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A Research Study of the Assessment of Escape Impairment by Irritant Combustion Gases in Postcrash Aircraft Fires

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16. Abstract

The primary objective of this research program was to assess the potential of representative combustion gases to impair human escape from a postcrash aircraft fire environment. A non-human primate model (juvenile savannah baboon) and an operant behavioral task were used to measure the individual effects of hydrogenchloride (HCL), carbonmonoxide (CO) and acrolein. A secondary objective was to evaluate the validity of laboratory tests with rodents to predict human escape impairment by combustion gases.

For HCl, despite severe irritant effects, all baboons were able to perform the escape task over the range of concentrations studied (190 to 17,290 ppm). Significant post-exposure effects were not observed at concentrations from 190 to 11,400 ppm; however, at the two highest concentrations, 16,570 and 17,290 ppm, the animals died 18 and 76 days past exposure, respectively. For CO, it was determined that a concentration of 6850 ppm caused escape impairment of the baboon for a five-minute exposure. The results of CO exposure with the rat and the baboon were remarkably similar. Although less definitive, the data suggest that laboratory tests with rodents may have usefulness in predicting the efffects of HCL atmospheres on human escape impairment.

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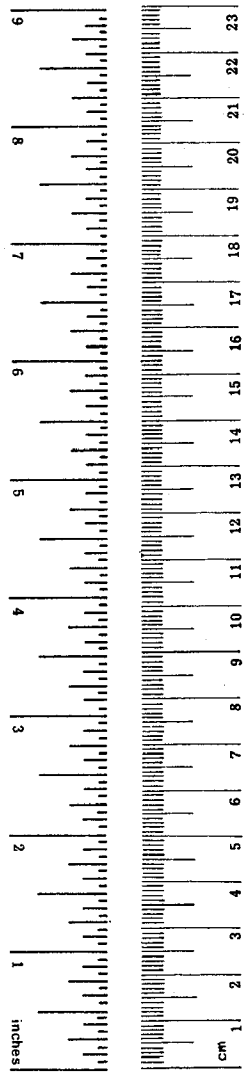
METRIC CONVERSION FACTORS

Approximate Conversions to Metric Measures

Symbol	When You Know	Multiply by	To Find	Symbol
	LENGTH			
in	inches	*2.5	centimeters	cm
ft	feet	30	centimeters	cm
yd	yards	0.9	meters	m
mi	miles	1.6	kilometers	km
	AREA			
in^2	square inches	6.5	square centimeters	cm^2
ft^2	square feet	0.09	square meters	m^2
yd^2	square yards	0.8	square meters	m^2
mi^2	square miles	2.6	square kilometers	km^2
	acres	0.4	hectares	ha
	MASS (weight)			
oz	ounces	28	grams	g
lb	pounds	0.45	kilograms	kg
	short tons (2000 lb)	0.9	tonnes	t
	VOLUME			
tsp	teaspoons	5	milliliters	ml
Tbsp	tablespoons	15	milliliters	ml
fl oz	fluid ounces	30	milliliters	ml
c	cups	0.24	liters	l
pt	pints	0.47	liters	l
qt	quarts	0.95	liters	l
gal	gallons	3.8	liters	l
ft^3	cubic feet	0.03	cubic meters	m^3
yd^3	cubic yards	0.76	cubic meters	m^3
	TEMPERATURE (exact)			
°F	Fahrenheit temperature	5/9 (after subtracting 32)	Celsius temperature	°C

*1 in = 2.54 (exactly). For other exact conversions and more detailed tables, see NBS Misc. Publ. 286, Units of Weights and Measures, Price $2.25, SD Catalog No. C13.10:286.



Approximate Conversions from Metric Measures

Symbol	When You Know	Multiply by	To Find	Symbol
	LENGTH			
mm	millimeters	0.04	inches	in
cm	centimeters	0.4	inches	in
m	meters	3.3	feet	ft
m	meters	1.1	yards	yd
km	kilometers	0.6	miles	mi
	AREA			
cm^2	square centimeters	0.16	square inches	in^2
m^2	square meters	1.2	square yards	yd^2
km^2	square kilometers	0.4	square miles	mi^2
ha	hectares (10,000 m^2)	2.5	acres	
	MASS (weight)			
g	grams	0.035	ounces	oz
kg	kilograms	2.2	pounds	lb
t	tonnes (1000 kg)	1.1	short tons	
	VOLUME			
ml	milliliters	0.03	fluid ounces	fl oz
l	liters	2.1	pints	pt
l	liters	1.06	quarts	qt
l	liters	0.26	gallons	gal
m^3	cubic meters	35	cubic feet	ft^3
m^3	cubic meters	1.3	cubic yards	yd^3
	TEMPERATURE (exact)			
°C	Celsius temperature	9/5 (then add 32)	Fahrenheit temperature	°F

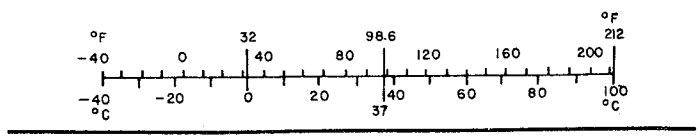


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TABLE OF CONTENTS
(Continued)

TABLE OF CONTENTS
(Continued)

LIST OF ILLUSTRATIONS

LIST OF ILLUSTRATIONS
(Continued)

LIST OF TABLES

LIST OF TABLES
(Continued)

EXECUTIVE SUMMARY

For passengers to survive a postcrash aircraft fire, their escape capability must not be severely impaired by toxic combustion gases during the few minutes available for egress. Little information exists regarding the concentrations at which human escape capability is impaired by these combustion gases, particularly the irritants. Most laboratory test methods have used loss of locomotor function or incapacitation of rodents as endpoints to measure the sublethal effects of the narcotic gases. In addition, respiratory rate depression of mice has been used as an index of incapacitation in studies of the irritant gases. However, the correlation between any of these effects in rodents and escape impairment of humans has not been established.

The primary objective of this research program was to assess the potential of representative combustion atmosphere irritant gases to impair human escape from a postcrash aircraft fire environment. For this assessment, a nonhuman primate model (the juvenile African savannah baboon) and an operant behavioral task were used to measure the effects of acrolein and hydrogen chloride (HCl) on escape performance. Carbon monoxide (CO) was also included as a test gas in order to enable comparison of the sensitivities of the baboon and the rat to a narcotic combustion gas. The behavioral task was designed to simulate human escape performance by requiring both mental functions (sensory recognition, memory, discrimination) and motor functions (locomotion, coordination) for successful escape of the animal. The animal was required, upon presentation of audio and visual cues, to select and operate an appropriate lever in order to open an escape door and then to exit through the door within a prescribed time. The escape performance task was presented to the animal after a 5-minute exposure to a steady concentration of the test gas as provided by a large volume recirculating mixing chamber. A computer system was used to train the animals to perform the task, to present the task for the exposure test and to record pertinent performance data, e.g., escape or failure, lever response time, escape time, number of correct and incorrect responses.

A secondary objective of this program was to evaluate the relevance and validity of presently used laboratory tests with rodents to predict human escape impairment by narcotic and irritant combustion gases. For this assessment, the effects of CO and HCl on the escape performance of the rat were determined using a shuttlebox and behavioral task similar in complexity to that used in the nonhuman primate studies. The results of these studies were compared with the primate escape performance data and human data (when available) to evaluate the rat as a model for the baboon and man. These results were also compared with available data obtained with other laboratory test methods with rodents to assess the predictive capability of these methods for human escape impairment.

In studies of the effects of CO, the gas concentration causing escape impairment for the baboon was determined to be 6850 parts per million (ppm) and, for the rat, 6780 ppm. The remarkable similarity in results for the two species provides substantial evidence that the escape performance of the baboon and of the rat is prevented by approximately the same dose of CO. Available human CO toxicity data are sufficiently close to these values to support the validity of the rat as a model for predicting human escape impair-

ment by CO. Also, comparable results have been obtained with rodents by a number of other laboratories. Therefore, the data indicate that laboratory test methods with rodents are reasonably capable of predicting the effects of CO atmospheres on human escape capability.

The effects of acrolein on the escape performance of the baboon were investigated at concentrations ranging from 12 to 2780 ppm. All of the animals were able to perform the escape task; however, the severe irritant effects of the gas were evident during exposure at all concentrations, with the two highest concentrations (1025 and 2780 ppm) resulting in severe pulmonary edema and lethality of the subjects at 24 and 1.5 hours, respectively, after exposure. Thus, the results demonstrated that 5-minute exposures to acrolein did not impair the escape capability of baboons, even at concentrations that caused severe pulmonary damage and post-exposure death. The effects of acrolein on the escape performance of rats were not investigated in this program; however, unpublished experimental data obtained from the FAA/Civil Aeromedical Institute indicate that 5-minute exposures to approximately 5,000 to 10,000 ppm of acrolein are required to incapacitate the rat and that these exposures are also within the lethal range. Baboons were not exposed to these high concentrations in order to minimize lethalities of these animals. The results with baboons are not inconsistent with these rat data and suggest that laboratory tests with rodents may be used to predict escape capability of humans exposed to acrolein.

The results of escape impairment tests in baboons with HCl were similar to those obtained with acrolein in that, despite severe irritant effects, all subjects were able to perform the escape task over the range of concentrations studied (190 to 17,290 ppm), even when exposed to the two highest concentrations (16,570 and 17,290 ppm) of the gas. Significant post-exposure effects were not observed at concentrations from 190 to 11,400 ppm, except for persistent slightly raspy breathing in the animal exposed to the latter concentration. In the animals exposed to the two highest concentrations, severe dyspnea was evident following exposure, with deaths of the animals occurring at 18 and 76 days post exposure. Thus, the data indicate that the threshold concentration of HCl (5-minute exposure) for severe post-exposure respiratory effects and lethality in the baboon is in the range between 11,400 and 16,570 ppm, although the escape capability of the animal was not impaired by exposure to as high as 17,290 ppm of the gas.

The results of escape performance tests with HCl in rats indicated that all of the rats were able to perform the escape task at the concentrations tested (11,800 to 87,660 ppm), except for the highest concentration which resulted in death of the animal during exposure. However, all exposures produced signs of severe irritation and, except for the two lowest concentrations (11,800 and 14,410 ppm), persistent respiratory effects and post-exposure lethality. Therefore, these data suggest that laboratory test methods with rodents may have utility in predicting the effects of HCl atmospheres on the escape performance capability of humans.

INTRODUCTION

TOXIC GASES IN AIRCRAFT FIRES

During the fifteen-year period from 1965 to 1979, there were 831 fatalities in U.S. carrier, turbine-powered aircraft accidents involving fire (reference 1). These accidents were impact-survivable crashes that were followed by fire. Of the 831 fatalities, approximately 480 were attributed to the effects of the fires. These effects are often classified as thermal (direct burns, hyperthermia), chemical (toxic gases, oxygen deficiency, smoke obscuration) or extrinsic (panic, stress, accidents) (reference 2). It is now recognized that the cause of death in most fires, whether in residences, commercial buildings or survivable aircraft accidents, is the inhalation of toxic gases (reference 3).

