By the end of the exposure to 110 ppm CO subjects showed a significant performance decrement. Christensen et al. (1977) were unsuccessful in replicating these findings. Other

investigations of the effects of CO on vigilance have reported only negative findings (Benignus et al. 1977, Putz et al., 1976).

Negative results have generally been reported on simple measures of coordination and hand steadiness following exposure to CO (Stewart et al., 1973; Wright et al., 1973; Fodor and Winneke, 1972; and Winneke, 1974). Only Bender et al. (1972) reported a small effect on the Purdue Pegboard test following exposure to 100 ppm CO for about 2.5 hr. One of the tests involved a concurrent verbal task which may have made performances on the pegboard test more difficult. O'Donnell et al. (1971) reported no changes on any measures of ataxia in the Pensacola Ataxia Battery.

Driving involves both vigilance and tracking. As with other investigations of the effects of CO on various dimensions of human performance, the findings on driving behavior have been inconclusive.

No effects were reported by Forbes et al. (1937) or Wright et al. (1973). Data suggesting that CO exposed subjects required more roadway viewing when driving under higher speeds has been suggested by McFarland et al. (1944). Rummo and Sarlanis (1974) found increased reaction time in subjects exposed to 800 ppm CO for 20 minutes prior to driving. They also reported no change in response time to dashboard warning lights. Similar findings were reported by Wright et al. (1973) and Stewart et al. (1973).

Beard and Wertheim exposed subjects performing an auditory time discrimination task to 0, 50, 100, 175 and 250 ppm CO for 2.5 hours. Significant impairments were found at all CO concentrations. Attempts to replicate these findings by O'Donnell et al. (1971) were unsuccessful, although Stewart et al. (1973) did find a small performance decrement.

4.0 SUMMARY

Investigations of the behavioral effects of carbon monoxide in animal models have used primarily simple schedules of reinforcement. The data suggests that the most disruptive effects of carbon monoxide occur on high rate schedules for example CRF, FR, and progressive ratio schedules,

while performance on DRL schedules which engender a low response rate remains intact until very high CO exposures. There are, however, several problems which must be considered before generalization are made based on the currently available data.

For the most part, the number of animals used in each study has been small. The approach of using small samples has been a tradition in operant psychology (Skinner, 1938). Each animal is used as its own control and changes in performance which occur as a function of an insult are evaluated against each individual animal's baseline performance. This approach can be very sensitive and provides a good deal of information about the effects of this insult on that animal's performances. The use of a small number of subjects precludes statistical analysis of the data. The animal studies to date, in general, suffer from the lack of statistical analysis. With toxicological studies where concern is more broad based this approach to be useful must be used in conjunction with large scale studies which allow for statistical analysis.

Comparisons across studies are difficult to make because exposure conditions have varied greatly and in some cases, the methodology and data have been inadequately presented. There are no basic parametric studies on the effects of CO on any single behavioral measure. While there is no question that these types of studies are boring and tedious, they are the scientific data base which more creative investigations are built on. Thus, with the CO literature in its current state one can only speculate as to the potential sensitivity of a particular measure.

The available literature does suggest a concentration range over which behavioral measures in rodents is disrupted. Exposure to 250-500 ppm CO appears to represent a threshold concentration. The exposure duration is somewhat more difficult to assess. If one accepts a relationship between COHb levels and CNS function based on Montgomery & Rubin's (1971) data one would not expect to see serious behavioral disruption with less than an hour exposure time at the lower CO concentrations. This agrees with most of the behavioral studies which have used exposure times of about an hour. Only Annau used very short exposure times and although he did report disruptions these data must be interpreted cautiously. As he has pointed out, the novelty

of the change in the environment with CO exposure may account for the disruptions he observed. Another factor which must be considered is the nature of the reinforcing stimulus. Brain stimulation was his reinforcer. Electrical brain stimulation appears to have several unique properties and has been shown to have effects different from those seen with other reinforcers when the organism is challenged.

This raises the important issue of the nature of the reinforcing stimulus. The potential interaction of the reinforcing stimulus and the exposure conditions might present serious problems. CO has been shown to decrease food and water intake, and weight gain in animals exposed to CO for 24 hours (Koob, 1974). Other studies over extended periods have not reported any long-term effects on these measures. Behavioral studies have used food reinforcement and although no effects have been specifically reported, there is no indication that anorexia could explain the behavioral effects observed. Consideration of effects of CO on food and water consumption or on body weight are important if food and water are to be used as the reinforcer in behavioral studies.

The aspects of human function which have been assessed in evaluating the effects of CO have included time discrimination, various aspects of the visual and auditory systems, motor behavior and some of the component processes involved in driving behavior. The data have been controversial and inconclusive.

The studies using human subjects suffer from many of the same problems observed with the animal studies. Only a small number of subjects have been used in each study; there is a lack of statistical analysis; and exposure parameters have varied greatly. In addition, the nature of the experimental conditions have varied so greatly across studies that these variables may account for the large discrepancies between the results of different investigations. While no definitive statements can be drawn from the data on human subjects, some of the observations are suggestive of the areas of behavior which might be affected as a consequence of exposure to CO.

Time discrimination appears to be resistant to disruption by CO. With the exception of Beard and Werthiem, no investigations have shown any disruption in time discrimination. The results of the data in rats using DRL performance is similar. DRL appears to be the most resistant to disruption by CO and this schedule has been used as an indicator of time discrimination in animals. This does not preclude the possibility that more sensitive test of time discrimination might be affected by exposure to CO.

Motor coordination, assessed by a number of different measures does not appear to be an aspect of human behavior that is disrupted by CO. The few studies that have been done on motor behavior, per se, in rats do not suggest it as very sensitive to disruption by CO. Vigilance data, both auditory and visual are suggestive of disruption by CO. Vigilance is influenced by the environmental conditions under which the studies are conducted with decreases in vigilance occuring earlier in environments which lack any other stimulation.

TABLE B3

TABULAR PRESENTATION
EFFECTS OF CARBON MONOXIDE ON BEHAVIORAL RESPONSES IN ANIMALS

Reference	Species	Exposure Conditions	CoHb	Behavioral Measure	Results
Koob et al (1974)	Rats	250,500,1000 ppm CO for 24 hours. Also exposed animals to 16,14% 10% O_2		Food & H_2O intake weight gain	Dose related decreases in all measures under both types of hypoxic conditions
Culver & Norton (1976)	Rats	Neonates: 1% CO until respiratory arrest Adults: .6% CO until respiratory arrest		Activity in residential maze	Neonates: Hyperactivity from 23 days to weeks, gone at 3 months. Adults: persistent hyperactivity from 6 weeks post-exposure except in 18 mo old males
Plevova & Frantik (1974)	Rats	200 ppm for 24 hrs 700 ppm for 30 min	22.8% 19.6%	Treadmill performance	Decreased under both exposure conditions
Musselman et al (1959)	Rats	50 ppm CO continuous for 3 months	1.8%	Treadmill activity	No effect
Malorny (1972)	Rats	300 ppm for 1 hr 500 ppm for 1 hr	8-13.5% 20-28%	Swimming	DEC swim time

TABLE B3 (continued)

Reference	Species	Exposure Conditions	CoHb	Behavioral Measure	Results
Goldberg and Chappell (1967)	Rats	250 ppm 55 min/day for 3 days		CRF	Slight INC on day 1, DEC on days 2&3, return to baseline day 4 (post-exposure)
		500 ppm 55 min/day for 3 days		CRF	DEC on days 1,2,3; INC over baseline day 4 (post-exposure)
		200 ppm for 1 hour pre-session and 1 hour during experimental (Groups 1 and 2 differed as to age and training history)		CRF	DEC below control animals
				Responding in extinction	DEC below control animals
				VR3 out of 10 responses	Group 1: DEC below control animals Group 2: No difference when compared to controls
Annau (1975)	Rats	250, 500 and 1000 ppm for 16 min		Self-stimulation (CRF)	Concentration-related DEC in responding
		500 and 1000 ppm for 2 hr		Self-stimulation (CRF)	DEC at both concentrations
Tiechner (1967)	Rats	500 ppm for 1 hour pre-session for 5 days		Runway	DEC starting speed and running speed
		500 ppm for 5 hours pre-session for 5 days		CRF	DEC responding

TABLE B3 (continued)

Reference	Species	Exposure Conditions	CoHb	Behavioral Measure	Results
Beard and Wertheim (1967)	Rats	250, 500, 750 and 1000 ppm for 48 min		FI 3-min FR 25 VI 25-min VR 25 VR 15	Dose related DEC on all schedules: slight DEC first apparent at 250 ppm
		100, 250, 500, 750 and 1000 ppm for 4? min		DRL with values of 2, 5,10,15,20 30-SEC	Dose-related DEC in response rate
Carter et al. (1973)	Rats	1000 ppm for 90 min		FR 15	90% decrease in response rate
Geller et al. (1979)	Rats	25,50,100,200,500 ppm for 1 hour pre-session and duration of 1 hour session	50 ppm: 13.9-19.6%	FI-2 min	DEC at 200 ppm
				FR 60	Slight INC at 50 ppm; Slight DEC at 100 and 200 ppm
				VI 2 min	INC in response rate in all animals in range of 25-200 ppm DEC responding at 500 ppm
Ator et al. (1976)	Rats	100,250,500,600,750 and 1000 ppm CO 30' pre-session & during 1 hr session	up to 240 min 250: 7-22 500:16-40 750:26-52 1000: -57	DRL 21-SEC	No effect until 750 ppm DEC response rates; IRTs not disrupted extended pausing

TABLE B3 (continued)

Reference	Species	Exposure Conditions	CoHb	Behavioral Measure	Results
Smith et al. (1976)	Rats	200,400 or 600 ppm CO 30 or 60 min before and during 45 min session		FCN schedule 20 responses on one lever followed by 1 response on second lever	INC rsp rate due to DEC local rate and extended pauses at 600, at 400 in some rats, and in one rat at 200
Merigan and McIntire (1976)	Rats	155,330,520 and 700 ppm 30 min pre-session and for duration of session		Progressive ratio 5 or 7	Decreased breaking point at 700 & 520 ppm; local rates DEC at 700 pause length INC
Zorn (1972)	Rats	150 ppm CO for 8 hours each night for 2, 4 and 10 weeks	10%	Shock-escape	Reduced Efficiency
Stupfel & Bouley (1970	Rats	550 ppm CO for 3 months or lifetime 95 hrs/week		Shuttle box Avoidance	Slight, but not significant decrease in avoidance responding
McMillan and Miller (1974)	Pigeons	380,490,1000,1410,1720 ppm for one hour pre-session and during one hour session	350ppm: 27% 900ppm: 45% 1700ppm: 55%	MULT FR30FI5	490 ppm: 15-20% DEC 1410 ppm: little responding 1720 ppm: no responding
Theodore et al. (1971)	Rhesus Monkeys	55 mg/m^3 : 100-105 days; 110,220,440 mg/m^3: 1 wk ea. 220 mg/m^3: 100 days; 440 mg/m^3: 99 days	110:7-10% 220:17-21% 440:27-34%	Complex avoidance task	No statistically significant effects; in one study 2/12 monkeys showed slight performance disruptions
Geller (1974)	Baboons	25,50,100 ppm for 6 hrs/day, 5 days at each exposure level		Match to sample discrimination task	Minimal effects

TABLE B4

LIST OF STUDIES OF CARBON MONOXIDE EFFECTS ON HUMAN PERFORMANCE BY TYPE OR PERFORMANCE TASK

Vision and Audition	
Beard and Grandstaff (1970)	Impairments in brightness difference thresholds, critical flicker fusion, visual acuity
Stewart et al (1970)	No effect
Wright et al (1973	No effects on night vision, glare recovery or depth perception
Fodor and Winneke (1972)	No effect on critical flicker fusion
Guest et al (1970)	No effect of CFF
Lilienthal and Fugett (1946)	No effect on CFF
O'Donnell et al (1971)	No effect on CFF
Ramsey (1972)	No effect on CFF
Vollmer (1946)	No effect on CFF
von Post-Lingen (1964)	No effect on CFF
Winneke (1974)	No effect on CFF
Seppanen et al (1977)	Decreased CFF in smokers
Auditory Vigilance	
Fodor and Winneke (1972)	Initial decrease, then increase
Groll-Knapp et al (1972)	Decreased
Haider et al (1975)	No effect
Winneke (1974)	No effect
Visual Vigilance	
Beard and Grandstaff (1970)	Decrease
Horvath et al (1971)	Signal identification deteriorated and monotony effect potentiated
Christensen et al (1977)	No effect

Visual Vigilance (cont'd)	
Benignus et al (1977)	No effect
Putz et al (1976	No effect
Coordination	
Stewart et al (1970)	No effect
Wright et al (1973)	No effect
Fodor and Winneke (1972)	No effect
Winneke (1974)	No effect
O'Donnell et al (1971)	No effect
Bender et al (1972)	Slight impairment on Purdue Pegboard test
Driving	
Forbes et al (1937)	No effect
Wright et al (1973)	No effect
McFarland (1973) et al (1944)	No effect on most measures but more roadway viewing time required when driving at high speeds.
Rummo and Sarlanis (1974)	Increased reaction time, no change in response time to dashboard warning lights
Time Discrimination	
Beard and Wertheim (1967)	Significant decreases
O'Donnell et al (1971)	No effect
Stewart et al (1973)	No effect

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APPENDIX C

LITERATURE REVIEW:

PHYSICAL, PHYSIOLOGICAL AND PSYCHOLOGICAL STRESSORS

A stressor can be defined from a biological perspective as any stimulus that activates the autonomic nervous system beyond its usual level of responsiveness (Candland, 1968). Whatever the stressor a characteristic bodily reaction is produced. Although the activation of the sympathetic division of the autonomic nervous system under conditions of stress is extensive and complicated, basic physiological responses which occur include increased heart rate, increased blood pressure, decreased blood flow to the skin and increased blood flow to skeletal muscles, increased blood sugar, and dilation of the pupils. Activation of the autonomic nervous system, in general, prepares the organism to respond most effectively. Historically, the sympathetico-adrenal medullary and pituitary-adreno-cortical systems have been emphasized as being of primary importance in the stress reaction but there is also substantial evidence to suggest that this reaction is more of a general, integral response that includes secretions from a number of endocrine glands (Archer and Blackman, 1971).