All commercial aircraft contain a wide variety of organic and polymeric materials that are capable of undergoing thermal decomposition and flaming combustion. These include foam seat cushions, upholstery, carpets, blankets, structural and decorative molded plastics, paneling and interior finishing, luggage, natural and synthetic clothing materials, and various other materials. Although most cabin interior materials are fire-resistant when exposed to a small ignition source, they do undergo thermal decomposition and burn upon exposure to a major fire.

Whenever materials are combusted, toxic airborne products are generated, the most prevalent of which is carbon monoxide (CO). In modern aircraft, high performance materials are extensively utilized to meet flammability performance requirements. Many of these materials have the chemical potential of generating a spectrum of other toxic decomposition products. Among these additional toxicants are hydrogen cyanide (HCN), halogen acids, nitrogen oxides and a wide variety of organic chemicals which may produce effects on sensory organs as well as on the respiratory and central nervous systems and other systems of the body.

SURVIVABILITY IN POSTCRASH AIRCRAFT FIRES

The key to survival in any fire is escape from the fire environment. In a postcrash aircraft fire, escape usually cannot be delayed because of the rapid development of the fire. Assuming sufficient time, unimpaired faculties and the absence of physical restraints, such as locked doors or blocked escape routes, escape should be possible. However, fire presents a combination of time-dependent threats to life because of a variety of physiological and behavioral effects resulting from the inhalation of heated air and fire gases. Restricted vision, disorientation, faulty judgement, loss of motor coordination, panic and physical incapacitation may occur. Delay or impairment of escape by any of these effects may lead to subsequent injury or death from further inhalation of toxic gases, the suffering of thermal burns and/or other possible factors.

Our current knowledge of the escape-impairing effects of combustion gases in humans is very limited. In studies of the hypoxia-producing toxicants (i.e., CO and HCN), laboratory test methods generally have utilized loss of gross locomotor function (i.e., incapacitation) of rodents as the end-point to measure the sublethal effects of the gases (references 3, 4, 5). Although the

mechanisms of action of both CO and HCN in the rodent and man appear comparable, the correlation between the incapacitation of rodents and impairment of escape in humans has not been established.

The role of sensory and pulmonary irritants in causing incapacitation has also been investigated primarily with rodents. These studies have shown that exposure of rodents to sensory irritants results in a reflex inhibition of respiratory rate whereas a temporary increase in respiratory rate occurs upon exposure to pulmonary irritants (references 6, 7). However, the relevance of these respiratory effects in the rodent to human escape impairment also has not been investigated.

In view of the potential for many aircraft interior materials to rapidly generate high concentrations of CO, HCN and various irritant gases in fires, experiments are needed to determine the concentrations of these gases at which human escape impairment is compromised. Only when this information is available will it be possible to assess the toxicological hazards of these materials in postcrash aircraft fires.

OBJECTIVES

This research program was designed to accomplish two major objectives. The primary objective was to use a nonhuman primate model to assess the potential of representative combustion atmosphere irritant gases to impair human escape from a postcrash aircraft fire environment. For this assessment, studies were conducted using a relevant behavioral task that simulates human escape performance. The secondary objective was to evaluate the relevance and validity of presently used laboratory test methods with rodents in predicting the potential of combustion gases to impair human escape capability. The effects of representative combustion gases were determined in the rodent using a behavioral task similar in complexity to that used in the nonhuman primate studies. These data, and available experimental data with other laboratory test methods, were compared with the primate escape impairment data in order to evaluate the predictive capability of these test methods.

TECHNICAL APPROACH

ASSESSMENT OF ESCAPE IMPAIRMENT BY IRRITANT COMBUSTION GASES IN PRIMATES

NONHUMAN PRIMATE ESCAPE IMPAIRMENT MODEL. The first phase of a two-phase experimental program was designed to assess the potential of representative combustion atmosphere irritant gases to impair human escape from a postcrash aircraft fire environment. For this assessment, a nonhuman primate model and an operant behavioral task were used to measure the effects of these gases on escape performance. The juvenile African savannah baboon (superspecies Papio cynocephalus) was selected as the animal model for a number of reasons. This animal is widely recognized as a surrogate of the human in biomedical research, and it is an animal of choice in inhalation toxicology studies because its respiratory system is similar to that of man. In addition, the juvenile baboon is a hardy animal and less likely than other laboratory primate species to develop respiratory complications after exposure to irritant gases. Another significant advantage of this animal is its ability to rapidly learn complex behavioral tasks that measure various sensory and cognitive functions.

An operant behavioral task was developed and test chambers were constructed in order to measure escape performance of the juvenile baboon. The behavioral paradigm was designed to simulate human escape performance by requiring both mental functions (sensory recognition, memory and discrimination) and motor functions (locomotion, coordination) for successful completion of the task. The animal was required, upon presentation of audio and visual cues, to select and operate an appropriate lever, which opened a door, and then to exit through the door within a prescribed time. A computer system was used to train animals to perform the behavioral task, to present the task for the exposure test, and to record pertinent performance data, e.g., response time, escape time, number of correct and incorrect responses.

GAS GENERATION SYSTEM. Escape performance of the nonhuman primates was measured after exposure of the animals to the test gas for a fixed period of time. In order to determine concentration-response relationships, it was advantageous to maintain a uniform test gas concentration during exposure and to avoid marked concentration gradients. Therefore, a large volume mixing chamber which continuously recirculated the test gas through the animal exposure chamber was selected in preference to a flow-through system. The latter type of system requires precise control and rapid mixing of the component gases and is more apt to result in transient exposures of animals to the test gas at higher than desirable concentrations. Other advantages of the large volume mixing chamber generation system are relatively easy and safe disposal of the highly toxic test gases and less consumption of these gases.

TEST GASES AND EXPOSURES. Three test gases, carbon monoxide (CO), acrolein (CH_2CHCHO) and hydrogen chloride (HCl) were selected for the program. Although the primary emphasis of the program was on the effects of irritant combustion gases on escape capability, CO was studied because it is the most prevalent combustion gas. Also, the effects of CO on escape performance of a nonhuman primate had to be established in order to evaluate the relevance and validity of presently used laboratory test methods with rodents in the second phase of the program. Acrolein is evolved during the thermal decomposition of cellulosic materials. This gas is a highly potent irritant even at low concentrations; it can act as a sensory irritant, pulmonary irritant and/or bronchoconstrictor. Hydrogen chloride, which is generated during thermal decomposition of polyvinyl chloride and other chlorinated polymers, has become of major concern in fires involving these materials. This gas is a potent sensory irritant, causing a reflex inhibition of respiration at low concentrations which are claimed to be incapacitating to humans within a few minutes (references 8, 9, 10, 11).

With CO, the nonhuman primate subjects were exposed to several concentrations of the gas to enable derivation of a dose-response relationship and EC_{50} value for escape impairment. With acrolein and HCl, however, the occurrence of post-exposure respiratory effects and lethalities precluded multiple exposures of subjects, except at the lower concentrations.

In each experiment, the escape performance of the animal was measured after a 5-minute exposure to a test gas. The selection of a 5-minute exposure time was based on an estimated upper limit for the time available to escape from a rapidly developing postcrash aircraft fire. In this situation, ignition of aircraft fuel could quickly cause combustion of aircraft interior materials and evolution of high concentrations of irritant and other combustion gases.

In order to survive, the escape capability of passengers must not be severely impaired by exposure to these gases prior to the stage where heat and flames become the critical factors.

EVALUATION OF RELEVANCE AND VALIDITY OF LABORATORY TEST METHODS WITH RODENTS

COMPARISON OF EFFECTS OF COMBUSTION GASES ON ESCAPE PERFORMANCE OF NONHUMAN PRIMATES AND RATS. The objective of the second phase of the program was to evaluate the validity and relevance of presently-used laboratory methods with rodents to predict the potential of combustion gases to impair human escape capability. To accomplish this objective, it was first necessary to compare the effects of both a narcotic and an irritant combustion gas on escape capability of the rodent and the nonhuman primate. To enable this comparison, an operant behavioral task of complexity similar to that used with the nonhuman primate was developed for tests with the rat. This task required the pressing of a lever by the animal upon presentation of audio and visual cues. Pressing of the lever opened a door of a modified shuttlebox, and the animal was required to exit through the door into an adjacent cage within a prescribed time in order to successfully perform the escape task.

The behavioral task for measurement of escape capability was presented to each rat after a 5-minute exposure to the test gas. Two test gases, CO and HCl, were investigated. With CO, the animals were exposed to a number of concentrations to enable derivation of a dose-response relationship and EC_{50} value for escape impairment. However, with HCl, each animal was exposed to only one concentration of the gas because most of the concentrations tested caused post-exposure respiratory effects and death. The data obtained with rats were compared with the baboon data to evaluate the sensitivity of the rodent in predicting human escape impairment by narcotic and irritant combustion gases, under the assumption that this nonhuman primate species is a valid surrogate of man.

EVALUATION OF LABORATORY TEST METHODS. Laboratory toxicity tests have used a variety of behavioral and physiological methods to measure the incapacitating effects of combustion gases in rodents. Considerable data relating CO concentrations and exposure times to the incapacitation of rodents are available in the literature; comparable data for acrolein or HCl are limited or unavailable. The scientific literature was reviewed and relevant data were compiled. These data, as well as unpublished experimental data obtained from various investigators, were compared with data for nonhuman primate escape impairment from this program in order to evaluate the relevance and validity of presently used laboratory test methods with rodents.

METHODOLOGY

ESCAPE PERFORMANCE TESTS WITH NONHUMAN PRIMATES

TEST FACILITY. The test facility for the primate studies was located on the first floor of Building No. 143 of the Department of Fire Technology. The facility consisted of three rooms: (1) the test room in which the gas mixing/exposure apparatus and escape performance test system were located; (2) the analytical laboratory in which the gas generation and monitoring equipment were located; and (3) the computer room which housed the computer system for control of the behavioral experiments and recording of data. Both

the analytical laboratory and the computer room were adjacent to the test room, which facilitated the installation of the necessary cables and gas lines between the three rooms.

The test room was a 20 x 16 x 11 ft high room with a concrete floor and painted gypsum walls; two personnel doors and one roll-up door provided access. A diagram of the room with the gas mixing/exposure apparatus, associated ducting and valves and the escape performance test system is shown in Figure 1.

GAS MIXING/EXPOSURE SYSTEM. An artist's drawing of the gas mixing/exposure system with the escape performance test apparatus is shown in Figure 2. The test system consisted of three elements: (1) The gas mixing chamber; (2) The gas bypass loop; and (3) The primate escape performance exposure chambers. The purpose of the system was to allow the recirculation of a premixed test gas atmosphere either through a gas bypass loop or the animal exposure chamber.

Gas Mixing Chamber. The gas mixing chamber was a cylinder, with a diameter of 5 ft and a height of 8 ft (approximately 157 ft^3), constructed of glass-reinforced fiberglass with an interior and exterior coating of acid-resistant, epoxy paint. Four ducts were connected to the chamber:

(1) a gas mixing outlet (to conduct test gas to the gas bypass loop or animal exposure chamber);

(2) gas mixing inlet (to return test gas to the mixing chamber from the gas bypass loop or the animal exposure chamber);

(3) a chamber exhaust (to exhaust test gas from the mixing chamber to the outside); and

(4) a fresh air inlet (to allow fresh air into the mixing chamber).

The ducts also were constructed of 1/2-in. thick, glass-reinforced fiberglass, coated on the interior and exterior surfaces with acid-resistant epoxy paint. The in-line valves within the ducts which controlled flow through the ducts were constructed of 1/2-in. thick polymethylmethacrylate coupled with a positive lock mechanism. Seal-seating material for the valves consisted of 3/4-in. rubber tubing. The pump for recirculating the test gas atmosphere through the system was an in-line, 18-in. diameter Speedline air-operated fan installed in the gas mixing inlet duct.

Gas Bypass Loop. The gas bypass loop served as part of the gas recirculation system until the test gas/air exposure atmosphere was routed to the escape performance exposure chambers. The loop consisted of a chamber and two 18-in. square ducts, and was approximately 42 ft^3 in volume. The gas bypass loop was also constructed of 1/2-in. thick, glass-reinforced fiberglass coated with acid-resistant epoxy paint.

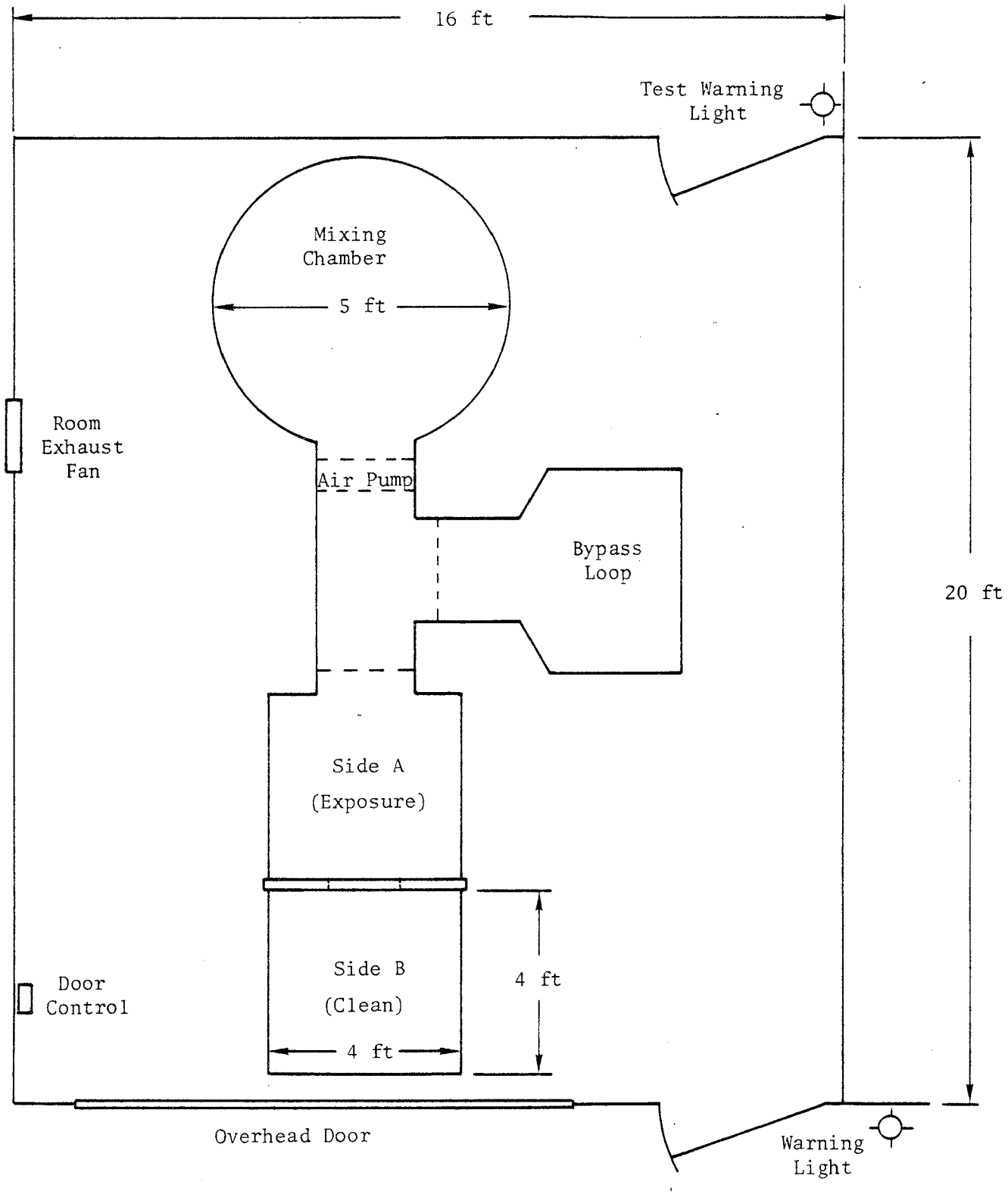

FIGURE 1. TEST ROOM FOR PRIMATE ESCAPE PERFORMANCE TESTS

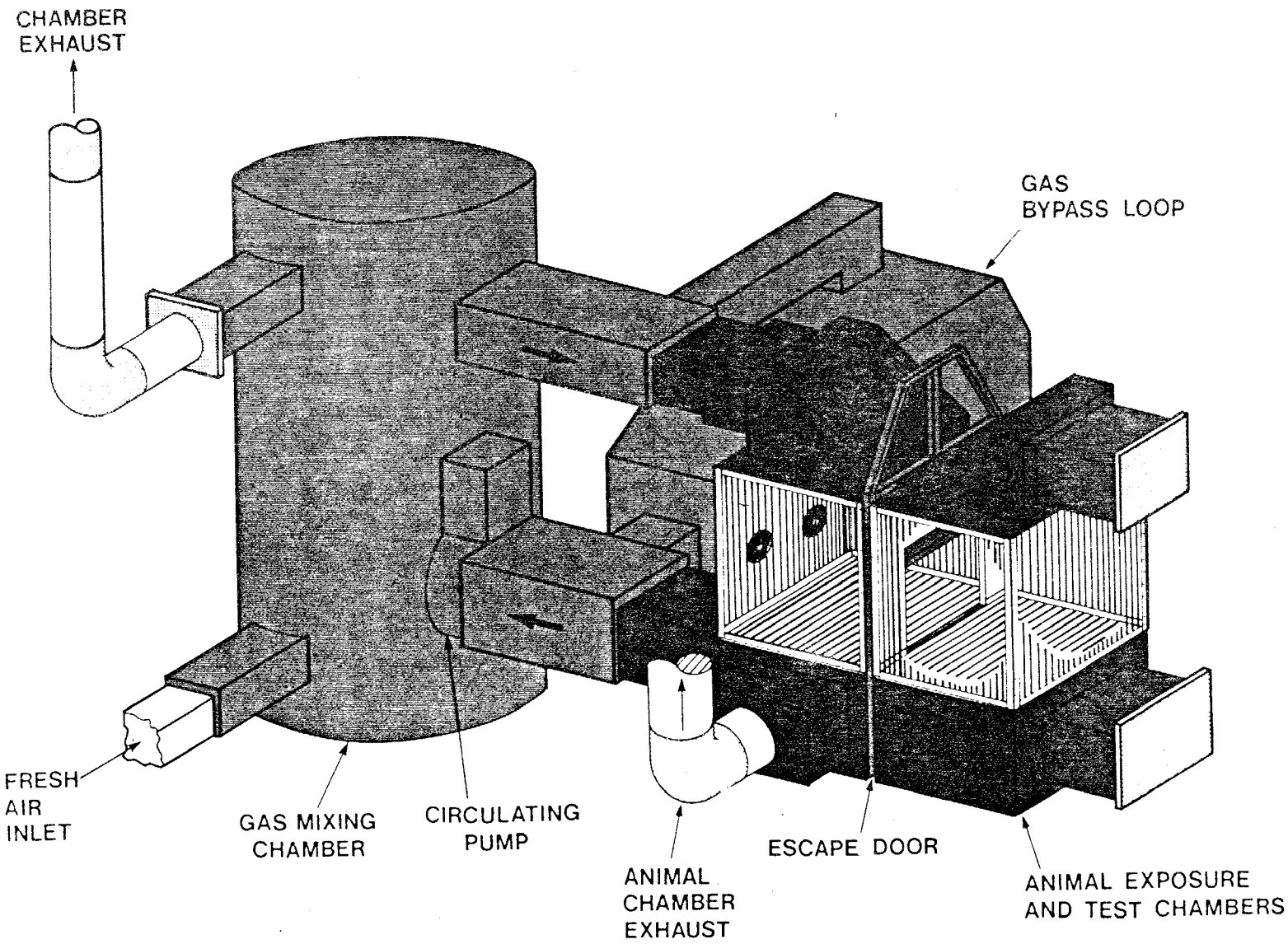

FIGURE 2. GAS MIXING/EXPOSURE SYSTEM AND ESCAPE PERFORMANCE TEST APPARATUS FOR PRIMATE TESTS

ESCAPE PERFORMANCE TEST SYSTEM

Test Apparatus. The primate escape performance test apparatus was a shuttlebox consisting of two identical chambers, each 46 x 46 x 86 in. high with a volume of approximately 2100 L. Each chamber was constructed of fiberglass, with one-half inch thick, 38-1/4 x 38-1/4-inch polymethylmethacrylate panels on the two sides to permit observation of the subjects during the tests. One chamber, designated "Side A, Exposure," served as the test chamber; it was equipped with ducts and valves to allow either the test gas/air atmosphere or fresh air to enter the chamber through the top and exit through the bottom. The second chamber, designated "Side B, Clean", was set up to allow only fresh air intake and exhaust. Both chambers also were equipped with water sprayers to enable the washing of irritant gases from both the animals and the chamber walls immediately after completion of the test. During a test, the two chambers were located side by side but separated by a vertically sliding escape door, 16-1/2 in. wide and 20 in. high, that was operated by an air-activated (40 psi) hydraulic ram. Within each chamber was a cage that was 34-1/4 in. wide, 37-1/4 in. deep and 36-1/2 in. high and was constructed of aluminum bars (5/8 in. diameter) placed on 3/4 in. centers. Constant current shockers provided 4 to 8 milliamps (ma) current to the aluminum bars; scrambling circuits were used to prevent the subjects from standing on bars of the same polarity in order to avoid the shock. The floor bars were mounted on an insulating framework which was pivoted at the end near the sliding escape door and rested on two microswitches located beneath the floor. These microswitches functioned to identify the chamber in which the animal was located by sensing its weight on the floor. Each cage also contained a "house light" to signal that a test session was in progress and two response levers mounted on the wall opposite the escape door. The response levers, which were located 16 in. above the floor and 18 in. apart, projected 2.5 in. from the cage wall. Circular hard rubber covers installed on the ends of the levers made the levers more prominent to the animals. Two cue lights, one red and one white, were mounted above each response lever on a circular 4-in. plate. During a trial, the white light was activated over one lever and the red light was activated over the other lever, with the sequence randomized by the behavioral control system. Downward pressure on either lever activated a microswitch and was recorded. However, depression of only the lever over which the white light was "on" caused the escape door to open; depression of the lever when its red light was "on" had no effect.