The main contributions to the biology of stress have been provided by Selye (1950). Selye distinguishes three basic stages in the physiological reaction to stress. The overall reaction of an organism to stressful experiences he calls the general-adaptation-syndrome. This general-adaptation-syndrome consists of three distinct stages. The first is the alarm reaction which consists of basic, heightened responsiveness of the autonomic nervous system; vasodialation occurs, blood pressure decreases, and in general bodily changes occur which prepare an organism to respond, With continued stress, these physiological changes are reversed, blood pressure and temperature increase, and the adrenal cortex is enlarged. During both stages adrenocorticotropic hormone (ACTH) is released. ACTH is released from the pituitary and stimulates the production of hormones from the adrenal cortex. The final stage of the general-adaptation-syndrome is exhaustion,

which occurs when the organism's ability to adapt to the stress is exhausted.

Thus, when investigating the effects of stressors, either alone or in combination, it is important to recognize the different physiological states which are induced with different degrees of stress. The nature of the effects observed may be affected by the magnitude and length of exposure to a specific stressor, by the pre-exposure physiological state of the organism, and by other stressors impinging at the same time.

From birth, human beings are surrounded by a multiplicity of environmental stressors. Throughout life it is impossible for the human to function without exposure to stressors. These stressors might be categorized as either physiological, which would include such variables as heat, cold, noise, and extreme exercise and psychological, which include much less well-defined variables and may be the consequence of physiological variables. Archer and Blackman (1971) used the term "psychological stressors" to refer to situations which "although not physically harmful in terms of causing tissue damage, evoke hormonal changes characteristic of the stress response originally described by Selye for physical stressors."

Stress has been studies from several aspects. Most often the contribution of stress to the pathology of disease states has been investigated. These have included the production of hypertension, (e.g. Lovidond, 1969, Pare, 1971; Weiss, 1971; Price, 1972), ulcers (e.g. Buckley et al., 1964; Hudak and Buckley, 1961; Rosecrans et al., 1966; Smookler and Buckley, 1969) cardiovascular disorders (e.g. Corley et al. 1973; Haft and Fani, 1973; Raab et al. 1968; Sobel et al. 1962) frequent sequelae of continuous exposure to stressors. A second area of intense investigation has been an attempt to determine the biochemical changes which occur following or concomitant with stressful events. (e.g. Anisman et al. 1978; Weiss et al. 1976).

Animal models of stress have involved using many of the environmental variables which have been recognized as human stressors. The most frequently used animal stressors include: restraint or immobilization, electric shock presentation, swim stress, noise, crowding, and isolation.

Extremes in environmental temperature have also been considered and are discussed in Appendix D.

Procedures which involve the forced physical confinement of an animal constitute a stressor. The magnitude of the response to restraint or immobilization is dependent on a number of variables including the severity of the restraint as well as intrinsic biological variables. Restraint can increase plasma coricosterone and decrease hypothalamic NE (Keim and Sigg, 1976). Immobilization has been used as a model of stress to examine its interactive effects with manganese, a neurotoxin which produces extrapyramidal disorders on neurochemical measures. The combinations of the two produced changes in neurotransmitters and their precursors in excess of the changes seen following either alone (Chandra et al., 1979). These findings suggest that the neurotoxic effect of manganese is enhanced in a physically stressful situation. This study illustrates an approach frequently used in studying the effects of stress. Stress is used as an independent variable in combination with a toxic agent to assess the potential additive effects of the two variables on endpoints which have typically been biochemical or physiological.

Exposure to inescapable shock has been one of the most frequently used stressors in animals. It has been used as an approach to the study of organic disease states and as a model of disturbed psychological function (e.g., Weiss, 1972).

Using operant conditioning techniques, Estes and Skinner (1941) attempted to study "fear" or "anxiety" in an animal model. Rats performing on a variable interval schedule for food or water reinforcement periodically received inescapable electric shock paired with a discrete auditory sound (a click). Shock presentations typically result in the animal ceasing to respond and displaying behavior characterized by crouching, defecation and immobility. Eventually presentation of the click alone results in the same behavior. This model of "anxiety" has been successfully used in behavioral pharmacology to differentiate drugs useful in the treatment of

human anxiety (Geller and Seifter, 1960).

Inescapable shock has been shown to produce deficits in subsequent training of an escape response (see reviews by Maier and Seligman, 1976; Weiss et al., 1976). Animals exposed to shock from which they are unable to escape fail to learn the appropriate response (Overmeir and Seligman, 1967; Seligman and Maier, 1967; Seligman et al., 1975). This model has been called "learned helplessness" and has been proposed as an animal model of depressive disorders (Seligman, 1975).

Because electric shock has suggested itself as a technique which in animals produces states of anxiety and depression, the biochemical effects correlated with shock have frequently been investigated. In general, these changes have involved decreases in norepinephrine, the neurotransmitter frequently postulated to be involved in depressive disorders.

In animal studies, swimming has been used both as a dependent variable in the assessment of various chemicals and as an independent variable in the study of stress. Some of the advantages of swimming as an experimental method in working with rodents are that the equipment is inexpensive and relatively easy to construct, swimming is a part of the response repertoire that develops fairly early in life, and where indicated performance can be forced by weighting of the animal. There are also some disadvantages. Good control of water temperature is necessary to assure that results are not confounded by effects of high or low temperatures or differences in temperatures between animals. Observation of the animals is necessary to assure that subjects are not lost due to drowning, however, it is feasible for one person to observe a number of animals at the same time.

As a dependent variable swimming has been used as a measure of endurance and as a measure of motor performance. The animals are allowed to swim with or without weights; swim speed, time to escape from the water, and/or time to exhaustion are measured and compared for control and treated animals or for control periods vs. periods after a treatment. The effects of tricholoroethylene exposures on swimming performance was investigated in rats by Grandjean (1963). Following 6 hours exposure to 800 ppm trichloroethylene, swimming performance was decreased in terms of swimming times. The development of coordinated swimming behavior in

immature rodents is a reasonably consistent sequence of events and disruption of this sequence has been used as a measure of effects of chemical agents (Schapiro et al., 1970; Preache and Gibson, 1976).

As an independent variable in animal studies forced swimming may be used as means of studying the effects of exercise or to induce physical fatigue or exhaustion. However, in these situations there would appear to be also elements of a psychological stressor. Survival is dependent upon either escaping from the water, swimming, or at least keeping afloat.

Porsolt et al. (1977, 1978) described a characteristic behavior pattern observed when rats or mice are placed unweighted in a restricted water filled space from which they cannot escape. There is an initial period of vigorous activity and thereafter they float in a characteristic immobile posture making only those movements necessary to keep their heads above water. These investigators have proposed the immobility posture as a model for screening antidepressants. They found that administration of drugs which increase central dopamine and norepinhephrine activity reduce the amount of time spent in the immobile posture, whereas those which diminish central dopamine and norephinephrine activity have the opposite effect (Porsolt et al. 1979).

Attempting to determine an animal model which adequately reflects stress as conceived in the human situation has resulted in a biochemical approach. Since "stress" is conceived to be related to such emotional responses as anxiety and depression, the neurotransmitters suspected of being involved in these affective states have been examined using animal models of stress. For example, four hours of swim stress was shown to decrease brain content of norepinephrine (Moore and Larivier, 1964). Grid shock caused a similar decrease, but there was no effect on brain NE with sound, tail shock or restraint stress.

Considering the effects of prenatal stress on behavior of the offspring, Archer and Blackman (1971) reviewed a substantial literature in which a variety of qualitatively different procedures were used to induce or mimic stress. These included handling, conditioned avoidance, crowding, swimming, tilting, and injections with epihephrine, norephinephrine, or hydrocortisone. They concluded that with the exception of the hormone

treatments, the specific nature of the stressor was not critical in determining the general direction of the response.

Thus, swimming would appear to be a reasonable choice for evaluating interactions of stress with chemical exposures. It involves physical stress, an aspect of stress which can be very important to subsequent behavioral performance and appears to have relevance as a psychological stressor. The biochemical data following swim stress suggest the neurotransmitter systems disrupted may be similar to those which are involved in human affective disorders. While it is impossible to attribute emotional states to animals, at least the relationship between neurotransmitter systems suggests similarities in underlying mechanisms. However, it should be noted that when considering the interactions of forced swimming and exposure to carbon monoxide, it will also be necessary to consider the effect of exercise on inhalation of carbon monoxide and carboxyhemoglobin formation.

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APPENDIX D

LITERATURE REVIEW:

EXTREMES IN AMBIENT TEMPERATURE AS A STRESS CONDITION AND INTERACTIONS WITH CHEMICALLY INDUCED TOXICITY

Behavioral toxicology is still in its early stages and it is not surprising that the effects of ambient temperature on the behavioral toxicity of environmental agents has received only limited investigation. The relative importance of this variable has long been a source of consideration to physiologists and pharmacologists, however, and their knowledge of temperature effects can be valuable in approaching the interactive effects of temperature and any toxic agent. Many books and reviews have dealt with temperature regulation and the physiological effects of heat and cold. For example, Hardy *et al.* (1971) have published a comprehensive volume on both the physiological and behavioral aspects of temperature regulation. The effects of temperature and other environmental stressors on humans is the subject of a book by Folinsbee *et al.* (1978) with the area of primary interest being physiological responsiveness. The interaction of temperature regulation and drugs has also received much attention (e.g. von Euler, 1961, Weihe, 1973, von Euler, 1964), however, the main emphasis of this work has been physiological and pharmacological changes in drug responsiveness excluding behavioral effects.

The processes of absorption, distribution, metabolism and elimination are responsible for the concentration of a drug or toxin at its biologic receptor. Each of these processes is to some extent temperature dependent and it is not unreasonable to expect changes in environmental temperature to influence toxic responses in both humans and animals (Ballard, 1972, 1974). Another factor which must be considered is that a specific agent may directly affect temperature regulation processes and thus may alter the responsiveness of the organism to heat and cold. This area has received extensive investigation using drugs which are known to specifically affect thermoregulatory processes (Fuhrman and Fuhrman, 1961). Carbon monoxide has not been reported to act directly on temperature regulation processes, either central or peripheral, and therefore such direct effects do not appear relevant to CO toxicity.

Consideration of the effects of ambient temperature can only be appreciated in the context of the thermoregulatory processes of mammalian species. Homeostatic mechanisms in mammals are well developed and they are able to maintain their body temperature over a wide range of ambient temperature variation. This is partially accomplished through behavioral temperature regulation, that is the active control of heat production and loss, utilizing food and water intake, posture and activity, huddling and aggregation or disaggregation. It is important to point out that the mode of regulation of body temperature differs between man and the common laboratory animal (e.g., rats, mice). Laboratory animals in confinement adapt to heat and cold metabolically. Man responds primarily through behavioral temperature regulation (Barnett *et al.* 1967; Hardy *et al.* 1971; Hart, 1971).

There are many examples of increased drug toxicity with increased ambient temperature (e.g., Askew, 1962; Furhman, 1963). The toxicity of sympathomimetic drugs such as amphetamine and methamphetamine, (Hardinge and Peterson, 1963; Müller and Vernekos-Danellio, 1969), epinephrine and norepinephrine, (Richards *et al.* 1970) cortisone (Scherr, 1952) and antihistamines (Keplinger and Lanier, 1959) has been shown to increase with increases in environmental temperature. The interaction of temperature and some toxins has also been addressed. The toxicity of organophosphate pesticides was increased with increased environmental temperature (Gohlke *et al.* 1973; Grigorowa and Binnewies, 1973). The toxicity of lead, a central nervous system toxin, was also greater with high environmental temperatures. Baetjer *et al.* (1960) and Baetjer and Horeguchi (1963) reported that rats and mice had increased mortality rates when exposed to high doses of lead and high environmental temperatures. This effect was due to increased retention of lead at the higher temperatures.

The effects of ambient temperature in determining the toxicity of carbon monoxide have received very limited investigation. Walters (1926) examined the effects of CO inhalation on the basal metabolism of rats and found that as the concentration of CO increased, metabolism decreased over a period of three to four hours. As metabolism decreased, body temperature also decreased. This was correlated with a decrease in symptoms of CO poisoning. There was no follow-up on this work, rather investigations of the effects of temperature on hypoxic hypoxia were pursued. Gellhorn (1937) investigated interaction between hypoxia, induced by decreasing inspired oxygen concentration, and carbon dioxide at various environmental temperatures. He showed that decreased body temperature and metabolism were dependent on the environmental temperature, with decreases occurring only at environmental temperatures below 27°C. Exposure to 32°C resulted in increased mortality at oxygen concentrations which had been tolerated at lower room temperatures. Annau and Dyer (1977) reported a similar effect with CO.

Gellhorn's studies used mice, rats and guinea pigs. Kottke *et al.* (1948) extended these studies to dog and man adding the observation that exposure to hypoxia in cold environments inhibited shivering. The addition of increased humidity was shown by Phillips *et al.* (1950) to protect mice exposed to hypoxia and low temperatures by reducing the energy requirements of the animals via a reduction in the rate of vaporization of moisture from the body. These studies show the importance of environmental temperature in determining the effects of hypoxia.

The thermoneutral zone is defined as the temperature region where basal metabolism in a resting animal is at its lowest. For most mammals this is between 28°C to 30°C. In a normal oxygen environment, any change in ambient temperature will increase metabolism. Changing the inspired O_2 concentration within the thermoneutral zone does not affect the animal's metabolism until very severe hypoxic conditions are reached (Hill, 1959).

When the ambient temperature is below the thermoneutral zone, lowering the inspired oxygen concentration lowers body temperature and metabolism resulting in increased survival due to lowered metabolic demands. When the ambient temperature is above the thermoneutral zone and the animal is made hypoxic, the rate of metabolism is increased due to the hyperthermia. This results in deaths at oxygen concentrations that can be tolerated at lower ambient temperatures.