Behavioral Control System. A behavioral control system, consisting of a Data General Nova 3 minicomputer equipped with a BRS/LVE Corporation INTERACT System, was used to program and control the escape performance test apparatus and to record performance data. Data recorded by the control system were:

(1) Time to first press of lever
(2) Time to first press of correct lever
(3) Time to exit chamber
(4) Number of correct and incorrect lever presses
(5) Number of intertrial lever presses
(6) Cumulative number of avoidances and escapes
(7) Number of shock pulses delivered

Escape Performance Paradigm. The behavioral paradigm for measurement of escape performance of primates was a standard signalled avoidance task (Figure 3). An audio cue, provided by a Sonalert buzzer, signalled the start of a trial. Simultaneously with the tone, the white light over one lever and the red light over the other lever were turned on to indicate to the subject which lever was the correct response, i.e., would open the escape door. A fully random schedule was used to determine whether the left or right lever was the "correct" lever for each test. Ten seconds after activation of the tone and lights, an electric shock was applied to the aluminum bars of the cage. The shock remained on for 20 seconds. If the subject responded by pressing the correct lever and moved through the door opening within 10 seconds after presentation of the audio and visual cues, the response was designated an "avoidance." If the animal pressed the correct lever and exited the cage after 10 seconds, but within 30 seconds, following presentation of the cues, the response was termed an "escape." The response was designated a "failure" if either the subject did not exit the test cage or exited after 30 seconds.

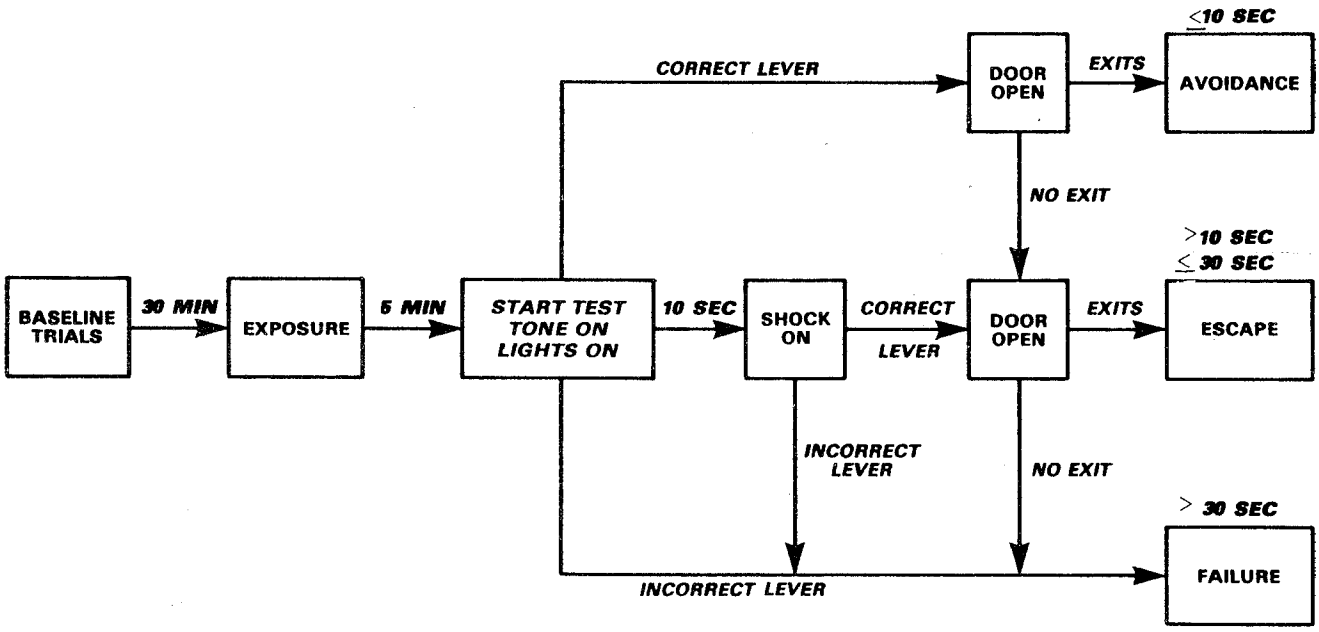

FIGURE 3. BEHAVIORAL PARADIGM FOR MEASUREMENT OF ESCAPE PERFORMANCE IN PRIMATES

Test Protocol. Each escape performance test was conducted in accordance with the check list shown in Table 1; some minor modifications were made for the different test gases. The location of the valves listed in Table 1 are shown in Figure 4. The test protocol may be divided into four parts: (1) initialization; (2) baseline; (3) exposure; and (4) post exposure. Because personnel were located in three separate rooms, a test coordinator supervised the performance of each test.

TABLE 1. TYPICAL ESCAPE PERFORMANCE TEST CHECK LIST

Time	Task
Initialization	room exhaust Fan on; test warning light on; air pump (blower) on Valves V9, V10 closed (exhaust duct closed) Valves V5, V6 open (mixing chamber open to bypass loop) Valves V3, V4 closed (animal chamber, Side A, isolated from gas mixing chamber) Valves V1, V2 open, Fan S1 on (animal chamber, Side A, venting) Valves V7, V8 open, Fan S2 on (animal chamber (Side B) venting) animal chamber door open; computer off; animal chambers disconnected
Pre Test	place animal into animal chamber (Side A), close door manually; connect Sides A & B; connect cables; activate computer
T-30 minutes	begin baseline trials, with intertrial interval (ITI) = 2 minutes, 15 seconds
T-10 minutes	introduce test gas into mixing chamber, measure and revise concentration as necessary; personnel don breathing masks
T-70 seconds	hold countdown; verify that gas concentration is satisfactory, calculate makeup gas needed for animal exposure; verify that animal is on Side A; check all systems, stopwatches, personnel assignments; release hold
T-60 seconds	synchronize countdown clocks; close Valves V1, V2, Fan S1 off (stop venting animal chamber, Side A)
T-0 (Begin Exposure)	start clocks, stopwatches; simultaneously, open Valve V3 & close Valve V5, then open Valve V4 & close Valve V6 (route gas to animal exposure chamber and isolate bypass leg); introduce makeup gas; press "run" button on computer, mark printout
During Test	make animal observations; draw samples for analyses; adjust continuing makeup flow of gas as needed
T + 5 minutes	begin avoidance trial
End of Test (or Abort)	Fan S1 on, open Valves V1, V2, close Valves V3, V4 (vent animal chamber, Side A, isolate animal chamber from gas mixing chamber); open Valves V5, V6 (activate bypass loop); allow 30 sec for animal chamber to ventilate; if animal on Side B, activate tone/shock to move him to Side A; once animal is in Side A, disconnect Sides A & B, remove computer cables, open door manually and remove animal; open Valves V9, V10 (exhaust mixing chamber)

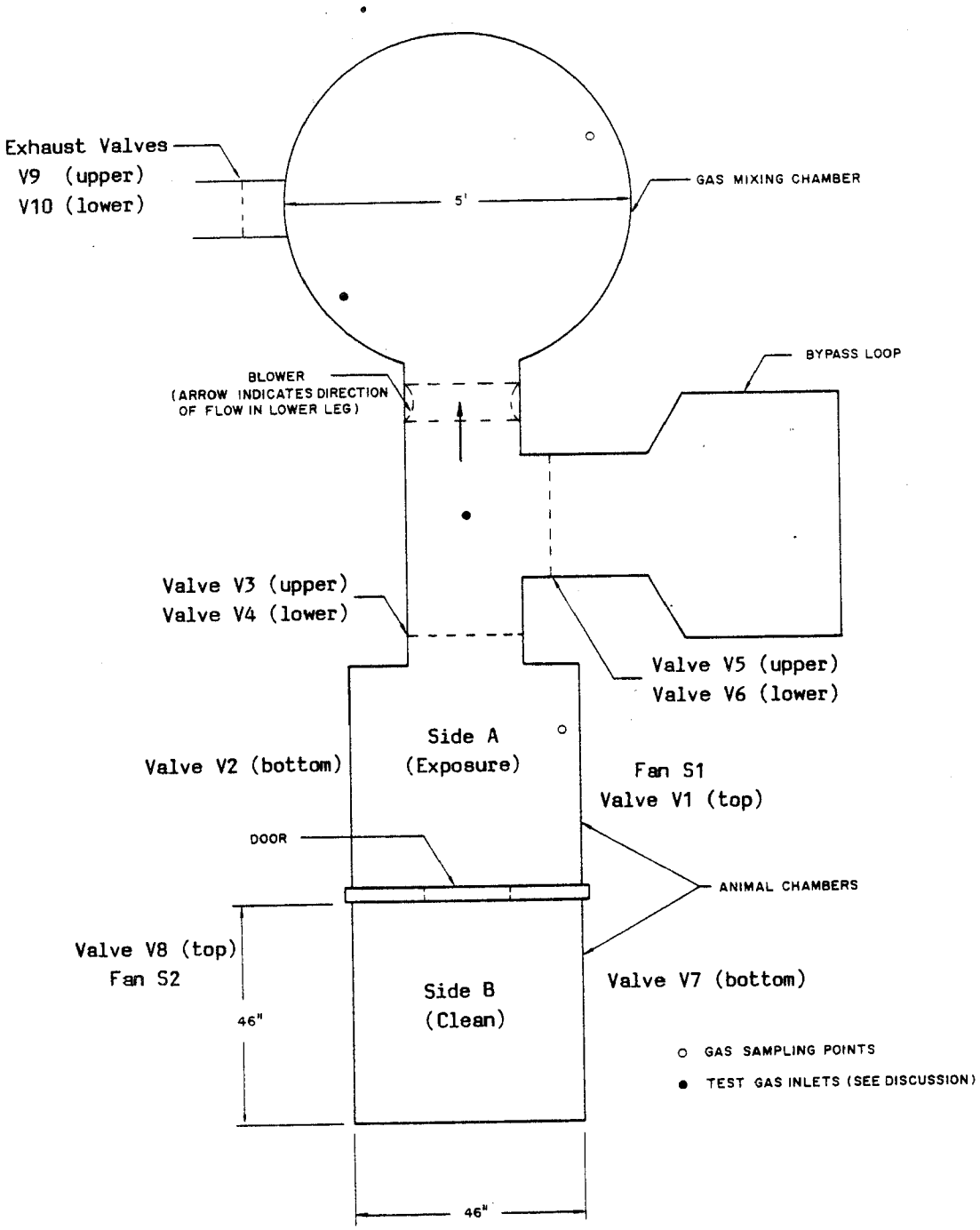

FIGURE 4. SCHEMATIC DIAGRAM OF GAS MIXING AND EXPOSURE SYSTEM

1. Initialization--The initialization phase consisted of preparation of the test room and the gas mixing/exposure apparatus for the test. The warning lights, the exhaust fan, the CO detector (when applicable), and the air pump were turned on. Also, appropriate valves were opened (or closed) to open the bypass loop to the mixing chamber and to prevent the flow of test gas to the animal exposure chambers. The animal chambers were vented with room air.

Upon completion of the initialization phase, the animal was transferred from its transport cage to the exposure chamber ("Side A") and the second chamber ("Side B") was placed in position adjacent to the exposure chamber. The chambers were locked in position and the computer was activated.

2. Baseline--The baseline phase was initiated 30 minutes prior to the estimated exposure time ("T minus 30 minutes"). The control variables for conducting the baseline trials were selected, and the task variables and other pertinent information (e.g., animal I.D. No., intertrial interval, shock pulse rate) were entered into the computer. During the 30-min baseline period, 10 to 12 escape performance trials were presented to the test subject. The last baseline trial was conducted in Side B so that the animal would return to the exposure chamber (Side A) for exposure to the test gas. During the baseline trials, the test gas atmosphere was generated in the gas mixing chamber and recirculated through the bypass loop. The concentration was revised as necessary to attain the desired level. Prior to the actual performance trial, the countdown was placed on "hold" at T minus 70 seconds. A primary purpose of the pause in the countdown was to verify the final concentration of gas needed for the beginning of the exposure. During this holding period, the exposure test parameters were entered into the computer and final checks were made with all personnel. Upon resumption of the countdown, the animal chamber was sealed off from the fresh air flow in preparation for the exposure.

3. Exposure--When the countdown reached zero, the exposure was begun. The appropriate valves were immediately closed (or opened) to isolate the bypass loop and to include the exposure chamber into the recirculating gas system. A quantity of additional gas had to be rapidly added to the system to make up for the dilution and losses caused by the animal exposure chamber. During the exposure phase, an observer continuously monitored the subject's behavior and symptoms and recorded his observations into a pocket recorder for later transcription onto the test record sheet. In the event an animal was visibly incapacitated, the time of incapacitation was recorded. The continuous analyzer for each gas indicated, during the period, the actual concentration of gas to which the animal was being exposed. At the same time, samples of the exposure atmosphere were being withdrawn for subsequent analyses.

4. Post Exposure--After exactly 5 minutes of exposure, the audio and visual cues were presented to start the escape performance trial. Upon completion of the trial, the gas exposure was terminated. The valves were thrown to stop the flow of test gas into the animal exposure chamber and to ventilate the chamber with fresh air. If the animal escaped to Side B of the chamber, he was induced to return to Side A after the gas had been removed. Once he was in Side A, the two chambers were disconnected and the animal was removed from the chamber through the door.

Once the animal was removed from the chamber and placed into its transport cage, it was immediately treated for effects due to the gas exposure. For the tests with CO, the animal was placed into an oxygen box (however, blood samples were first obtained from most of the unconscious animals); for tests with acrolein and HCl, the animal was washed with water prior to transfer to the oxygen box. (In some tests, the animal was flushed with water

by a nozzle at the top of the chamber before being removed from the chamber; in other tests, a hose was used to wash the animal outside of the exposure chamber). After oxygen therapy was completed and post-exposure observations were made, the subject was returned to the animal facility for observation and treatment.

ESCAPE PERFORMANCE TEST SUBJECTS

Animal Subjects. Nine juvenile (ages 2 to 3 years) male baboons (superspecies Papio cynocephalus) were used in a series of 49 exposure tests (CO = 32, acrolein = 9 and HCl = 8). The animal numbers, dates of birth and body weights at the times of the tests are shown in Table 2. All of these animals were obtained from the breeding colony at the Southwest Foundation for Biomedical Research, San Antonio, Texas. A complete examination of each animal was made by the project veterinarian to ensure that the animal was healthy and free of respiratory problems. Most of the CO tests were conducted in October, 1982; the acrolein tests were conducted in late March and early April, 1983; and the HCl tests were accomplished during the last quarter of 1983.

Animal Training. Training of the animals to perform the escape performance paradigm generally required training sessions of approximately 30 minutes in duration three times a week for a total of from five to eight weeks. Prior to testing of an animal, the subject was trained until it attained a 90-percent or greater avoidance rate for two sessions prior to the test. Almost every animal "avoided" successfully 95 percent of the time in the pre-exposure trial conducted prior to the exposure test. Whenever weeks or months elapsed since a training session or a test, refresher training sessions were conducted until the animal again attained a satisfactory performance level.

TABLE 2. BABOON SUBJECTS USED IN ESCAPE PERFORMANCE TESTS

Animal Number	Date of Birth	Body Weight (kg) at Time of First Exposure Test		
		CO	Acrolein	HCl
500	07/30/80	7.4 (4)*	13.2 (2)	11.8 (1)
527	08/04/80	7.1 (6)	9.5 (1)	10.9 (2)
532	08/08/80	6.9 (6)	8.6 (2)	--
565	09/09/80	7.3 (5)	9.1 (2)	9.6 (1)
575	09/22/80	6.4 (6)	8.2 (1)	--
634	11/03/80	6.2 (5)	8.4 (1)	10.9 (1)
833	11/02/81	--	--	6.8 (1)
844	11/25/81	--	--	5.5 (1)
861	12/16/81	--	--	5.9 (1)
	Mean ±	6.9 ±	9.5 ±	8.8 ±
	S.D.	0.5	1.9	2.6

* ( ) = Number of times animal tested.

Animal Care and Treatment

1. Animal Care--The animals were cared for by the Department of Laboratory Animal Sciences at Southwest Research Institute in accordance with guidelines of the American Association of Laboratory Animal Care and regulations of the United States Department of Agriculture.

Prior to testing, the baboons were housed in individual cages with water available ad libitum and were fed Purina baboon ration (supplemented with fruit) once daily. They were located in an open-air environment on a concrete floor with an all-weather roof. Heat and or ventilating fans were used in extreme weather conditions.

2. Post-Test Medical Care--The baboons were removed from the test chambers immediately after the test gas was exhausted and placed in a chamber into which O_2 was added at 10 L/min. The animals remained in this chamber for observation and initial recovery from the gas effects. Once they returned to a normal state, they were removed from the oxygen chamber and moved to an intensive care room in the Animal Sciences area. In this area, the baboons could receive further O_2 therapy plus corticosteroids to reduce the inflammatory response to the gas and antibiotics to prevent infection to the sensitized respiratory system.

The baboons were observed daily for clinical signs, and appropriate medical care was administered until recovery was noted by the veterinarian. Medical records of the care and treatment were maintained during the study.

GENERATION AND ANALYSES OF EXPOSURE ATMOSPHERES

Introduction. The design and configuration of the test system (gas mixing chamber, bypass loop and animal exposure chambers) have been previously described in general terms; the use of this system (Figure 4) for gas generation and analysis is described more fully below:

(1) Initially, the valves to the animal chambers were closed, the valves to the bypass loop were open and the blower was on.

(2) Gas was introduced into the mixing chamber in the immediate vicinity of the blower in accordance with procedures given below for each gas.

(3) Gas concentration in the mixing chamber/bypass loop was monitored as described below for each gas. Concentration was adjusted to achieve the desired level.

(4) Exposure of the animal to the gas was initiated by simultaneously opening the valves to the animal chamber and closing the valves to the bypass loop.

(5) Supplemental gas was rapidly introduced into the mixing chamber to make up for the dilution caused by including the animal chamber volume into the test system. This was a one-

time dilution which was a predictable value (except for HCl, see later discussion).

(6) If necessary, additional gas was monitored into the mixing chamber in order to maintain a steady concentration over the duration of the test.

The actual volumes of the various segments of the test system were determined by metering into the system a known amount of gas and then analyzing the concentration. This was considered to be a better measure of the volumes than that calculated from the dimensions. The volumes of the segments were determined to be as follows:

Mixing chamber/bypass loop = 5700 L,
Mixing chamber/animal chamber = 6600 L;

Therefore,

Mixing chamber (alone) = 4500 L,
Bypass loop (plus ducts) = 1200 L,
Animal chamber (plus ducts) = 2100 L.

The dilution which occurred upon switching from the mixing chamber/bypass loop to the mixing chamber/animal chamber was calculated from the above data to be about 32 percent by volume (since the bypass loop is cut out of the system upon switching over to the animal chamber, the dilution is 2100/6600).

The different techniques required for generation and analysis of each of the gases are detailed in the following sections. Continuous analysis procedures were used for each gas, supplemented (except for CO) by non-continuous laboratory analyses. Sampling during the test runs was performed directly from the animal exposure chamber.

Carbon Monoxide. Carbon monoxide (CO) was the easiest of the three gases to generate and to monitor. Because it has little or no tendency to react with moisture or wall surfaces, there was no difficulty in handling CO (other than its toxicity) . Also, a reliable continuous analyzer and various compressed gas mixtures (including analyzed calibration gases) were commercially available.

A schematic diagram of the CO generation and analysis set-up is presented in Figure 5. Generation of CO atmospheres for testing was accomplished using compressed gas cylinders of pure CO. The gas was metered through a calibrated flowmeter directly into the inlet side of the blower (see Figure 4). All transfer lines were 1/4-in. polyolefin plastic tubing. The concentration of CO was continuously monitored from the mixing chamber/bypass loop as shown in Figure 5. The CO analyzer was a Beckman 865 non-dispersive infrared analyzer. The analyzer was calibrated each day with an appropriate concentration calibration gas. The calibration gas was introduced into the analyzer in the same manner as the test gas (i.e., through the same control valve and flowmeter and with no back-pressure on the analysis cell).

The test system was not 100-percent air tight. This was determined by creating a stable concentration of CO in either the mixing/bypass loop or the mixing/animal chamber section and monitoring the concentration continuously. Since the analyzer removed a negligible amount of gas from the system, the

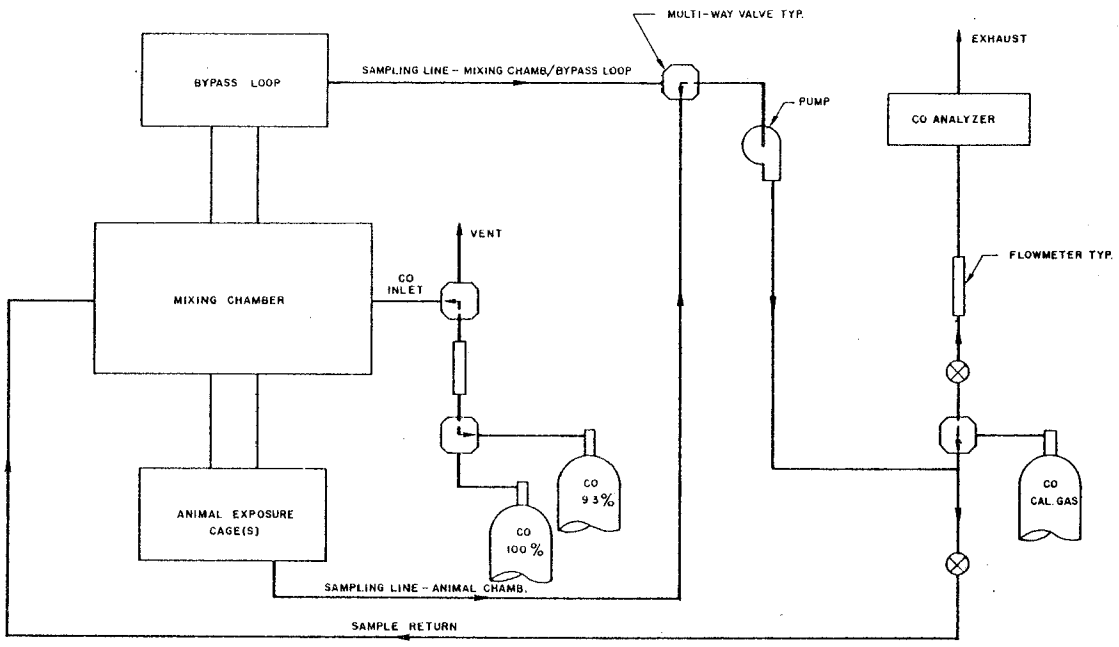

FIGURE 5. SCHEMATIC DIAGRAM OF THE CO GENERATION AND ANALYSIS SET-UP

nature and extent of the leaks could be determined. It had been previously ascertained by use of a "Halogen Leak Detector" (TIF Instruments) that there were some leaks in the test system at certain seams and in the animal chamber at the points of hardware penetration through the walls. These were sealed with a silicone caulking compound; however, there continued to be a small rate of leakage around the valves into the exhaust system. The percent leakage rate was determined to be approximately 0.4 percent/min for the mixing/bypass loop and 0.8 percent/min for the mixing/animal chamber section. This was not considered to be a serious error; however, a low concentration of CO (approximately 10 percent) was bled into the test system to make up for the leakage.