Few studies have investigated behavioral disruption following changes in ambient temperature and exposure to hypoxic conditions. Annau (1976) investigated the effects of exposure to 8% oxygen on self-stimulation rates whem ambient temperature was either 20°C or 30°C. Under training conditions, the chamber temperature was 20°C for one group and 30°C for another group. Although Annau does not report a statistically significant effect on responding during training, the data suggest that response rates were lower in the group trained at 30°C. Exposure to 8% O_2 for 24 hours produced a significant increase in response rates for the first 12 hours of exposure in the animals exposed to an ambient temperature of 20°C. In contrast, animals exposed to 8% O_2 in ambient temperature of 30°C had significantly decreased response rates during the first 12 hours of exposure. Both groups returned to control levels when O_2 was returned to 21%. These temperature related effects were not replicated when hypoxia was induced by CO exposure.

The physiological concomitants of this effect were also examined by Annau. Rats were implanted with thermistors in the lateral hypothalamus and the peritoneal cavity. Exposure to 8% O_2 or 0.1% CO had no effect on peritoneal temperature in either 20°C or 30°C temperatures. Brain temperature, however, was decreased during exposure to both 8% O_2 and 0.1% CO at 20°C. There was also a decrease in brain temperature at 30°C but this effect was much smaller.

Annau has also found decreases in body temperature following exposure to 0.1% CO in restrained animals that are related to ambient temperatures. In addition, CO exposed animals show a lower LD_{50} when exposure occurs in higher ambient temperatures.

These data illustrate that the toxicity of agents can be affected by environmental temperature and for some toxins increased behavioral disruption would be predicted as a consequence of the interaction between temperature and toxin. The effects of extremes in ambient temperature on conditioned behavior in animals has not been an area of extensive investigation. The paucity of data in this area may be explained by the fact that man and animals are capable of adapting to both heat and cold stress. Homeothermia is, of course, better controlled in man than in laboratory animals and man's metabolic rate does not change greatly with exposure to cold and heat (Buskirk *et al.* 1957, Stein *et al.* 1949). Such findings may seem to mitigate the importance of the interaction of high environmental temperature and exposure to environmental toxins. The significance of the potentially behavioral disruptive effects must be viewed in the context of the interaction. The combined exposure of heat and toxin may further compromise the integrity of the nervous system and make appropriate function impossible. Combustion toxicology has shown concern with this and examples from this area may provide good models. The combustion of many products especially plastics results in the release of toxic gases in combination with heat stress from the actual fire conditions. Although examination of behavioral disruptions has only recently been the subject of investigation and the approach has differed, the findings are applicable to investigations of heat stress and environmental toxins.

McGuire and Annau (1980) investigated the effects of brief exposures to heat stress (50°C for 5 min, followed by 10 min at 35°C) as a heat control in a combustion product's study. Behavioral measures included avoidance performance and a licking response maintained on a variable ratio schedule for water reinforcement. Heat stress decreased responses and increased shocks on the avoidance schedule. Although the overall response rate changes on the licking schedule were not statistically significant, there was a slight response rate decrease during heat exposure. These data suggest that even brief exposures to intense heat can produce behavioral disruption. It would appear that heat is a variable which is often overlooked but should be of concern because of its behavioral disruption.

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APPENDIX E

ANIMAL WEIGHTS AND AGES AT EVALUATION

AD-A146 576 DEVELOPMENT OF BEHAVIORAL TOXICOLOGY METHODOLOGY FOR INTERACTIVE EXPOSURE REGIMENS(U) IIT RESEARCH INST CHICAGO IL M M PREACHE ET AL. DEC 83 IITRI-L06131-18 3/3

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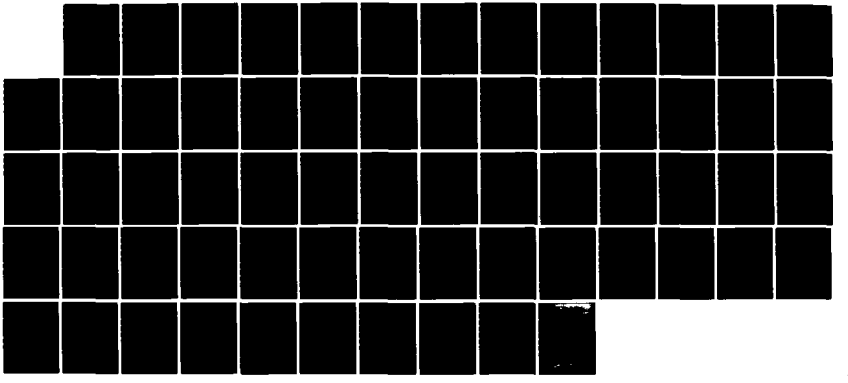

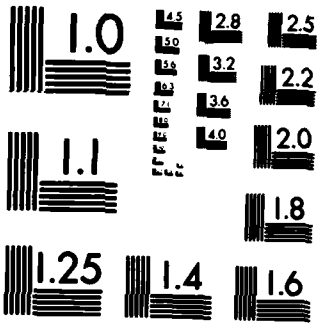

TABLE E1

ANIMAL WEIGHTS AND AGES AT EVALUATION

Experiment	Mean (Range) of Weights at Testing	Age at Testing
Fore-and hindlimb grip strength with 5 g weightings	352 (273-450)	31 weeks
Hindlimb extensor response	345 (286-429)	33 weeks
Fore-and hindlimb grip strength with 10 g weightings; phenobarbital control	362 (299-437)	17 weeks
CO effects on VR5-FR15	332 (273-446)	37 weeks
CO + swim stress: FR30-FR30	324 (296-357)	49 weeks
CO + heat stress: FR30-FR30	384 (367-407)	48 weeks
CO - COHb determinations	327 (294-381)	34 weeks
CO + swim/heat stress COHb determinations	356 (330-376)	35 weeks

APPENDIX F

METHOD FOR COHB DETERMINATION

Spectrophotometric Determination of Carboxyhemoglobin in Blood

1. Principle of the Method

Absorbance measurements are made in the Soret region at a blood dilution of approximately 1:1000. The diluent contains sodium hydrosulphite, $Na_2S_2O_4$, which removes dissolved oxygen from solution, preventing the displacement of CO from Carboxyhemoglobin (COHb), thus providing the two component system COHb-Hb for absorbance measurements at 420 and 432 nm.

At 420 nm. the absorbance of COHb is about double that of Hb; at 432 nm. the absorbance of Hb is almost threefold that of COHb. Absorbance measurements made at these two wavelengths are very sensitive to small changes in the relative proportions of COHb and Hb present in the two component system.

2. Reagents All chemicals ACS grade or better

2.1 Deionized or distilled water

2.2 Tris(hydroxymethyl)aminomethane, "THAM", Fisher Scientific Co, Catalog no. T-395

2.2.1 <u>0.01 M Diluent:</u> Dissolve 1.21 g of "THAM" in 1 liter of distilled or deionized water. This solution is used directly as diluent in the COHb determination.

2.3 Sodium hydrosulphite, solid , Fisher Scientific Co, Catalog No. S-310

2.4 Carbon Monoxide, compressed, about 99% pure, Matheson Gas Product

2.5 Potassium Cyanide, KCN

2.6 Potassium Ferricyanide, $K_3Fe(CN)_6$

3. Apparatus and Equipment

3.1 UV/VIS Spectrophotometer. In this laboratory, we used a Perkin-Elmer Model "Lambda 1"

3.2 Set of four matched 1.0 cm glass cuvets with Teflon stoppers

3.3 Disposable glass capillary pipets (3 microliter capacity) MICROCAPS, Fisher Scientific Cat No. 21-170C

3.4 Teflon Mixing Aids

3.4.1 Prepared by cutting 1/8 inch diameter Teflon rod into pieces 1/16" long. Two pieces are added to each cuvet for mixing (to avoid scratching the cuvets)

3.5 Polyethylene Tubing 1.14 mm ID Fisher Scientific Cat No. 14-170-1

3.6 Plastipak disposable syringes (20 ml) with 18 gauge needles

3.7 Glass Beads

3.8 Parafilm

3.9 Test tube. Should hold 20-30 ml when filled to the very top. Add 10 to 20 glass beads, and fill with water until meniscus wells up over the top of the tube. Measure the volume of water added, and let this volume be called V.

3.10 Spoons made from paper clips and polyethylene tubing. Insert one end of paper clip into the tubing, making sure that the fit is secure. Prepare two spoons, one capable of delivering 10 mg, the other should deliver 2V mg of sodium hydrosulphite. This is done simply by trimming the tubing, and weighing the amount of solid delivered. These will prove to be very convenient.

3.11 Analytical Balance

3.12 Centrifuge with refrigerated rotor (5° C)

3.13 Centrifuge tubes

3.14 10 ml volumetric pipet

3.15 100 ml volumetric flask

3.16 1 liter volumetric flask

4. Procedure for COHb Estimation

4.1 Each cuvet in a given series (one blank and three samples) must contain an identical concentration of $Na_2S_2O_4$ in the diluent.

4.1.1 Add 10 to 20 glass beads to the test tube described in 3.9 above. This test tube will contain V ml of diluent when filled to the very top, so that the meniscu wells up over the wall of the tube. Take care to dislodge any trapeped air bubbles.

4.1.2 Using the spoon calibrated to deliver 2V milligrams of $Na_2S_2O_4$, transfer the hydrosulphite to the tube, pouring the solid into the center of the meniscus above the tube wall.

4.1.3 Immediately cover the tube with Parafilm, displacing excess fluid, and invert several times letting the glass beads fall from one end of the tube to the other. No air should be present in the tube.

4.1.4 Draw the oxygen free diluent into a syringe fitted with an 18 gauge neede extended with a piece of polyethylene tubine long enough to reach the bottome of the test tube

4.1.5 Expel any air entering the syringe at the start of this transfer, before filling the syringe completely with diluent.

4.1.6 Having added the Teflon mixing aids in each cuvet, place the top of the polyethylene tube at the very bottom of the cuvet and fill it with diluent from the bottom up, until the meniscus wells up above the top of the cuvet.

4.2 Dislodge any air bubbles trapped in the cuvet on the walls or the Teflon pieces.

4.3 Replace any diluent lost in 4.2 so that the meniscus is rounded above the top of the cuvet.

4.4 Carefully insert the stopper in the blank cuvet, trapping no air and express the excess fluid around the edge of the stopper.

4.5 Transfer the blood samples to the remaining three cuvets as follows:

4.5.1 If the Red Blood Cells have settled to the bottom, mix the sample briefly on a vortex mixer, in order to assure a homogenous sample.

4.5.2 Fill the three microliter capillary pipet with sample,

and insert the tip into the middle or bottom of the sample cuvet and expel the sample.

4.6. When all samples have been delivered to the cuvets, insert the stoppers, taking care to exclude all air, and expressing the excess fluid out around the stopper.

4.7 Mix the comtents of each cuvet by shaking briskly in such a manner that the Teflon pieces pass from one end to the other.

4.8 It is extremely important that mixing be complete. Incomplete mixing can result in unstable absorbance readings.

4.9 Wipe excess diluent from the outside of each cuvet, using Kimwip

4.10 Allow cuvets to stand for about 10-15 minutes and measure the absorbance at 420 and 432 nm.

4.11 The cuvets and all Teflon pieces should then be thoroughly rinsed with de-ionized water, but not necessarily dried before the next set of measurements.

5. Calculations

5.1 Beer's law is usually stated as:

$A = \epsilon \cdot l \cdot c$

where A is the absorbance, ϵ is the molar absorptivity of the absorbing species, l is the light path length in centimeters, and c is the concentration of the absorbing species in moles per liter.

5.2 For the two component system COHb-Hb at each wavelength, Beer's law gives:

$$(1)\quad A_{420} = \left[\epsilon^{Hb}_{420} (1-x) + \epsilon^{COHb}_{420}\, x \right] l \cdot c$$

$$(2)\quad A_{432} = \left[\epsilon^{Hb}_{432} (1-x) + \epsilon^{COHb}_{432}\, x \right] l \cdot c$$

Here x is the fraction of total hemoglobin present as COHb, the remainder being present as Hb. The wavelengths for absorbance measurements are shown as subscripts. The absorbing species are shown as superscripts. The total hemoglobin concentration (c) is in moles of hemoglobin iron per liter, and l is the path length in centimeters.

Simultaneous solution of equations (1) and (2) gives the percentage of total hemoglobin present as COHb by equation (3)

$$(3)\quad \%COHb = \frac{100 \cdot A_{432} \cdot \epsilon^{Hb}_{420} - A_{420} \cdot \epsilon^{Hb}_{432}}{A_{420}(\epsilon^{COHb}_{432} - \epsilon^{Hb}_{432}) - A_{432}(\epsilon^{COHb}_{420} - \epsilon^{Hb}_{420})}$$

5.3 Since both photometer response and wavelength calibration vary with different spectrophotometers, it is recommended that for highest accuracy, the molar absorptivities for COHb and Hb be determined in the instrument actually being used for the analysis and with the blood of the species being tested. This is described below.

6.5.4 Prepare four dry cuvets , each containing two Teflon mixing aids.

6.5.5 Add diluent to the blank cuvet,taking care to exclude all air. The liquid should well up in a meniscus over the top of the cuvet.

6.5.6 Add the CO saturated secondary dilution to the three sample cuvets.

6.5.7 Transfer 10mg of $Na_2S_2O_4$ to each cuvet. Pour the solid directly into the center of the meniscus at the top of each cuvet.

6.5.8 Carefully insert the stoppers in the cuvets, trapping no air, and express the excess fluid out around the stopper

6.5.9 Invert the cuvets several times in order to ensure thorough mixing.

6.5.10 Wipe excess fluid from the sides of the cuvets and allow to stand for 15 minutes.

6.5.11 Measure the absorbance at 420 and 432 nm versus the blank

6.5.12 Calculate the molar absorptivity of COHb as follows:

(4) $$\epsilon^{COHb} = A/(l \cdot c)$$

Here A is the measured absorbance of the COHb solution, taken as the mean of three readings. By saturating the aliquot of secondary dilution with CO, all other forms of hemoglobin have been converted to COHb. Here c is the concentration of hemoglobin iron determined above in step 6.3

6.6 Determine the molar absorptivity of deoxyhemoglobin, Hb, as follows:

6.6.1 NOTE: Do not use the same Teflon pieces that have been exposed to high CO concentrations during the determination of the molar absorptivity of COHb, except as noted in 6.5.3 above.