The output of the CO analyzer was monitored directly by computer and logged onto floppy disk. Simultaneously, the mV output of the analyzer was traced onto a recorder. After each test, a printout of the computer record was made.

Acrolein. The final set-up for generation and analysis of acrolein is pictured schematically in Figure 6. Considerable trial and error was required to establish the locations of valves and sampling ports and to verify that a

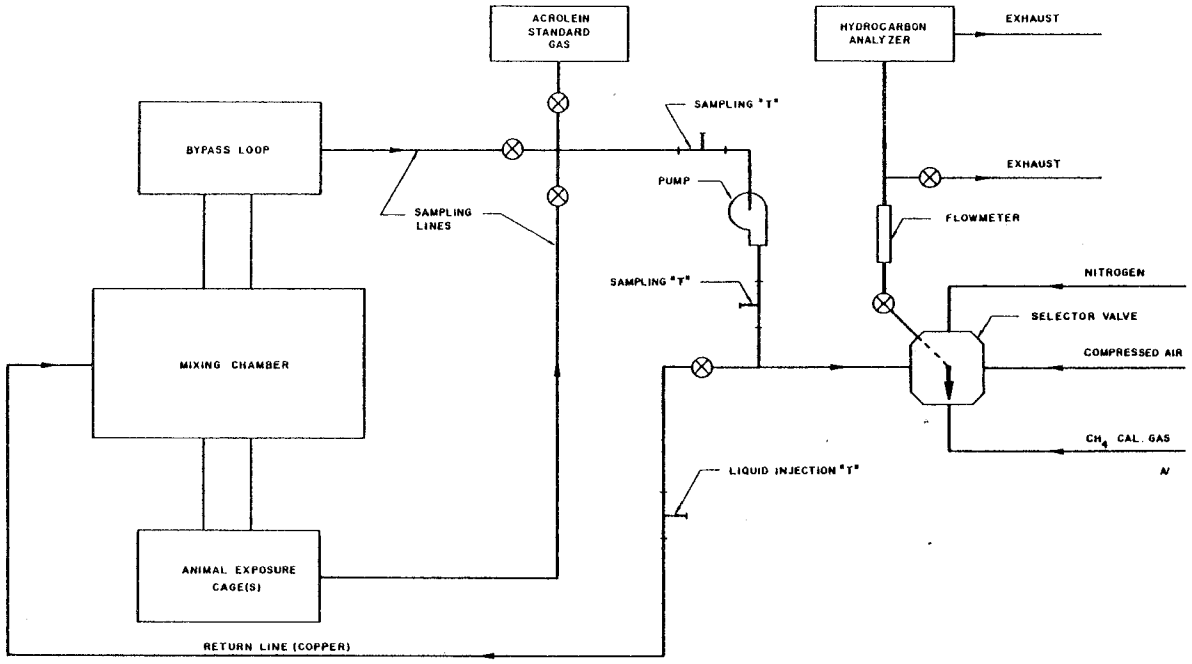

FIGURE 6. SCHEMATIC DIAGRAM OF THE ACROLEIN GENERATION AND ANALYSIS SET-UP

simple hydrocarbon (HC) analyzer was adequate for measuring acrolein gas quantitatively. For the test runs, direct gas samples of acrolein were extracted and injected into a gas chromatograph (GC) for analysis (gas chromatography is an accepted analytical technique for acrolein). Comparison of a series of gas chromatographic analyses (ppm) with the output of the hydrocarbon analyzer (mV) was sufficient to define a calibration curve for the continuous analyzer. Also, mixtures of acrolein in air were prepared in large Tedlar bags to serve as daily "standards" (verified by GC) for checking the response of the HC analyzer.

Acrolein is a liquid at room temperature (boiling point = 53 C); however, it is easily vaporized. Introduction of gaseous acrolein to the test chamber was performed in the following manner:

(1) The quantity of liquid acrolein at room temperature that was required to produce a given gas concentration in the test chamber volume (mixing chamber/bypass loop) was calculated using the ideal gas law (PV = nRT). For example, at 760-mm Hg atmospheric pressure (P), and 25 C/298 K temperature (T), one gram-molecular weight of acrolein (n) will occupy 24.5 L of volume (V), since the gas constant (R) for these units is 62.4. One gram-molecular weight of acrolein is 56.1 g. The density of acrolein at 20 C is 0.8 g/cc. Thus, for the 5700-L mixing chamber/bypass loop, 1000 ppm acrolein would require approximately 16.3 cc of liquid acrolein.

(2) The calculated quantity of acrolein was loaded into a glass syringe from a serum vial which had been stored under refrigeration.

(3) The liquid acrolein was injected through a septum (the "liquid injection T" in Figure 6). The acrolein quickly evaporated in the copper line due to the recirculating air flow from the pump.

The liquid dose of acrolein corresponded closely to the predicted gaseous concentration. The rate of loss of acrolein in the test system was no greater than that for CO (i.e., due to leakage alone).

The test procedure as outlined above was established using acetone as a substitute for acrolein. Acetone has very similar physical and chemical properties to acrolein (molecular weight, density, boiling point and its tendency to attack plastic materials). However, it does not have the irritant effect of acrolein and was therefore much easier to handle. The method of introducing the liquid into the test system to create a desired gas concentration was an especially critical part of the test procedure for acrolein.

Once the calibration curve for acrolein was established (mV output of hydrocarbon analyzer as a function of ppm acrolein as determined by GC), the HC analyzer was used to define the concentration of acrolein during the test run. The output of this analyzer was recorded by the computer in the same manner as was done for CO.

Hydrogen Chloride. Generation and monitoring of hydrogen chloride (HCl) gas in the test system was more difficult than either of the other two gases. Dry HCl attracts moisture from the air, resulting in deposition onto surfaces and consequential loss from the atmosphere. It was decided not to attempt to generate an aerosol, but to maintain a stable atmosphere as best as possible under ambient conditions. Attempts to dehydrate the volume of the test chamber proved to be ineffective.

Problems with HCl loss were anticipated; however, the magnitude of loss of HCl was considerably greater than expected. Experiments in a 50-l laboratory acrylic box showed that reasonably stable concentrations of HCl could be achieved when the relative humidity was less than 50-percent. At higher moisture levels, yellow droplets appeared on the walls and there was a greater loss of HCl than under dry conditions. In general, even under low humidity conditions, only about 50 percent of the HCl introduced into the chamber was found in the box atmosphere. Dry HCl was metered into the system from a compressed gas cylinder through a Teflon and glass flowmeter. The regulator and flowmeter were kept as dry as possible between test runs by purging them with nitrogen.

Sampling and analysis of HCl were performed by three different techniques which may be summarized as follows:

(1) Batch sampling was performed using dry soda-lime absorption tubes. Chloride ion in the aqueous extract from the soda lime was analyzed by titration.

(2) Intermittent gas sampling was achieved using a 60-mL syringe containing 5-mL of absorbing solution. Direct analysis of

chloride ion in solution was performed either by ion-selective electrode (ISE) or, using a different absorbing solution, by ion-chromatography (IC).

(3) Continuous sampling and analysis was accomplished using a recirculating pump and a Hall Conductivity Detector. This detector was calibrated immediately prior to a run by technique No. 2 above.

Techniques No. 1 and 2 were relied upon for a quantitative assessment of HCl (however, the soda lime technique was considered to be our primary method, with the syringe technique used as a backup). While the continuous analysis method (#3) was used to establish and maintain a constant level of HCl during the test runs.

Generation of HCl was accomplished by the same procedure as for CO (i.e., as shown in Figure 5); however, using a compressed gas cylinder of pure HCl. Maintenance of the HCl concentration was accomplished by reducing the flow rate (rather than using a dilute gas as with CO). The continuous monitoring system for HCl is shown schematically in Figure 7. A high volume recirculating pump drew a portion of the atmosphere from the chamber through heated sampling lines. Most of this was recirculated, while a very small volume of sample (usually 1-4 cc/min) was extracted for analysis. This sample was diluted with pure, dry air (flowing at approximately 40 cc/min), and the mixture then flowed through the conductance cell concurrent with a low volume (4 mL/min) of n-propyl alcohol as the solvent. The effluent solvent stream was not recirculated because of the high concentration of chloride ion (the pure solvent was run through an ion-exchange bed).

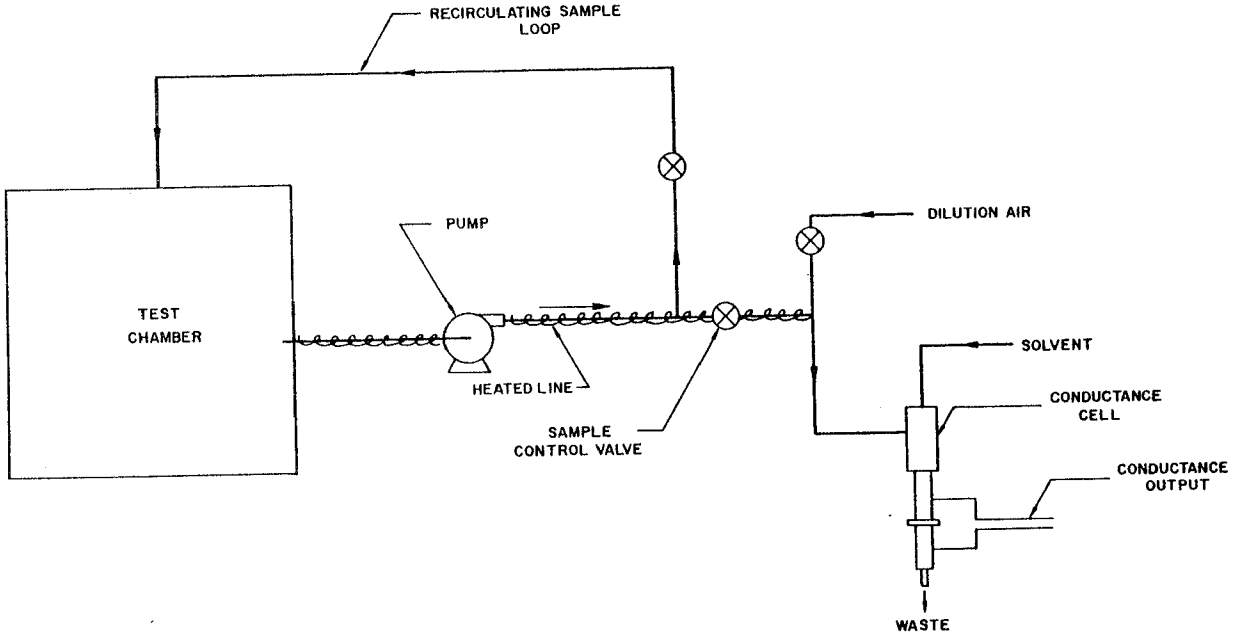

FIGURE 7. SCHEMATIC DIAGRAM OF THE CONTINUOUS MONITORING SYSTEM FOR HCl

This continuous measurement device was useable for both the 50-L laboratory box and for the full-scale test chamber. For the earlier HCl runs, the continuous monitor was calibrated prior to the test run, using the 50-L box. Later, the continuous monitor was calibrated directly from the full-scale gas mixing chamber. In either case, HCl was introduced step-wise into the volume being sampled and a comparison was made between the mV output from the conductivity detector and the ppm analysis from the syringe/ISE method described above. The calibration curve was determined for the particular range of HCl concentration to be tested. When used for actual test runs, the heated sampling line was connected first to the mixing chamber for generation and stabilization of the HCl concentration, and then it was changed to the animal chamber for the exposure period. Supplemental analyses of HCl in the animal exposure chamber were performed using both the soda-lime tubes and the syringe technique.

ESCAPE PERFORMANCE TESTS WITH RATS

ESCAPE PERFORMANCE TEST SYSTEM

Test Apparatus and Paradigm. A modified commercially available shuttlebox (Lafayette Instrument Company, Model No. 85103) was used. The shuttlebox consisted of two identical chambers (12-1/4 in. wide, 8 in. deep, 7-3/4 in. high) separated by a vertically sliding partition driven by an electric motor. Two levers were mounted in each chamber on each side of the partition and a white cue light was mounted above each lever. The floor and top of each shuttlebox chamber consisted of a grid of electrically isolated bars for the administration of an electric shock to the test animal. The floor of each chamber was equipped with a microswitch which was activated when the animal moved into the chamber. Floor pivots and adjustable counterbalances were located opposite the microswitches.

The escape performance paradigm was similar to that used in the primate studies (shown in Figure 3), but the visual cues differed. For the rat, each trial was initiated with the presentation of a tone (Sonalert buzzer) and the lighting of the white light over each of the two levers. (For the primate, red and white cue lights were used in order to train the animal to discriminate between levers.) Pressing of either lever by the rat opened the partition and allowed the animal to escape into the adjacent chamber. Ten seconds after the presentation of the audio and visual cues, a shock of 4 to 8 ma was administered to the bars. The shock pulsed for 20 seconds, at which time the trial was terminated. If the animal pressed a lever and moved into the "escape" side of the box within 10 seconds, the response was designated an "avoidance." If the exit of the subject occurred during the 20-second shock, the response was designated an "escape." The trial was a "failure" if the animal did not move into the adjacent side at all or did so after 30 seconds following the presentation of the cues. Programming and control of the shuttlebox and recording of performance data were accomplished by means of the same behavioral control system used in the primate studies.

EXPOSURE SYSTEMS AND TEST PROTOCOLS

Carbon Monoxide. Rats were exposed to CO in the primate exposure test system because this system was readily adaptable for use with the rat shuttlebox. Also, the primate tests had demonstrated that the system was capable of

generating an excellent square-wave pattern of the desired test gas concentration. To utilize this system for the rat tests, the rodent shuttlebox was placed inside the exposure cage (Side A) of the primate system and connected to the computer located in an adjacent room. Once this was accomplished, the test protocol was essentially the same as that followed in the primate tests. Each animal was given 30 minutes of pre-exposure trials prior to initiation of the 5-minute exposure to the test gas. At the end of 5 minutes of exposure, the animal was presented with the escape performance task. Although CO was also present in the escape side of the shuttlebox, the gas is colorless, odorless and tasteless and would not interfere with an animal's training to enter the escape side. However, the presence of an irritant gas in the escape side might interfere with the animal's escape performance.

Hydrogen Chloride. For the HCl tests with rats, an alternative smaller exposure system was used in place of the primate exposure system for a number of reasons. With the latter system, HCl would have been present in both sides of the rat shuttlebox, unless major modifications were made. The presence of high concentrations of HCl in the escape side of the shuttlebox might have interfered with the escape performance of the rat. Also, the smaller exposure system required less HCl and facilitated disposal of the test atmospheres. Therefore, the shuttlebox was interfaced with a more suitable exposure system, consisting of a 300-liter acrylic chamber, 2 x 2 x 2.5 ft high (Figure 8). Adjacent compartments of the shuttlebox were separated into an "exposure side" and a "clean side." Airtight connections between the shuttlebox and the test gas chamber were ensured by application of silicone-based sealant and grease. Exposed electrical connections of the exposure side of the shuttlebox were protected from acid gas corrosion by application of plastic air-setting sealant. A 12.7-cm fan situated in the chamber aided in the uniform mixing of the test gas atmosphere. Access to the exposure chamber was facilitated by a 2-ft square polymethylmethacrylate panel. The test protocol, providing 30 minutes of pre-exposure trials and 5 minutes of exposure to the test atmospheres prior to presentation of the escape performance task, was the same as in other tests. However, the requirements for valve switching in the primate system were eliminated by the use of this alternative system.

ESCAPE PERFORMANCE TEST SUBJECTS

Animal Subjects. Seventeen male Sprague-Dawley rats, obtained from the Timco Breeding Laboratories, Houston, Texas, were used in these tests. The supplier certified that the animals were free of major respiratory pathogens. All animals were quarantined in a suitable facility for a period of at least two weeks during which they were observed for signs of illness. Animals that were considered unsuitable because of symptoms, body weight or other reasons were not used in the study.

The animal numbers, dates of birth, and body weights of the subjects are listed in Table 3. Also shown are the test gas or gases to which each animal was exposed and the number of times each animal was tested. Twenty tests were conducted with CO and 14 were conducted with HCl; acrolein was not tested with rats.

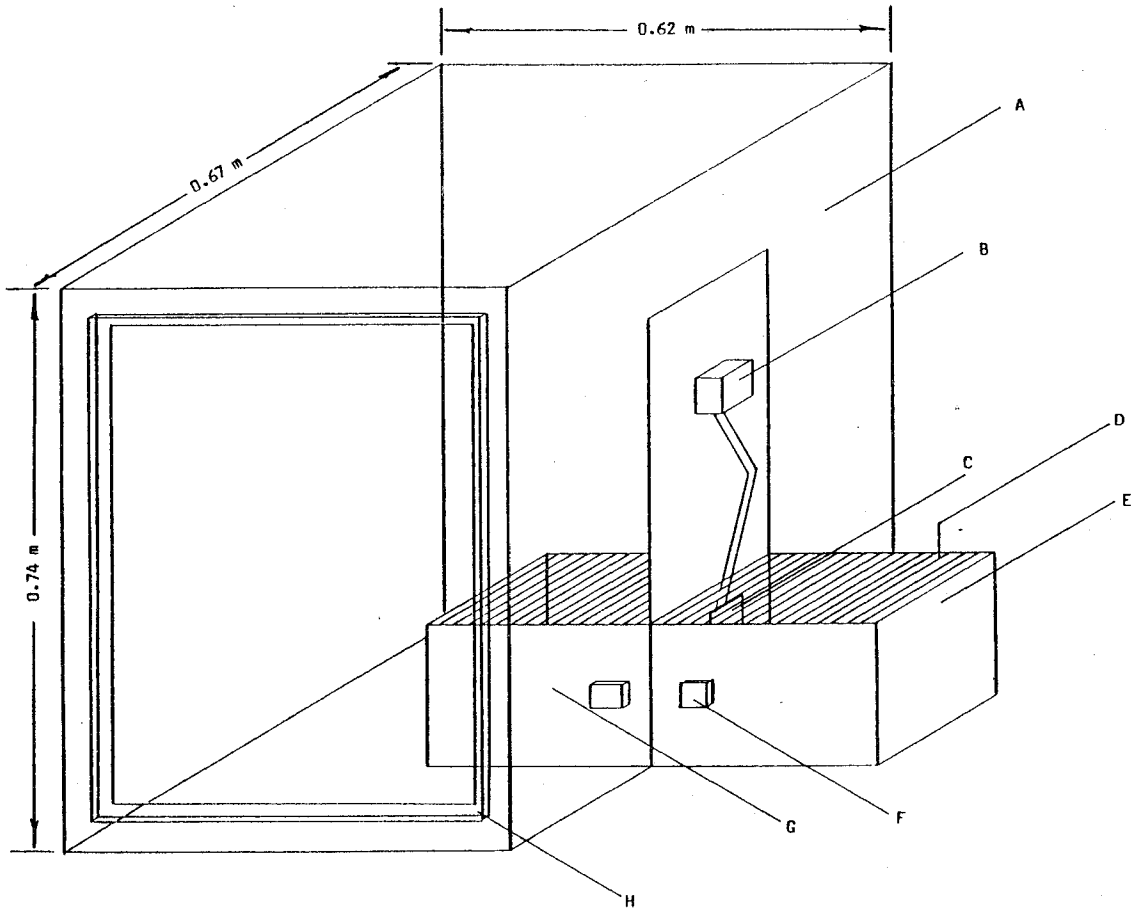

FIGURE 8. EXPOSURE AND ESCAPE PERFORMANCE TEST SYSTEM FOR THE RAT

A, Test Gas Exposure Chamber; B, Escape Door Motor;
C, Escape Door; D, Electric Grid; E, Shuttlebox "Clean Side;"
F, Response Lever Housing; G, Shuttlebox "Exposure Side;"
H, Removable Access Panel

Animal Training. Initially, training of subjects to discriminate between a red light over one lever and a white light over the second lever was attempted. However, the training did not result in a satisfactory percentage of animals that could discriminate; therefore, this requirement was eliminated. A second problem was encountered in attempting to train animals to a high level of "avoidance" performance for the CO tests. After approximately 4 months of daily 30 to 60-minute sessions, some animals avoided successfully in more than 90 percent of the trials, but others usually responded only when shock was administered ("escape"). All animals achieved a satisfactory (greater than 95 percent) level of "escape" performance. For the HCl tests, the training of animals consisted of approximately six weeks of daily sessions of 30 to 60 minutes duration. All animals attained a satisfactory "escape" performance level prior to testing.