6.6.2 Prepare four dry stoppered cuvets, each containing two Teflon mixing bars.

6.6.3 Fill the blank cuvet with diluent, and fill the remaining three cuvets with the secondary blood dilution (6.1.4) wut (without CO)

6. Evaluation of Molar Absorptivities

6.1 Obtain a control sample of blood from the appropriate species, with low COHb content.

6.1.1 Anticoagulate the blood with heparin or disodium EDTA

6.1.2 Prepare a primary dilution of approximately 1:151 by adding 0.2 ml of whole blood to 30 ml of deionized water.

6.1.3 Mix the solution to hemolyze the blood completely, and centrifuge at 5°C and 1500 g for 15 minutes to obtain the clear supernatant

6.1.4 Prepare an accurate secondary dilution by diluting 10.0 ml of the clear primary dilution to 100.0 ml with the diluent. Use volumetric glassware for accuracy.

6.2 Treat the remainder of the primary dilution with 2-3 mg of KCN, and 3-4 mg of $K_3Fe(CN)_6$. Mix, and allow 2 hours for complete conversion to CNMetHb.

6.3 Measure the absorbance of CNMetHb at 540 nm at which the molar absorptivity per mole of hemoglobin iron of CNMetHb is equal to 1.10×10^4 $mol^{-1}cm^{-1}$

6.4 Calculate c, the concentration of the secondary dilution used to measure ϵ^{COHb} and ϵ^{Hb} at both wavelengths.

6.5 Determine the molar absorptivity of COHb as follows:

6.5.1 Transfer 20-30 ml of the secondary dilution to a glass stoppered flask and saturate the solution with CO by bubbling compressed CO through the solution for 10 min.

6.5.2 Replace the stopper, and mix the solution with the gas phase.

6.5.3 NOTE: Use extreme caution to avoid CO contamination of the remainder of the secondary dilution used for the determination of the molar absorptivity of Hb at both wavelengths. Teflon pieces (mixers and cuvet stoppers) will tend to dissolve CO when in contact with the pure gas. Such dissolved CO could leach out of the Teflon into the sample when COHb measurements are made. This contamination can be avoided by heating the Teflon piece at 100°C overnight before re-use in other aspects of this procedure.

6.6.4 Add 10 mg of $Na_2S_2O_4$ to each cuvet, including the blank, insert the stoppers, and mix thoroughly.

6.6.5 Allow the solutions to stand for 15 minutes and then measure the absorbance at 420 and 432 nm versus the blank.

6.6.6 The hemoglobin in this solution is present as Hb, and the COHb of the original blood. For animals, the COHb content is about 0.5%; for non-smoking humans it is above 1.0%.

6.6.7 Calculate ϵ^{Hb} from equation (5)

$$\epsilon^{Hb} = [A - (f\epsilon^{COHb} l c)] / [(1-f) l c]$$

Here A is the measure absorbance of the Hb solution, as taken by the mean of three readings, f is the fraction of the total hemoglobin assumed (or known) to be present as COHb, and ϵ^{COHb} is the molar absorptivity of COHb as determined in 6.5 at the same wavelength.

7. This method is adapted from a paper by Rodkey et al:

Rodkey,F.L. et al, Spectrophotometric Measurement of Carboxyhemoglobin and Methemoglobin in Blood, Clin.Chem.25,1388(1979)

APPENDIX G

NUMERICAL VALUES FOR COHb DETERMINATIONS

TABLE G1

INDIVIDUAL ANIMAL CARBOXYHEMOGLOBIN LEVELS AT VARIOUS TIMES AFTER EXPOSURE TO CO

Percent Carboxyhemoglobin

CO Level (ppm)	Minutes after initiation of a 60-Minute Exposure						
	0	70-73	75-80	90	90-97	130-136	250-254
700	-*	27.62				9.02	0.76
700	0.29		29.35			10.12	0.37
700	-	35.52				7.60	0.28
700	-			14.73		5.09	-
700	-		33.53			13.06	1.36
700	-		23.50			4.73	0.31
1250	-				33.33	14.56	1.52
1250	-				23.87	9.00	1.06
1250	0.53		27.60			8.30	0.16
1250	0.14	39.02				7.28	0.18
1250	0.18	38.46				11.85	No Sample
1250	-	40.86				10.74	1.15

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* A dash (-) indicates values too low for detection.

Each value is the mean of duplicate samples.

TABLE G2

MEAN (± SE) COHB LEVELS FOLLOWING CO EXPOSURE WITH AND WITHOUT HEAT OR SWIM STRESS

% COHb

	450 ppm			700 ppm		
Time*	CO Alone	Swim	Heat	CO Alone	Swim	Heat
2	32 ± 1.0	38 ± 0.6	32 ± 1.1	42 ± 1.2	47 ± 2.1	43 ± 1.4
15	25 ± 0.8	28 ± 0.4	28 ± 0.9	32 ± 1.5	33 ± 0.9	36 ± 0.9
30	31 ± 0.9	24 ± 1.1	21 ± 0.3	21 ± 1.9	27 ± 1.5	27 ± 0.3

* Minutes after the end of exposure.

TABLE G3

INDIVIDUAL ANIMAL VALUES FOR COHb DETERMINATIONS

Percent Carboxyhemoglobin

Time*	CO only	CO ± Swim	CO ± 29.5 degrees C
Exposure to 450 ppm CO			
2-min	32.6	37.6	33.9
	32.5	35.7	32.3
	29.5	37.0	30.2
15-min	23.2	28.4	28.5
	26.1	----	28.7
	24.4	27.6	26.0
30-min	18.7	25.8	20.7
	21.5	23.0	21.2
	21.4	22.2	20.2
Exposure to 700 ppm CO			
2-min*	43.6	50.5	42.7
	42.3	46.0	41.4
	39.6	43.4	46.2
15-min	33.2	33.8	35.9
	34.7	33.6	38.1
	29.6	31.0	35.1
30-min	30.3	29.5	27.4
	24.0	24.2	26.7
	29.1	26.7	27.8
Controls	1.0		1.2
	1.0		1.0
	1.7		
	1.2		

Each value is the mean of duplicate samples.

* Minutes after termination of exposure.

APPENDIX H

INDIVIDUAL ANIMAL'S DATA
FOR REPONSES ON VR5 COMPONENT OF CHAIN VR5 FR15 SCHEDULE

TABLE H1

INDIVIDUAL ANIMAL'S DATA FOR RESPONSES ON VR5 COMPONENT OF CHAIN VR5 FR15 SCHEDULE FOR FIVE WEEKLY EXPOSURES and CO PLUS SWIMMING - 0 PPM

Date of Exposure	Animal No.	Mean of 3 days pre-treatment	Treatment 0 ppm	Treatment as % control
3/17/82	51	1296	1282	98.9
	83	2504	2119	84.6
	63	1886	1815	96.2
	116	1466	1350	92.1
	107	1517	1603	105.7
Mean				95.5
SE				3.5
3/24/82	51	1301	1245	95.7
	83	2378	2275	95.7
	63	2263	2701	119.4
	116	1555	1753	112.7
	107	1790	1739	97.2
Mean				104.1
SE				5.1
3/31/82	51	1194	1178	98.7
	83	2288	2218	96.9
	63	2336	2130	91.2
	116	1544	1800	116.6
	107	1576	1556	98.7
Mean				100.4
SE				4.3
4/7/82	51	1351	1480	109.5
	83	2449	2101	85.8
	63	2407	2545	105.7
	116	1422	1420	99.8
	107	1766	1782	100.9
Mean				100.3
SE				4.0
4/14/82	51	1401	1680	120.6
	83	2205	2037	92.4
	63	2445	1190	48.7
	116	1482	1431	96.6
	107	1783	1792	100.5
Mean				91.8
SE				11.8
4/21/82**	51	1511	354	23.4
	83	2296	1210	52.7
	63	2016	82	4.1
	116	1990	1745	87.7
	107	1802*	330	18.3
Mean				37.2
SE				14.9

* 4/16/82 data eliminated because of equipment problems
** 20 min swimming prior to exposure

TABLE H2

INDIVIDUAL ANIMAL'S DATA FOR RESPONSES ON VR5 COMPONENT OF CHAIN VR5 FR15 SCHEDULE FOR FIVE WEEKLY EXPOSURES AND CO PLUS SWIMMING - 200 PPM

Date of Exposure	Animal No.	Mean of 3 days pre-treatment	Treatment 200 ppm	Treatment as % control
3/17/82	91	565	566	100.2
	64	2181	2543	116.6
	122	1377	1435	104.2
	117	2136	2365	110.7
	87	1144	1242	108.6
Mean				108.1
SE				2.8
3/24/82	91	701	725	103.4
	64	2828	2754	97.4
	122	1388	1429	103.0
	117	2656	2373	89.3
	87	1318	1157	87.8
Mean				96.2
SE				3.3
3/31/82	91	669	573	85.6
	64	2803	2659	94.9
	122	1371	1270	92.6
	117	2560	2570	100.4
	87	1276	1225	96.0
Mean				93.9
SE				2.4
4/7/82	91	734	998	136.0
	64	3234	3065	94.8
	122	1321	1177	89.1
	117	2803	2828	100.9
	87	1220	1169	95.8
Mean				103.3
SE				8.4
4/14/82	91	906	926	102.2
	64	3666	3414	93.1
	122	1200	710	59.2
	117	2586	2977	115.1
	87	1273	1281	100.6
Mean				94.0
SE				9.4
4/21/82*	91	781	529	67.7
	64	3253	1	0.0
	122	999	853	85.4
	117	2970	2822	95.0
	87	1339	2	0.2
Mean				49.7
SE				20.7

* 20 min swimming prior to exposure

TABLE H3

INDIVIDUAL ANIMAL'S DATA FOR RESPONSES ON VR5 COMPONENT OF CHAIN VR5 FR15 SCHEDULE FOR FIVE WEEKLY EXPOSURES AND CO PLUS SWIMMING - 700 PPM

Date of Exposure	Animal No.	Mean of 3 days pre-treatment	Treatment 700 ppm	Treatment as % control
3/17/82	112	2561	1734	67.7
	103	1329	1248	93.9
	74	1140	897	78.7
	92	1504	1695	112.7
	108	1016	948	93.3
	49	1675	1658	98.9
Mean				90.9
SE				6.4
3/24/82	112	2647	1867	70.5
	103	1610	1525	94.7
	74	853	738	86.5
	92	1594	1617	101.4
	108	1009	932	92.4
	49	1640	1659	101.2
Mean				91.1
SE				4.7
3/31/82	112	2503	2150	85.9
	103	1711	1574	92.0
	74	1018	1056	103.7
	92	1203	1523	126.6
	108	981	909	92.7
	49	1862	1964	105.5
Mean				101.1
SE				6.0
4/7/82	112	2426	1836	75.7
	103	1738	1591	91.5
	74	1226	1336	109.0
	92	1687	1531	90.8
	108	991	968	97.7
	49	1936	1861	96.1
Mean				93.5
SE				4.4
4/14/82	112	2600	2449	94.2
	103	1472	1276	86.7
	74	1407	1267	90.0
	92	1616	1426	88.2
	108	1146	1209	105.5
	49	1919	1860	96.9
Mean				93.6
SE				2.9
4/21/82*	112	2692	84	3.1
	103	1517	0	00.0
	74	1653	709	42.9
	92	1593	1308	82.1
	108	1546	18	1.2
	49	1797	1308	72.8
Mean				25.9
SE				14.8

* 20 min swimming prior to exposure

TABLE H4

INDIVIDUAL ANIMAL'S DATA FOR RESPONSES ON VR5 COMPONENT OF CHAIN VR5 FR15 SCHEDULE FOR FIVE WEEKLY EXPOSURES AND CO PLUS SWIMMING - 1250 PPM

Date of Exposure	Animal No.	Mean of 3 days pre-treatment	Treatment 1250 ppm	Treatment as % control
3/17/82	105	2281	1094	47.96
	127	1252	562	44.9
	50	1351	705	52.2
	96	1169	484	41.4
	109	1567	686	43.8
Mean				46.1
SE				1.9
3/24/82	105	2754	1357	49.3
	127	1490	671	45.0
	50	1475	760	51.5
	96	1153	562	48.7
	109	1569	622	39.6
Mean				46.8
SE				2.1
3/31/82	105	2375	1686	71.0
	127	1638	603	36.8
	50	1173	586	50.0
	96	1131	428	37.8
	109	1303	439	33.7
Mean				45.9
SE				6.9
4/7/82	105	2849	1026	36.0
	127	1679	669	39.8
	50	1473	789	53.6
	96	1113	425	38.2
	109	1499	591	39.4
Mean				41.4
SE				3.1
4/14/82	105	2371	1300	54.8
	127	1479	635	42.9
	50	1368	711	52.0
	96	1196	476	40.0
	109	1568	560	35.7
Mean				45.1
SE				3.6
4/21/82*	105	2262	397	17.6
	127	1907	351	18.4
	50	1358	1	0.1
	96	1171	0	0.
	109	1576	2	0.1
Mean				7.2
SE				4.4

* 20 min swimming prior to exposure

TABLE H5

INDIVIDUAL ANIMAL'S DATA FOR RESPONSES ON FR15 COMPONENT OF CHAIN VR5 FR15 SCHEDULE FOR FIVE WEEKLY EXPOSURE and CO PLUS SWIMMING - 0 PPM

Date of Exposure	Animal No.	Mean of 3 days pre-treatment	Treatment 0 ppm	Treatment as % control
3/17/82	51	4616	4565	98.9
	83	7932	7382	93.1
	63	3787	3871	102.2
	116	6939	6320	91.1
	107	4038	4220	104.5
Mean				98.0
SE				2.6
3/24/82	51	4338	3598	82.9
	83	7815	6845	87.6
	63	3612	4197	116.2
	116	6385	5964	93.4
	107	4303	4142	96.2
Mean				95.3
SE				5.7
3/31/82	51	3961	3776	95.3
	83	6947	6804	97.9
	63	4285	4282	99.9
	116	6338	6038	95.3
	107	4134	4380	106.0
Mean				98.9
SE				2.0
4/7/82	51	4305	4078	94.7
	83	7275	6188	85.1
	63	4915	4389	89.3
	116	6030	5933	98.4
	107	4723	4866	103.0
Mean				94.1
SE				3.2
4/14/82	51	3765	3697	98.2
	83	7103	6174	86.9
	63	4960	7159	144.3
	116	6124	6389	104.3
	107	5166	5125	99.2
Mean				97.2
SE				3.3
4/21/82**	51	3972	886	22.3
	83	7057	3816	54.1
	63	5441	4	0.07
	116	6595	5271	79.9
	107	4880*	889	18.2
Mean				34.9
SE				14.2

* 4/16/82 data eliminated because of problem with box
** 20 min swimming prior to exposure

TABLE H6

INDIVIDUAL ANIMAL'S DATA FOR RESPONSES ON FR15 COMPONENT OF CHAIN VR5 FR15 SCHEDULE FOR FIVE WEEKLY EXPOSURES AND CO PLUS SWIMMING - 200 PPM

Date of Exposure	Animal No.	Mean of 3 days pre-treatment	Treatment 200ppm	Treatment as % control
3/17/82	91	2622	3309	126.0
	64	3565	3095	86.8
	122	3635	3943	108.5
	117	6294	6153	97.8
	87	4403	4367	99.2
Mean				103.7
SE				6.6
3/24/82	91	2947	2569	87.2
	64	3841	3634	94.6
	122	3797	3766	99.2
	117	7025	7449	106.0
	87	4672	4306	92.2
Mean				95.8
SE				3.2
3/31/82	91	2611	2847	109.0
	64	3700	3224	87.1
	122	3683	3736	101.4
	117	7205	6401	88.8
	87	4724	4073	86.2
Mean				94.5
SE				4.6
4/7/82	91	3213	3368	104.8
	64	3790	3601	95.0
	122	3960	4154	104.9
	117	7947	7592	95.5
	87	4167	4221	101.3
Mean				100.3
SE				2.1
4/14/82	91	3660	3979	108.7
	64	3514	3687	104.9
	122	4308	4768	110.7
	117	7503	8102	108.0
	87	4584	4246	92.6
Mean				103.6
SE				3.4
4/21/82*	91	3862	1411	36.5
	64	4031	0	00.0
	122	4688	3037	64.8
	117	8707	7452	85.6
	87	4756	45	1.0
Mean				37.6
SE				17.0

* 20 min swimming prior to exposure

TABLE H7

INDIVIDUAL ANIMAL'S DATA FOR RESPONSES ON FR15 COMPONENT OF CHAIN VR5 FR15 SCHEDULE FOR FIVE WEEKLY EXPOSURES AND CO PLUS SWIMMING - 700 PPM

Date of Exposure	Animal No.	Mean of 3 days pre-treatment	Treatment 700 ppm	Treatment as % control
3/17/82	112	8094	5151	63.6
	103	4574	4335	94.8
	74	3171	2740	86.
	92	2850	2853	100.
	108	4300	3769	87.7
	49	6276	6569	104.7
Mean				89.5
SE				5.9
3/24/82	112	8663	6865	79.2
	103	5094	4825	94.7
	74	2288	2595	113.4
	92	3093	3199	103.4
	108	5576	5496	98.6
	49	6618	6760	102.1
Mean				98.6
SE				4.7
3/31/82	112	8136	6569	80.7
	103	5253	4932	93.9
	74	2771	2750	99.2
	92	2939	2357	80.2
	108	5623	5277	93.8
	49	5828	5757	98.8
Mean				91.1
SE				3.5
4/7/82	112	8524	4745	55.7
	103	5252	5019	95.6
	74	3276	3427	104.6
	92	2935	3226	110.0
	108	5114	4811	94.1
	49	5985	5122	85.6
Mean				90.9
SE				7.8
4/14/82	112	7577	6036	79.7
	103	5268	4567	86.7
	74	3294	4034	122.5
	92	3025	3205	106.
	108	5056	4925	97.4
	49	5526	5435	98.4
Mean				98.4
SE				6.1
4/21/82*	112	7941	193	2.4
	103	5927	0	00.0
	74	4187	2538	60.6
	92	3171	2334	73.6
	108	5115	11	0.2
	49	5190	4086	78.7
Mean				27.4
SE				14.9

* 20 min swimming prior to exposure

TABLE H8

INDIVIDUAL ANIMAL'S DATA FOR RESPONSES ON FR15 COMPONENT OF CHAIN VR5 FR15 SCHEDULE FOR FIVE WEEKLY EXPOSURES AND CO PLUS SWIMMING - 1250 PPM

Date of Exposure	Animal No.	Mean of 3 days pre-treatment	Treatment 1250 ppm	Treatment as % control
3/17/82	105	4877	2139	43.9
	127	4077	1804	44.0
	50	6027	2703	44.8
	96	3305	1566	47.4
	109	3964	1846	46.6
Mean				45.3
SE				0.7
3/24/82	105	4803	2218	46.2
	127	4723	2129	45.1
	50	5871	3185	54.2
	96	3233	1508	46.6
	109	4215	1966	46.6
Mean				47.7
SE				1.7
3/31/82	105	3882	2016	51.9
	127	4979	2003	40.2
	50	4727	2725	57.6
	96	3179	1403	44.1
	109	4512	1962	43.5
Mean				47.5
SE				3.2
4/7/82	105	4051	2287	56.5
	127	4936	2055	41.6
	50	5904	2874	48.7
	96	2947	1226	41.6
	109	4414	1804	40.9
Mean				45.9
SE				3.0
4/14/82	105	3819	2648	69.3
	127	4594	1928	42.0
	50	5589	3483	62.3
	96	3180	1343	42.2
	109	4328	1818	42.0
Mean				51.6
SE				5.9
4/21/82*	105	4279	970	22.7
	127	5495	1229	22.4
	50	5565	0	0.0
	96	2994	0	0.0
	109	4260	0	0.0
Mean				9.1
SE				5.5

* 20 min swimming prior to exposure

TABLE H9

INDIVIDUAL ANIMAL'S DATA FOR NUMBER OF REINFORCER PRESENTATIONS ON CHAIN VR5 FR15 SCHEDULE FOR FIVE WEEKLY EXPOSURES AND CO PLUS SWIMMING - 0 PPM

Date of Exposure	Animal No.	Mean of 3 days pre-treatment	Treatment 0 ppm	Treatment as % control
3/17/82	51	153	154	100.6
	83	417	360	86.3
	63	185	172	92.9
	116	205	190	92.7
	107	217	229	105.5
Mean				95.6
SE				3.4
3/24/82	51	160	145	90.6
	83	412	388	94.2
	63	181	219	121.0
	116	210	230	109.5
	107	249	241	96.8
Mean				102.4
SE				5.6
3/31/82	51	141	147	104.3
	83	395	391	99.0
	63	201	194	96.5
	116	212	230	108.5
	107	231	243	105.2
Mean				102.7
SE				2.2
4/7/82	51	159	169	106.3
	83	421	367	87.2
	63	216	204	94.4
	116	196	198	101.0
	107	255	275	107.8
Mean				99.3
SE				3.8
4/14/82	51	160	170	106.2
	83	391	351	89.8
	63	214	149	69.6
	116	203	202	99.5
	107	287	287	100.0
Mean				93.0
SE				6.4
4/21/82**	51	164	3	1.8
	83	399	199	49.4
	63	210	0	00.0
	116	253	194	76.7
	107	286*	50	17.5
Mean				29.1
SE				14.8

* 4/16/82 data eliminated because of problem with box
** 20 min swimming prior to exposure

TABLE H10

INDIVIDUAL ANIMAL'S DATA FOR NUMBER OF REINFORCER PRESENTATIONS ON CHAIN VR5 FR15 SCHEDULE FOR FIVE WEEKLY EXPOSURES AND CO PLUS SWIMMING - 200 PPM

Date of Exposure	Animal No.	Mean of 3 days pre-treatment	Treatment 200 ppm	Treatment as % control
3/17/82	91	64	74	115.6
	64	195	181	92.8
	122	151	159	105.3
	117	320	349	109.1
	87	175	192	109.7
Mean				106.5
SE				3.8
3/24/82	91	84	83	98.8
	64	214	212	99.1
	122	152	156	102.6
	117	392	367	93.6
	87	210	184	87.6
Mean				96.3
SE				2.6
3/31/82	91	77	70	90.9
	64	222	197	88.7
	122	148	144	97.3
	117	380	363	95.5
	87	203	194	95.6
Mean				93.6
SE				1.6
4/7/82	91	88	118	134.1
	64	239	226	94.6
	122	148	139	93.9
	117	418	421	100.7
	87	199	196	98.5
Mean				104.4
SE				7.6
4/14/82	91	109	119	109.2
	64	228	232	101.8
	122	147	105	71.4
	117	384	428	111.5
	87	212	211	99.5
Mean				98.9
SE				7.2
4/21/82*	91	96	54	56.2
	64	255	0	00.0
	122	136	99	72.8
	117	435	412	94.7
	87	223	0	00.0
Mean				44.7
SE				19.3

* 20 min swimming prior to exposure

TABLE H11

INDIVIDUAL ANIMAL'S DATA FOR NUMBER OF REINFORCER PRESENTATIONS ON CHAIN VR5 FR15 SCHEDULE FOR FIVE WEEKLY EXPOSURES AND CO PLUS SWIMMING - 700 PPM

Date of Exposure	Animal No.	Mean of 3 days pre-treatment	Treatment 700 ppm	Treatment as % control
3/17/82	112	369	249	67.5
	103	221	212	95.9
	74	124	102	82.3
	92	158	172	108.9
	108	150	134	89.3
	49	225	225	100.0
Mean				90.7
SE				5.9
3/24/82	112	253	285	112.6
	103	263	239	90.9
	74	95	90	94.7
	92	163	170	104.3
	108	150	142	94.7
	49	227	232	102.2
Mean				99.9
SE				3.3
3/31/82	112	369	309	83.7
	103	270	248	91.8
	74	113	113	100.0
	92	142	144	101.4
	108	161	150	93.2
	49	249	256	102.8
Mean				95.5
SE				3.0
4/7/82	112	367	243	66.2
	103	274	242	88.3
	74	130	134	103.1
	92	160	157	98.1
	108	160	156	97.5
	49	252	240	95.2
Mean				91.4
SE				5.4
4/14/82	112	373	330	88.5
	103	235	204	86.8
	74	144	144	100.0
	92	153	152	99.3
	108	174	195	112.1
	49	251	244	97.2
Mean				97.3
SE				3.7
4/21/82*	112	382	7	1.8
	103	250	0	00.0
	74	174	70	40.2
	92	160	112	70.0
	108	239	0	00.0
	-	235	164	69.8**
Mean				22.4
SE				12.9

* 20 min swimming prior to exposure
** Value not used, animal detached weight during swimming

TABLE H12

INDIVIDUAL ANIMAL'S DATA FOR NUMBER OF REINFORCER PRESENTATIONS ON CHAIN VR5 FR15 SCHEDULE FOR FIVE WEEKLY EXPOSURES AND CO PLUS SWIMMING - 1250 PPM

Date of Exposure	Animal No.	Mean of 3 days pre-treatment	Treatment 1250 ppm	Treatment as % control
3/17/82	105	216	99	45.8
	127	219	97	44.3
	50	191	99	51.8
	96	187	76	40.6
	109	168	74	44.0
Mean				45.3
SE				1.8
3/24/82	105	232	102	44.0
	127	266	119	44.7
	50	198	103	52.0
	96	189	90	47.6
	109	172	70	40.7
Mean				45.8
SE				1.9
3/31/82	105	188	111	59.0
	127	293	108	36.9
	50	161	81	50.3
	96	183	72	39.9
	109	156	55	35.3
Mean				44.3
SE				4.5
4/7/82	105	216	90	41.7
	127	300	119	39.7
	50	213	111	52.1
	96	183	69	37.7
	109	176	66	37.5
Mean				41.7
SE				6.0
4/14/82	105	201	119	59.2
	127	265	114	43.0
	50	200	108	54.0
	96	195	80	41.0
	109	170	60	35.3
Mean				46.5
SE				9.8
4/21/82*	105	214	36	16.8
	127	341	61	17.9
	50	205	0	0.0
	96	192	0	0.0
	109	168	0	0.0
Mean				6.9
SE				4.2

* 20 min swimming prior to exposure

TABLE H13

INDIVIDUAL ANIMAL'S DATA FOR RESPONSES ON VR5 COMPONENT OF CHAIN VR 5 FR15 SCHEDULE FOR FIVE DAILY EXPOSURES - 0 PPM

Date of Exposure	Animal No.	Mean of 3 days pre-treatment	Treatment 0 ppm	Treatment as % control
5/17/82	51	1356	1310	96.6
	83	2326	2461	105.8
	63	1573	1766	112.3
	116	1885	2485	131.8
	107	1672	1788	106.9
Mean				110.7
SE				5.9
5/18/82	51	1356	1668	123.0
	83	2326	2268	97.5
	63	1573	2359	150.0
	116	1885	2196	116.5
	107	1672	1625	97.2
Mean				116.8
SE				9.7
5/19/82	51	1356	1284	94.7
	83	2326	2252	96.8
	63	1573	2179	138.5
	116	1885	1915	101.6
	107	1672	1668	99.8
Mean				106.3
SE				8.1
5/20/82	51	1356	1366	100.7
	83	2326	2456	105.6
	63	1573	2598	165.2
	116	1885	2030	107.7
	107	1672	1631	97.5
Mean				115.3
SE				12.6
5/21/82	51	1356	1451	107.0
	83	2326	2648	113.8
	63	1573	2404	152.8
	116	1885	2361	125.3
	107	1672	1649	98.6
Mean				119.5
SE				9.4

TABLE H14

INDIVIDUAL ANIMAL'S DATA FOR RESPONSES ON VR5 COMPONENT OF CHAIN VR5 FR15 SCHEDULE FOR FIVE DAILY EXPOSURES - 200 PPM

Date of Exposure	Animal No.	Mean of 3 days pre-treatment	Treatment 200 ppm	Treatment as % control
5/17/82	91	985	1053	106.9
	64	2050	2853	139.2
	122	905	1201	132.7
	117	2560	2460	96.1
	87	1195	1369	114.6
Mean				117.9
SE				8.0
5/18/82	91	985	1232	125.1
	64	2050	2809	137.0
	122	905	1125	124.3
	117	2560	2751	107.5
	87	1195	1219	102.0
Mean				119.2
SE				6.4
5/19/82	91	985	1094	111.1
	64	2050	3288	160.4
	122	905	1206	133.3
	117	2560	2603	101.7
	87	1195	1183	99.0
Mean				121.1
SE				11.5
5/20/82	91	985	1091	110.8
	64	2050	3207	156.4
	122	905	1165	128.7
	117	2560	2880	112.5
	87	1195	1167	97.7
Mean				121.2
SE				10.1
5/21/82	91	985	1020	103.6
	64	2050	3322	162.0
	122	905	1080	119.3
	117	2560	2675	104.5
	87	1195	1283	107.4
Mean				119.4
SE				11.0

TABLE H15

INDIVIDUAL ANIMAL'S DATA FOR RESPONSES ON VR5 COMPONENT OF CHAIN VR5 FR15 SCHEDULE FOR FIVE DAILY EXPOSURES - 700 PPM

Date of Exposure	Animal No.	Mean of 3 days pre-treatment	Treatment 700 ppm	Treatment as % control
5/17/82	112	2798	2283	81.6
	103	1255	968	77.1
	74	1572	1807	114.9
	92	1582	2077	131.3
	108	1479	1686	114.0
	49	1669	1892	113.4
Mean				105.4
SE				8.7
5/18/82	112	2798	2702	96.6
	103	1255	1204	95.9
	74	1572	1815	115.4
	92	1582	2020	127.7
	108	1479	1245	84.2
	49	1669	2006	120.2
Mean				106.7
SE				6.9
5/19/82	112	2798	2493	89.1
	103	1255	1465	116.7
	74	1572	1514	96.3
	92	1582	2108	133.2
	108	1479	1747	118.1
	49	1669	1994	119.5
Mean				112.2
SE				6.6
5/20/82	112	2798	2605	93.1
	103	1255	1537	122.5
	74	1572	1698	108.0
	92	1582	2309	146.0
	108	1479	1831	123.8
	49	1669	1989	119.2
Mean				118.8
SE				7.3
5/21/82	112	2798	2674	95.6
	103	1255	1622	129.2
	74	1572	1687	107.3
	92	1582	2287	144.6
	108	1479	1823	123.3
	49	1669	2176	130.4
Mean				121.7
SE				7.2

TABLE H16

INDIVIDUAL ANIMAL'S DATA FOR RESPONSES ON VR5 COMPONENT OF CHAIN VR5 FR15 SCHEDULE FOR FIVE DAILY EXPOSURES AND CO PLUS SWIMMING - 1250 PPM

Date of Exposure	Animal No.	Mean of 3 days pre-treatment	Treatment 1250 ppm	Treatment as % control
5/17/82	105*	2235	168	7.5
	127	1883	453	24.1
	50	1318	670	50.8
	96	1027	493	48.0
	109	924	385	41.2
Mean				41.2
SE				5.4
5/18/82	105	2235	1060	47.4
	127	1883	606	32.2
	50	1318	660	50.1
	96	1027	516	50.2
	109	924	286	31.0
Mean				42.2
SE				4.3
5/19/82	105	2235	1157	51.8
	127	1883	739	39.2
	50	1318	793	60.2
	96	1027	494	48.1
	109	924	406	43.9
Mean				48.6
SE				3.6
5/20/82	105	2235	1381	61.8
	127	1883	677	35.9
	50	1318	941	71.4
	96	1027	602	58.6
	109	924	604	65.4
Mean				58.6
SE				6.1
5/21/82	105	2235	1582	70.8
	127	1883	787	41.8
	50	1318	1009	76.6
	96	1027	660	64.3
	109	924	946	102.4
Mean				71.2
SE				9.8

* Data not included in computation of mean due to malfunction of feeder.

TABLE H17

INDIVIDUAL ANIMAL'S DATA FOR RESPONSES ON FR15 COMPONENT OF CHAIN VR5 FR15 SCHEDULE FOR FIVE DAILY EXPOSURES - 0 PPM

Date of Exposure	Animal No.	Mean of 3 days pre-treatment	Treatment 0 ppm	Treatment as % control
5/17/82	51	3475	3499	100.7
	83	6948	7279	104.8
	63	4284	3486	81.4
	116	6116	6127	100.2
	107	4888	4993	102.1
Mean				97.8
SE				4.2
5/18/82	51	3475	3616	104.0
	83	6948	6748	97.1
	63	4284	5141	120.0
	116	6116	6158	100.7
	107	4888	4795	98.1
Mean				104.0
SE				4.2
5/19/82	51	3475	3902	112.3
	83	6948	6918	99.6
	63	4284	4702	109.8
	116	6116	6376	104.3
	107	4888	4708	96.3
Mean				104.5
SE				3.0
5/20/82	51	3475	4087	117.6
	83	6948	7922	114.0
	63	4284	4982	116.3
	116	6116	6247	102.1
	107	4888	4698	96.1
Mean				109.2
SE				4.3
5/21/82	51	3475	3892	112.0
	83	6948	7861	113.1
	63	4284	4893	114.2
	116	6116	6239	102.0
	107	4888	5115	104.6
Mean				109.2
SE				2.5

TABLE H18

INDIVIDUAL ANIMAL'S DATA FOR RESPONSES ON FR15 COMPONENT OF CHAIN VR5 FR15 SCHEDULE FOR FIVE DAILY EXPOSURES - 200 PPM

Date of Exposure	Animal No.	Mean of 3 days pre-treatment	Treatment 200 ppm	Treatment as % control
5/17/82	91	3720	3981	107.0
	64	3805	4262	112.0
	122	4211	3982	94.6
	117	8201	9026	110.1
	87	4399	4361	99.1
Mean				104.6
SE				3.3
5/18/82	91	3720	4856	130.5
	64	3805	4278	112.4
	122	4211	4016	95.4
	117	8201	8593	104.8
	87	4399	4212	95.7
Mean				107.8
SE				6.5
5/19/82	91	3720	4491	120.7
	64	3805	3814	100.2
	122	4211	3932	93.4
	117	8201	8787	107.1
	87	4399	4408	100.2
Mean				104.3
SE				4.7
5/20/82	91	3720	4312	115.9
	64	3805	3973	104.4
	122	4211	4028	95.7
	117	8201	8960	109.3
	87	4399	4710	107.1
Mean				106.5
SE				3.3
5/21/82	91	3720	4463	120.0
	64	3805	4132	108.6
	122	4211	4217	100.1
	117	8201	8388	102.3
	87	4399	4738	107.7
Mean				107.7
SE				3.4

TABLE H19

INDIVIDUAL ANIMAL'S DATA FOR RESPONSES ON FR15 COMPONENT OF CHAIN VR5 FR15 SCHEDULE FOR FIVE DAILY EXPOSURES - 700 PPM

Date of Exposure	Animal No.	Mean of 3 days pre-treatment	Treatment 700 ppm	Treatment as % control
5/17/82	112	8166	5799	71.0
	103	4841	3566	73.7
	74	3904	4282	109.7
	92	2701	3010	111.4
	108	4556	4525	99.3
	49	4952	5266	106.3
Mean				95.2
SE				7.4
5/18/82	112	8166	8058	98.7
	103	4841	5150	106.4
	74	3904	4220	108.1
	92	2701	3153	116.7
	108	4556	4466	98.0
	49	4952	4984	100.6
Mean				104.8
SE				2.9
5/19/82	112	8166	6750	82.7
	103	4841	5421	112.0
	74	3904	4979	127.5
	92	2701	3129	115.8
	108	4556	5087	111.7
	49	4952	4784	96.6
Mean				107.7
SE				6.5
5/20/82	112	8166	8182	100.2
	103	4841	5498	113.6
	74	3904	5060	129.6
	92	2701	3115	115.3
	108	4556	5560	122.0
	49	4952	5860	118.1
Mean				116.5
SE				4.0
5/21/82	112	8166	8423	103.1
	103	4841	5280	109.1
	74	3904	4731	121.2
	92	2701	3265	120.9
	108	4556	6058	133.0
	49	4952	5803	117.2
Mean				117.4
SE				4.2

TABLE H20

INDIVIDUAL ANIMAL'S DATA FOR RESPONSES ON FR15 COMPONENT OF CHAIN VR5 FR15 SCHEDULE FOR FIVE DAILY EXPOSURES - 1250 PPM

Date of Exposure	Animal No.	Mean of 3 days pre-treatment	Treatment 1250 ppm	Treatment as % control
5/17/82	105	3605	261	7.2
	127	5378	1455	27.1
	50	4933	2300	46.6
	96	2773	1307	47.1
	109	4488	1707	38.0
Mean				39.7
SE				4.2
5/18/82	105	3605	2013	55.8
	127	5378	1912	35.6
	50	4933	3171	64.3
	96	2773	1372	49.5
	109	4488	1984	44.2
Mean				49.9
SE				4.9
5/19/82	105	3605	2782	77.2
	127	5378	2038	38.7
	50	4933	2795	56.7
	96	2773	1693	61.1
	109	4488	2476	55.2
Mean				57.9
SE				2.8
5/20/82	105	3605	3061	84.9
	127	5378	2006	37.3
	50	4933	3616	73.3
	96	2773	1741	62.8
	109	4488	2398	53.4
Mean				62.3
SE				8.2
5/21/82	105	3605	2892	80.2
	127	5378	2089	38.8
	50	4933	3666	74.3
	96	2773	1855	66.9
	109	4488	3297	73.5
Mean				66.7
SE				7.3

TABLE H21

INDIVIDUAL ANIMAL'S DATA FOR NUMBER OF REINFORCER PRESENTATIONS ON CHAIN VR5 FR15 SCHEDULE FOR FIVE DAILY EXPOSURES - 0 PPM

Date of Exposure	Animal No.	Mean of 3 days pre-treatment	Treatment 0 ppm	Treatment as % control
5/17/82	51	147	148	100.7
	83	402	427	106.2
	63	179	159	88.8
	116	238	282	118.5
	107	273	272	99.6
Mean				102.8
SE				4.8
5/18/82	51	147	173	117.7
	83	402	391	97.3
	63	179	238	133.0
	116	238	274	115.1
	107	273	259	94.9
Mean				111.6
Se				7.0
5/19/82	51	147	152	103.4
	83	402	394	98.0
	63	179	233	130.2
	116	238	252	105.9
	107	273	270	98.9
Mean				107.3
SE				5.9
5/20/82	51	147	157	106.8
	83	402	440	109.5
	63	179	256	143.0
	116	238	264	110.9
	107	273	274	100.4
Mean				114.1
SE				7.4
5/21/82	51	147	164	111.6
	83	402	466	115.9
	63	179	245	136.9
	116	238	284	119.3
	107	273	279	102.2
Mean				117.2
SE				5.7

TABLE H22

INDIVIDUAL ANIMAL'S DATA FOR NUMBER OF REINFORCER PRESENTATIONS ON CHAIN VR5 FR15 SCHEDULE FOR FIVE DAILY EXPOSURES - 200 PPM

Date of Exposure	Animal No.	Mean of 3 days pre-treatment	Treatment 200 ppm	Treatment as % control
5/17/82	51	115	119	103.5
	64	201	256	127.4
	122	126	152	120.6
	117	391	392	100.3
	87	196	218	112.6
Mean				112.6
SE				5.1
5/18/82	91	115	146	127.0
	64	201	245	121.9
	122	126	147	116.7
	117	391	424	108.4
	87	196	202	103.1
Mean				115.4
SE				4.3
5/19/82				
	91	115	136	118.3
	64	201	239	118.9
	122	126	147	116.7
	117	391	420	107.4
	87	196	202	103.1
Mean				112.9
SE				3.2
5/20/82	91	115	132	114.8
	64	201	248	123.4
	122	126	145	115.1
	117	391	451	115.3
	87	196	202	97.0
Mean				113.1
SE				4.3
5/21/82	91	115	130	113.0
	64	201	258	128.4
	122	126	144	114.3
	117	391	419	107.2
	87	196	222	113.3
Mean				115.2
SE				3.5

TABLE H23

INDIVIDUAL ANIMAL'S DATA FOR NUMBER OF REINFORCER PRESENTATIONS ON CHAIN VR5 FR15 SCHEDULE FOR FIVE DAILY EXPOSURES - 700 PPM

Date of Exposure	Animal No.	Mean of 3 days pre-treatment	Treatment 700 ppm	Treatment as % control
5/17/82	112	396	296	74.7
	103	204	144	70.6
	74	164	188	114.6
	92	153	171	111.8
	108	233	245	105.2
	49	220	242	110.0
Mean				97.8
SE				8.9
5/18/82	112	396	393	99.2
	103	204	200	98.0
	74	164	192	117.1
	92	153	184	120.3
	108	233	202	86.7
	49	220	240	109.1
Mean				105.1
SE				5.7
5/19/82	112	396	345	87.1
	103	204	244	119.6
	74	164	174	106.1
	92	153	181	118.3
	108	233	271	116.3
	49	220	235	106.8
Mean				109.0
SE				5.5
5/20/83	112	396	384	97.0
	103	204	260	127.5
	74	164	195	118.9
	92	153	192	125.9
	108	233	280	120.2
	49	220	258	117.3
Mean				117.8
SE				4.9
5/21/82	112	396	395	99.7
	103	204	265	129.9
	74	164	197	120.1
	92	153	191	124.8
	108	233	290	124.5
	49	220	277	125.9
Mean				120.8
SE				4.8

TABLE H24

INDIVIDUAL ANIMAL'S DATA FOR NUMBER OF REINFORCER PRESENTATIONS ON CHAIN VR5 FR15 SCHEDULE FOR FIVE DAILY EXPOSURES - 1250 PPM

Date of Exposure	Animal No.	Mean of 3 days pre-treatment	Treatment 1250 ppm	Treatment as % control
5/17/82	105*	201	11	5.5
	127	355	80	23.9
	50	206	102	49.5
	96	170	81	47.6
	109	121	52	43.0
Mean				41.0
SE				5.2
5/18/82	105	201	101	50.2
	127	335	109	32.5
	50	206	106	51.4
	96	170	85	50.0
	109	121	42	34.7
Mean				43.8
SE				4.2
5/19/82	105	201	118	58.7
	127	335	130	38.8
	50	206	126	61.2
	96	170	85	50.0
	109	121	60	49.6
Mean				51.7
SE				3.9
5/20/82	105	201	145	72.1
	127	335	120	35.8
	50	206	151	73.3
	96	170	99	58.2
	109	121	76	62.8
Mean				60.4
Se				6.8
5/21/82	105	201	152	75.6
	127	335	129	38.5
	50	206	160	77.7
	96	170	106	62.4
	109	121	113	93.4
Mean				69.5
SE				9.2

APPENDIX I

BASELINE PERFORMANCE FOR FR30-FR30 SCHEDULE

L06131

TABLE II

BASELINE PERFORMANCE*

Exposure Group	Week 1 Mean ± S.E.M.	Week 1 Range	Week 2 Mean ± S.E.M.	Week 2 Range	Week 3 Mean ± S.E.M.	Week 3 Range
Responses on Lever for Light Presentation						
0 ppm	3857 ± 380	2135 - 6518	3870 ± 528	905 - 7349	3808 ± 435	874 - 6766
200 ppm	3841 ± 307	1178 - 7795	3620 ± 314	1057 - 7716	3639 ± 360	1350 - 8092
700 ppm	3701 ± 359	860 - 7719	3564 ± 391	441 - 7772	3531 ± 391	414 - 7744
1250 ppm	3865 ± 430	2052 - 7539	3631 ± 427	1708 - 7107	3523 ± 465	1591 - 7074
Responses on Lever for Food Presentation						
0 ppm	4071 ± 468	2345 - 7576	3926 ± 527	1164 - 7437	3905 ± 452	1022 - 6716
200 ppm	3993 ± 394	1546 - 9147	3520 ± 506	1450 - 7355	3518 ± 514	1474 - 7333
700 ppm	3976 ± 611	991 - 7720	3629 ± 605	425 - 7702	3910 ± 668	331 - 7539
1250 ppm	4006 ± 359	1844 - 9400	3677 ± 503	1572 - 8168	3640 ± 470	1636 - 7711
Number of Reinforcers						
0 ppm	109 ± 11	63 - 192	108 ± 15	26 - 209	107 ± 12	26 - 191
200 ppm	108 ± 15	36 - 210	100 ± 15	34 - 194	100 ± 15	41 - 194
700 ppm	105 ± 16	22 - 217	100 ± 17	10 - 221	101 ± 17	10 - 221
1250 ppm	107 ± 15	49 - 237	98 ± 14	44 - 220	97 ± 14	42 - 220

*Data from the three days prior to exposure

APPENDIX J

TABULAR SUMMARY AND STATISTICAL ANALYSES FOR FR30-FR30 SCHEDULE OF REINFORCEMENT AFTER EXPOSURE TO CO AND/OR SWIM STRESS

TABLE J3

P-VALUES FOR INDIVIDUAL EFFECTS AND INTERACTIONS

		Time To First Response	Total Responses On Lever For Light	Total Responses On Lever For Food	Number Of Rein-forcers
1	GR MEAN	0.00051	0.00000	0.00000	0.00000
2	REP	0.88031	0.57518	0.56713	0.71105
3	REP	0.08352	0.12208	0.37243	0.28950
4	DOSE (0 vs 200)	0.69901	0.70151	0.91250	0.66197
5	DOSE (0 vs 700)	0.68251	0.00000	0.00000	0.00000
6	DOSE (0 vs 1250)	0.00073	0.00000	0.00000	0.00000
7	SWIM	0.00148	0.00807	0.00016	0.00013
8	DOSE/SWIM (Swim x 0 vs 200)	0.74351	0.22862	0.77078	0.33371
9	DOSE/SWIM (Swim x 0 vs 700)	0.79729	0.21553	0.47563	0.21706
10	DOSE/SWIM (Swim x 0 vs 1250)	0.00062	0.14203	0.11513	0.04530

Where p value shown is 0.00000, significance level was beyond the fifth decimal place.

TABLE J1

MEAN NUMBER OF RESPONSES AND REINFORCERS ON AN FR30-FR30 SCHEDULE OF REINFORCEMENT AFTER EXPOSURE TO CO AND/OR SWIM STRESS

Responses on Lever for Light Presentation

	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
0 ppm	3921 ± 458	3836 ± 613	3892 ± 304
0 ppm + Swim	2454 ± 1214**	2891 ± 854**	3536 ± 1008
200 ppm	4236 ± 685	3387 ± 402	3555 ± 689
200 ppm + Swim	1816 ± 658	3091 ± 604	2160 ± 765
700 ppm	1194 ± 194	1449 ± 317	2008 ± 335
700 ppm + Swim	1043 ± 254	430 ± 183	548 ± 319
1250 ppm	210 ± 64	384 ± 109	268 ± 63
1250 ppm + Swim	3 ± 1	2 ± 1	18 ± 9

Responses on Lever for Food Presentation

	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
0 ppm	4062 ± 632	3788 ± 534	4181 ± 422
0 ppm + Swim	3021 ± 1651**	2376 ± 530**	3648 ± 968
200 ppm	4581 ± 760	3127 ± 360	3637 ± 771
200 ppm + Swim	2051 ± 736	3916 ± 940	2678 ± 1030
700 ppm	1581 ± 347	1383 ± 339	2216 ± 552
700 ppm + Swim	1146 ± 266	776 ± 415	1065 ± 646
1250 ppm	240 ± 73	406 ± 122	363 ± 79
1250 ppm + Swim	9 ± 5	2 ± 0	23 ± 16

Number of Reinforcers

	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
0 ppm	107 ± 14	105 ± 17	109 ± 9
0 ppm + Swim	64 ± 32**	62 ± 17**	90 ± 31
200 ppm	124 ± 19	87 ± 11	99 ± 20
200 ppm + Swim	49 ± 19	96 ± 18	57 ± 20
700 ppm	34 ± 5	40 ± 10	56 ± 10
700 ppm + Swim	30 ± 8	11 ± 5	16 ± 10
1250 ppm	5 ± 2	11 ± 4	8 ± 2
1250 ppm + Swim	0 ± 0	0 ± 0	0 ± 0

** Data for one animal was excluded due to an injury prior to the session.

TABLE J2

EFFECTS OF CARBON MONOXIDE AND SWIM FATIGUE ON PERFORMANCE ON A CHAIN FR30-FR30 SCHEDULE*

Responses on Lever for Light Presentation

	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
0 ppm	105 ± 4	101 ± 3	101 ± 3
0 ppm + Swim	68 ± 34**	70 ± 11**	121 ± 34
200 ppm	100 ± 1	105 ± 4	102 ± 5
200 ppm + Swim	59 ± 19	88 ± 23	70 ± 26
700 ppm	39 ± 4	43 ± 4	56 ± 6
700 ppm + Swim	25 ± 6	12 ± 4	26 ± 14
1250 ppm	6 ± 1	9 ± 2	9 ± 2
1250 ppm + Swim	0 ± 0	0 ± 0	0 ± 0

Responses on Lever for Food Presentation

	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
0 ppm	103 ± 3	98 ± 4	105 ± 2
0 ppm + Swim	74 ± 37**	68 ± 8**	115 ± 26
200 ppm	100 ± 2	106 ± 4	107 ± 5
200 ppm + Swim	72 ± 22	95 ± 18	82 ± 30
700 ppm	42 ± 3	37 ± 4	55 ± 8
700 ppm + Swim	26 ± 5	20 ± 7	45 ± 25
1250 ppm	6 ± 1	10 ± 2	10 ± 2
1250 ppm + Swim	0 ± 0	0 ± 0	1 ± 1

Number of Reinforcers

	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.
0 ppm	102 ± 3	98 ± 3	102 ± 2
0 ppm + Swim	65 ± 33**	61 ± 9**	106 ± 35
200 ppm	101 ± 2	102 ± 3	104 ± 5
200 ppm + Swim	63 ± 27	90 ± 22	67 ± 23
700 ppm	40 ± 3	39 ± 4	55 ± 6
700 ppm + Swim	25 ± 5	12 ± 4	26 ± 14
1250 ppm	5 ± 1	9 ± 2	9 ± 2
1250 ppm + Swim	0 ± 0	0 ± 0	0 ± 0

* Data are plotted as percent baseline
Baseline is the mean of the three days prior to exposure.

** Data for one animal was excluded due to an injury prior to the session.

Table J4

Effects of CO and CO + Swim Stress On the Total Responses On Lever 1 (For Light Presentation) during 10-minutes Intervals

	FACTORS-	1 (DOSE)	3 (SWIM)								
1	LEVELS-	(1 0000)	(1 NO)								
	N = 23.										
	MEANS	T1	= 667.43	T2	= 702.04	T3	= 692.17	T4	= 685.61	T5	= 662.48
		T6	= 668.39								
2	LEVELS-	(1 0000)	(2 YES)								
	N = 12.										
	MEANS	T1	= 332.17	T2	= 436.17	T3	= 431.50	T4	= 459.58	T5	= 470.67
		T6	= 463.00								
3	LEVELS-	(2 200)	(1 NO)								
	N = 23.										
	MEANS	T1	= 659.91	T2	= 629.00	T3	= 651.74	T4	= 627.65	T5	= 628.13
		T6	= 575.78								
4	LEVELS-	(2 200)	(2 YES)								
	N = 11.										
	MEANS	T1	= 431.18	T2	= 457.55	T3	= 480.36	T4	= 489.91	T5	= 483.45
		T6	= 529.18								
5	LEVELS-	(3 700)	(1 NO)								
	N = 19.										
	MEANS	T1	= 585.32	T2	= 564.26	T3	= 350.58	T4	= 114.21	T5	= 61.316
		T6	= 40.000								
6	LEVELS-	(3 700)	(2 YES)								
	N = 10.										
	MEANS	T1	= 464.40	T2	= 372.90	T3	= 147.00	T4	= 59.300	T5	= 2.3000
		T6	= 0.70000								
7	LEVELS-	(4 1250)	(1 NO)								
	N = 21.										
	MEANS	T1	= 337.62	T2	= 2.9048	T3	= 0.33333	T4	= 0.0	T5	= 0.95238D-01
		T6	= 0.0								
8	LEVELS-	(4 1250)	(2 YES)								
	N = 10.										
	MEANS	T1	= 12.000	T2	= 0.20000	T3	= 0.100000 00	T4	= 0.30000	T5	= 0.100000 00
		T6	= 0.40000								

1 - 0 ppm
2 - 0 ppm + swim stress
3 - 200 ppm CO
4 - 200 ppm CO + swim stress
5 - 700 ppm CO
6 - 700 ppm CO + swim stress
7 - 1250 ppm CO
8 - 1250 ppm CO + swim stress

T1 - T6 are successive 10 min intervals of the performance session beginning with the 10 min interval ending 25 min after the start of exposure. Values shown are means for the intervals.

Table J5

Effects of CO and CO + Swim Stress On the Total Responses On Lever 2 (For Food Presentation) During 10-minute Intervals

FACTORS- 1 (DOSE) 3 (SWIM)

1	LEVELS- (1 0000) (1 NO) N = 23.										
	MEANS	T1	= 653.09	T2	= 700.00	T3	= 677.82	T4	= 641.13	T5	= 625.96
		T6	= 591.52								
2	LEVELS- (1 0000) (2 YES) N = 12.										
	MEANS	T1	= 332.08	T2	= 338.25	T3	= 421.42	T4	= 447.50	T5	= 505.92
		T6	= 481.25								
3	LEVELS- (2 200) (1 NO) N = 23.										
	MEANS	T1	= 625.91	T2	= 680.65	T3	= 668.43	T4	= 629.74	T5	= 607.35
		T6	= 547.87								
4	LEVELS- (2 200) (2 YES) N = 11.										
	MEANS	T1	= 237.55	T2	= 323.00	T3	= 379.55	T4	= 446.18	T5	= 494.09
		T6	= 460.55								
5	LEVELS- (3 700) (1 NO) N = 19.										
	MEANS	T1	= 605.58	T2	= 560.26	T3	= 326.32	T4	= 100.21	T5	= 31.263
		T6	= 23.579								
6	LEVELS- (3 700) (2 YES) N = 10.										
	MEANS	T1	= 281.00	T2	= 263.60	T3	= 104.30	T4	= 21.100	T5	= 1.6000
		T6	= 1.5000								
7	LEVELS- (4 1250) (1 NO) N = 21.										
	MEANS	T1	= 292.19	T2	= 1.5238	T3	= 0.47619	T4	= 0.0	T5	= 0.95238D-01
		T6	= 0.19048								
8	LEVELS- (4 1250) (2 YES) N = 10.										
	MEANS	T1	= 6.9000	T2	= 0.100000 00	T3	= 0.100000 00	T4	= 0.80000	T5	= 0.30000
		T6	= 0.30000								

1 - 0 ppm
2 - 0 ppm + swim stress
3 - 200 ppm Co
4 - 200 ppm CO + swim stress
5 - 700 ppm CO
6 - 700 ppm CO + swim stress
7 - 1250 ppm CO
8 - 1250 ppm CO + swim stress

T1 - T6 are successive 10 min intervals of the performance session beginning with the 10 min interval ending 25 min after the start of exposure. Values shown are means for the intervals.

Table J6

Effects of CO and CO + Swim Stress on the Number of Reinforcers Obtained During 10-minute Intervals

FACTORS-
1 (DOSE) 3 (SWIM)

1 LEVELS- (1 0000) (1 NO)
N = 23.

MEANS											
	T1	= 17.217	T2	= 19.435	T3	= 19.217	T4	= 18.043	T5	= 17.913	
	T6	= 16.696									

2 LEVELS- (1 0000) (2 YES)
N = 12.

MEANS											
	T1	= 6.9167	T2	= 8.9167	T3	= 10.250	T4	= 11.333	T5	= 12.333	
	T6	= 12.083									

3 LEVELS- (2 200) (1 NO)
N = 23.

MEANS											
	T1	= 15.652	T2	= 18.391	T3	= 18.478	T4	= 17.696	T5	= 16.870	
	T6	= 15.565									

4 LEVELS- (2 200) (2 YES)
N = 11.

MEANS											
	T1	= 7.0000	T2	= 9.6364	T3	= 11.636	T4	= 12.818	T5	= 13.182	
	T6	= 13.273									

5 LEVELS- (3 700) (1 NO)
N = 19.

MEANS											
	T1	= 15.579	T2	= 15.474	T3	= 9.5263	T4	= 2.8947	T5	= 0.94737	
	T6	= 0.68421									

6 LEVELS- (3 700) (2 YES)
N = 10.

MEANS											
	T1	= 7.7000	T2	= 7.7000	T3	= 3.2000	T4	= 0.80000	T5	= 0.0	
	T6	= 0.0									

7 LEVELS- (4 1250) (1 NO)
N = 21.

MEANS											
	T1	= 7.9524	T2	= 0.476190-01	T3	= 0.0	T4	= 0.0	T5	= 0.0	
	T6	= 0.0									

8 LEVELS- (4 1250) (2 YES)
N = 10.

MEANS											
	T1	= 0.100000 00	T2	= 0.0	T3	= 0.0	T4	= 0.0	T5	= 0.0	
	T6	= 0.0									

1 - 0 ppm
2 - 0 ppm + swim stress
3 - 200 ppm CO
4 - 200 ppm CO + swim stress
5 - 700 ppm CO
6 - 700 ppm CO + swim stress
7 - 1250 ppm CO
8 - 1250 ppm CO + swim stress

T1 - T6 are successive 10 min intervals of the performance session beginning with the 10 min interval ending 25 min after the start of exposure. Values shown are means for the intervals.

APPENDIX K

BASELINE PERFORMANCE FOR FR30-FR30 PERFORMANCE PRIOR TO HEAT STRESS

TABLE K1

BASELINE PERFORMANCE FOR FR30 FR30 SCHEDULE PRIOR TO HEAT STRESS*

Exposure Group	Week 1 Mean ± S.E.M.	Week 1 Range	Week 2 Mean ± S.E.M.	Week 2 Range
Responses on Lever for Light Presentation				
0 ppm	4356 ± 401	2264 - 6737	4426 ± 378	2384 - 6549
200 ppm	4379 ± 488	2591 - 9256	4494 ± 515	2835 - 9644
450 ppm	4687 ± 481	2779 - 8332	4522 ± 410	2794 - 7398
700 ppm	4141 ± 301	2272 - 5777	4075 ± 313	2265 - 5621
Responses on Lever for Food Presentation				
0 ppm	4283 ± 345	2410 - 6190	4374 ± 314	2485 - 6030
200 ppm	4808 ± 461	3242 - 9476	4696 ± 451	3002 - 8980
450 ppm	5055 ± 491	3018 - 9226	4768 ± 454	2945 - 8860
700 ppm	4347 ± 351	2488 - 5985	4276 ± 391	2173 - 6070
Number of Reinforcers				
0 ppm	123 ± 10	73 - 187	125 ± 9	75 - 181
200 ppm	132 ± 13	79 - 257	134 ± 14	83 - 260
450 ppm	136 ± 14	80 - 271	132 ± 12	85 - 244
700 ppm	120 ± 10	60 - 165	119 ± 11	64 - 171

* Data from the three days prior to exposure.

APPENDIX L

TABULAR SUMMARY FOR FR30-FR30 SCHEDULE OF REINFORCEMENT AFTER EXPOSURE TO CO AND/OR HEAT STRESS

TABLE L1

EFFECTS OF CARBON MONOXIDE AND HEAT STRESS ON PERFORMANCE ON A CHAIN FR30-FR30 SCHEDULE EXPRESSED AS A PERCENT OF BASELINE

Responses on Lever for Light Presentation

	Mean ± S.E.M.	Mean ± S.E.M.
0 ppm	106 ± 5	96 ± 3
0 ppm + Heat	47 ± 9	63 ± 5
200 ppm	100 ± 5	103 ± 2
200 ppm + Heat	52 ± 10	54 ± 9
450 ppm	96 ± 2	103 ± 6
450 ppm + Heat	35 ± 3	51 ± 3
700 ppm	38 ± 5	51 ± 4
700 ppm + Heat	23 ± 3	33 ± 4

Responses on Lever for Food Presentation

	Mean ± S.E.M.	Mean ± S.E.M.
0 ppm	105 ± 5	99 ± 3
0 ppm + Heat	48 ± 8	67 ± 6
200 ppm	98 ± 6	104 ± 2
200 ppm + Heat	54 ± 10	62 ± 8
450 ppm	92 ± 2	101 ± 6
450 ppm + Heat	40 ± 2	50 ± 2
700 ppm	42 ± 6	54 ± 4
700 ppm + Heat	27 ± 4	33 ± 4

Number of Reinforcers

	Mean ± S.E.M.	Mean ± S.E.M.
0 ppm	105 ± 4	98 ± 3
0 ppm + Heat	46 ± 9	66 ± 5
200 ppm	99 ± 4	104 ± 2
200 ppm + Heat	53 ± 10	55 ± 9
450 ppm	94 ± 2	98 ± 3
450 ppm + Heat	35 ± 3	51 ± 3
700 ppm	39 ± 5	52 ± 3
700 ppm + Heat	23 ± 3	33 ± 4

Baseline is the mean of the three days prior to exposure.

TABLE L2

MEAN NUMBER OF RESPONSES AND REINFORCERS ON AN FR30-FR30 SCHEDULE OF REINFORCEMENT AFTER EXPOSURE TO CO AND/OR HEAT STRESS

Responses on Lever for Light Presentation

	Mean ± S.E.M.	Mean ± S.E.M.
0 ppm	4293 ± 511	4441 ± 660
0 ppm + Heat	2036 ± 401	2705 ± 317
200 ppm	3668 ± 324	5358 ± 982
200 ppm + Heat	2421 ± 407	2122 ± 442
450 ppm	4329 ± 526	4684 ± 584
450 ppm + Heat	1665 ± 270	2191 ± 170
700 ppm	1683 ± 246	1899 ± 202
700 ppm + Heat	844 ± 119	1445 ± 203

Responses on Lever for Food Presentation

	Mean ± S.E.M.	Mean ± S.E.M.
0 ppm	3945 ± 280	4607 ± 562
0 ppm + Heat	2187 ± 456	2767 ± 356
200 ppm	4293 ± 557	5277 ± 777
200 ppm + Heat	2650 ± 416	2681 ± 452
450 ppm	4282 ± 299	4883 ± 733
450 ppm + Heat	2147 ± 394	2242 ± 123
700 ppm	2019 ± 309	1900 ± 234
700 ppm + Heat	997 ± 159	1630 ± 252

Number of Reinforcers

	Mean ± S.E.M.	Mean ± S.E.M.
0 ppm	117 ± 9	130 ± 19
0 ppm + Heat	58 ± 13	78 ± 8
200 ppm	111 ± 10	157 ± 22
200 ppm + Heat	76 ± 12	66 ± 14
450 ppm	127 ± 9	128 ± 22
450 ppm + Heat	47 ± 8	66 ± 4
700 ppm	52 ± 8	52 ± 3
700 ppm + Heat	23 ± 4	45 ± 6

APPENDIX M

TABULAR SUMMARY FOR NUMBER OF REINFORCERS OBTAINED AND NUMBER OF TIME OUTS DURING REACTION TIME TESTING FOLLOWING CO AND/OR HEAT STRESS

Table M1

INDIVIDUAL ANIMALS' DATA FOR REINFORCERS OBTAINED ON REACTION TIME TASK

Animal No.	Mean of 3 Days Pre-Treatment	Treatment	Treatment as % Baseline	Means of 3 Days Pre-Treatment	Treatment	Treatment % of Baseline
		0 ppm			0 ppm and Heat	
529	171	164	96	161	144	89
538	12	11	92	9	23	256
537	88	67	76	60	82	137
491	35	27	77	37	39	105
513	55	64	116	57	107	188
506	96	88	92	107	157	147
			91.5			153.7
			± 6.0			± 24.8
		450 ppm			450 ppm and Heat	
516	20	17	85	22	30	136
534	26	58	223	29	62	214
548	105	109	104	56	93	166
505	67	62	92	30	16	53
542	102	97	95	132	96	73
490	145	103	71	178	84	47
			111.7			114.8
			± 22.7			± 27.7
		700 ppm			700 ppm and Heat	
535	85	40	47	91	23	25
527	70	28	40	70	50	71
507	112	40	36	70	68	97
533	92	47	51	89	67	75
500	26	14	54	22	20	91
544	118	91	77	178	125	70
			50.8			71.5
			± 5.9			± 10.3

Table M2

INDIVIDUAL ANIMALS' DATA FOR TIME OUTS ON THE REACTION TIME TASK

Animal No.	Mean of 3 Days Pre-Treatment	Treatment	Treatment as % Baseline	Means of 3 Days Pre-Treatment	Treatment	Treatment % of Baseline
		0 ppm			0 ppm and Heat	
529	19	20	105	20	24	120
538	36	39	108	37	37	100
537	30	33	110	34	32	94
491	35	37	106	35	35	100
513	33	31	94	33	27	82
506	30	30	100	28	23	82
			103.8 ± 2.4			96.3 ± 5.8
		450 ppm			450 ppm and Heat	
516	37	37	100	37	35	95
534	36	29	81	35	32	91
548	24	27	112	34	29	85
505	32	32	100	35	37	106
542	27	26	96	23	14	61
490	21	21	100	17	10	59
			98.2 ± 4.1			82.8 ± 7.7
		700 ppm			700 ppm and Heat	
535	29	9	31	28	9	32
527	32	12	38	33	11	33
507	28	27	96	33	33	100
533	30	21	70	30	16	53
500	38	24	63	39	25	64
544	25	15	60	18	12	67
			59.7 ± 9.5			58.2 ± 10.3

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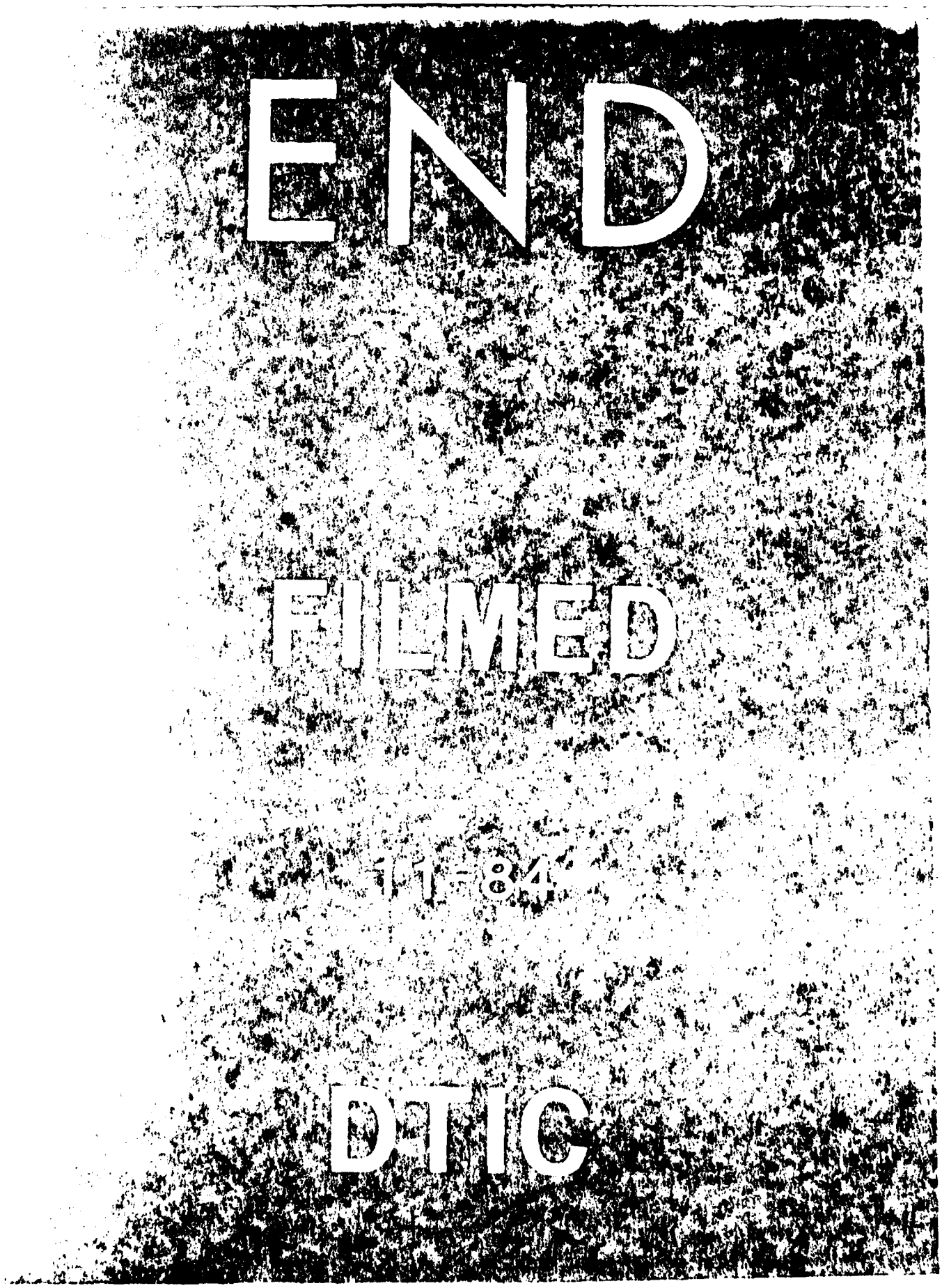
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