TABLE 3. RAT SUBJECTS USED IN ESCAPE PERFORMANCE TESTS

Animal Number	Date of Birth	Body Weight (gm) at Time of First Exposure Test	
		CO	HCl
2	12/20/82	346 (4)*	471 (1)
3	"	372 (1)	--
5	"	373 (4)	501 (1)
6	"	360 (2)	452 (1)**
7	"	390 (4)	--
9	"	372 (4)	453 (1)
10	"	362 (1)	--
44	10/03/83	--	385 (1)
45	"	--	389 (1)
46	"	--	365 (1)
47	"	--	363 (1)**
51	"	--	389 (1)
52	"	--	446 (1)
54	"	--	393 (1)
57	"	--	435 (1)
58	"	--	452 (1)
60	"	--	391 (1)
	Mean ± S.D.	368 ± 14	420 ± 43

* ( ) Number of times tested.

** Experiment aborted because of equipment malfunction.

Animal Care. The rats were housed in individual cages with water and commercial rodent chow available ad libitum except during the training sessions and tests. The animals were cared for in accordance with guidelines of the American Association of Laboratory Animal Care and regulations of the United States Department of Agriculture.

GENERATION AND ANALYSIS OF EXPOSURE ATMOSPHERES. The overall procedure followed for generation and analysis of the gas atmospheres for the rats was the same as that described for the primates. However, for the HCl tests, certain changes in the test procedure were necessary to accommodate use of the new 300-L chamber.

Generation of the HCl atmospheres was by the same route as for the larger chamber, using a cylinder of pure HCl, a system of valves and a rotameter. However, 100 percent of the test concentration had to be created in the exposure chamber at the start of each test, rather than the 30 percent makeup needed for the other setup. This was partially balanced by the fact that the total volume was only 300 L (instead of about 6000 L). The major drawback of

this procedure was not having a definite indicator of the stability of HCl for the particular test chamber conditions until the test run was underway. The other (full-scale) chamber permitted an assessment of the relative stability of HCl in the mixing chamber/bypass loop prior to introduction of gas into the animal chamber. As indicated previously, ambient humidity was a major factor in the loss of dry HCl from the atmosphere. Calibration of the Hall detector was performed prior to the animal test run in the same manner as described for primates by metering HCl into the chamber in increments and measuring the detector response. This was a further disadvantage in the rat studies because the exposure chamber had to be cleaned after the calibration run, before the animal exposure. This occasionally resulted in problems with the Hall detector monitoring system, due to changes in gas or solvent flow rates.

Analyses of HCl were performed by the same techniques as in the primate studies. The continuous monitor was relied upon mostly for an immediate indication of concentration and HCl stability. The soda-lime technique was considered to be the primary sampling and analysis method, with the syringe/ion-selective electrode technique as a secondary measure of HCl concentration.

BEHAVIORAL DATA ANALYSES

INTRODUCTION. Given the limited scope of the data available, primary emphasis was placed on descriptive rather than inferential statistics. Because the objectives of the research program emphasized identification of concentrations of chemicals which produce behavioral deficits, data analysis also focused on determination of dose response relationships using linear regression techniques and probit analysis.

STATISTICAL ANALYSES

Linear Regression. Conventional least squares procedures were used to "best fit" function, $Y = mX + b$, to the various data sets. (For these analyses, Y equals a behavioral measure and X equals a chemical measure.) The value of m gives the slope of the line and b is the intercept. The Pearson product moment correlation coefficient was computed, and the significance of the observed r value was evaluated by testing the slope against the null hypothesis that the slope equaled zero. A p value of less than 0.05 led to rejection of the null hypothesis and acceptance of the equation as being statistically significant.

The strength of the association between two variables is indicated by the magnitude of the r coefficient which can range from 0.00 to 1.00; 0 means no relationship and 1.0 means essentially absolute identity. Either positive or negative r values can occur, depending upon the direction of the relationship between the two variables. If a subject's scores on one variable increase as scores on the other increase, a positive correlation exists; if scores on one variable get smaller as scores on the other variable get larger, a negative correlation exists.

As the number of degrees of freedom (the number of data pairs minus 2) increases, the absolute magnitude of the correlation coefficient required for statistical significance decreases. For example, $r = 0.63$ is required for $p < 0.05$ with $df = 8$ (10 pairs of points), but with 24 pairs of points ($df = 22$) an r value of only 0.40 will be significant at the $p < 0.05$ level.

In these analyses, when correlation coefficients were not significant, inspection of the scatterplots usually indicated apparently random scatter of points. Only in a few instances did the scatter plots suggest a curvilinear relationship between variables. Such data, which can make sense, could be analyzed using non-linear regression equations, but in this case there were not enough data to make such an effort productive.

Chi Square. It was possible to use chi square statistics to evaluate the observed distribution of events such as escape or fail for significance.

Primary Data. The single trial behavioral paradigm employed provided a limited data set: the primary measurement was the animal's ability to perform the escape task within a prescribed time. Successful escape performance included both avoidance and escape responses, requiring exit from the chamber within 10 and 30 seconds, respectively, from presentation of the cues. When an animal became visibly incapacitated, the time to this effect was recorded. In addition, other performance parameters were measured, including time to exit, time to first lever press, time to first correct lever press, number of correct and incorrect lever presses and number of lever presses during the intertrial interval.

Secondary Data. Animal performance on the test trial provided several types of data which might indicate more subtle graded, rather than pass-fail, effects on behavior. These measures were: 1) time (sec) to first responses, 2) time (sec) to first correct response, 3) number of correct responses, 4) number of incorrect responses, and 5) mean number of responses per minute during the intertrial interval (ITI). Data on these measures also were collected during the baseline trial period just prior to conduct of the exposure test.

z Scores. Inferential statistical procedures for comparing pre-test (baseline) performance over several trials with test performance on a single trial do not exist. However, the z-score can be used to describe performance on the single test trial with the average performance on the numerous pretest trials. A z score transforms scores so that descriptive comparisons can be made and arithmetic operations can be performed. A z score is the mean minus the particular score with the difference divided by the standard deviation of the mean. For example, if a subject had an average pretest escape time of 4.1 seconds with a standard deviation of 1.1 seconds, and if the post-exposure escape time was 9.4 seconds, then the z score is $(4.1 - 9.4)/1.1$ or $- 4.82$. The normal probability distribution can be used to evaluate how likely such a score is liable to occur; a z score with an absolute value of 1.65 or larger would occur in less than 5 percent of the trials. Thus, in this case, we would assume that the animal's escape time following exposure probably was slower than normal.

RESULTS

CARBON MONOXIDE

EFFECTS ON ESCAPE PERFORMANCE OF PRIMATES

Range-Finding Tests. The results of the four range-finding experiments with CO are summarized in Table 4. In these preliminary tests, average CO concentrations of 4740 and 6450 ppm did not prevent performance of the escape

task, but at concentrations of 7420 and 7434 ppm, the two test subjects were unable to perform the task. In the 7420 ppm experiment, the subject collapsed at approximately 19 seconds after initiation of the escape trial; in the 7434 ppm experiment, the animal collapsed after 3.75 minutes of exposure.

TABLE 4. SUMMARY OF RANGE-FINDING EXPERIMENTS: EFFECT OF CARBON MONOXIDE ON ESCAPE PERFORMANCE OF THE BABOON

Test I.D. No.	Animal I.D. No.	Average CO Concentration (ppm)	Integrated CO Dose (ppm·min)	Test Result	Time of Avoid/ Escape Response (sec)
CO1B	527	4740	23,700	Avoid	6:13
CO3B	634	6450	32,270	Avoid	5:09
CO4B	532	7434	30,830	Fail	--
CO2B	575	7420	37,080	Fail	--

Escape Impairment: Dose-Response Relationship and EC_{50} Value. The results of the range-finding studies suggested a concentration range of 6400 to 8000 ppm as appropriate for dose-response experiments to determine the effective escape impairment concentration for CO in baboons. Therefore, a series of experiments was conducted at five concentrations in this range. The dose-response data from these experiments are shown in Tables 5 and 6. The five concentrations are designated in these tables as "nominal CO concentrations" and represent the concentrations which the investigators attempted to generate and maintain during the 5-minute exposure. The values in the average CO concentration column were obtained by dividing the corresponding integrated CO dose (the integrated area under the concentration-time plot) by the exposure time (5 minutes). The average concentration of CO obtained in each of the experiments is less than the nominal concentration because of the time required for the chamber to reach the desired concentration. However, with few exceptions, the average concentrations are within 5 percent of the nominal values. A typical concentration versus time curve for CO is shown in Figure 9.

Six juvenile baboons were used in the dose-response experiments. Each of these animals was exposed to each of four nominal concentrations of CO (6400, 7200, 7500 and 8000 ppm). In addition, four of these animals were exposed to a fifth nominal concentration, 6800 ppm, of CO; additional experiments at this concentration were not conducted because the four other concentrations were considered adequate to establish a dose-response curve. Although the results of the 6800 ppm (nominal) experiments are shown, these data were not used in deriving the EC_{50} value.

TABLE 5. SUMMARY OF CARBON MONOXIDE DOSE-RESPONSE DATA ON ESCAPE PERFORMANCE IN THE BABOON

Test I.D. No.	Animal I.D. No.	Average CO Concentration (ppm)*	Integrated CO Dose (ppm·min)	Test Result	Observed Time of Incapacitation, (min)	Pre-Exposure Mean Avoid/Escape Time ± S.D. (sec)	Test Avoid/Escape Time, (sec)
			Nominal CO Concentration: 6400 ppm				
CO31B	532	6,060	30,318	Fail	5:00		
CO29B	527	6,130	30,645	Fail	4:55		
CO30B	565	6,160	30,785	Avoid		6.70 ± 3.10	2.54
CO32B	575	6,070	30,345	Avoid		9.19 ± 3.60	4.61
CO28B	500	6,170	30,870	Avoid		5.51 ± 0.97	**
CO27B	634	6,130	30,670	Avoid		7.99 ± 2.02	5.34
Mean ± S. D.		6120 ± 47	30,606 ± 227			7.35 ± 1.59	4.16 ± 1.45
			Nominal CO Concentration: 6800 ppm				
CO23B	532	***					
CO24B	527	6,520	32,590	Fail	4:45		
CO26B	565	6,520	32,600	Fail	5:00		
CO25B	575	6,450	32,240	Avoid		10.42 ± 5.99	4.03
Mean ± S.D.		6500 ± 40	32,477 ± 205			10.42 ± 5.99	4.03 ± 0
			Nominal CO Concentration: 7200 ppm				
CO17B	532	6,850	34,240	Fail	4:50		
CO20B	527	6,800	34,020	Fail	4:50		
CO22B	565	6,840	34,200	Fail	4:50		
CO21B	575	6,860	34,280	Escape		5.80 ± 1.86	12.61
CO19B	500	6,870	34,350	Avoid		5.95 ± 2.16	5.82
CO18B	634	6,800	34,000	Avoid		6.78 ± 1.01	7.20
Mean ± S.D.		6840 ± 30	34,180 ± 142			6.18 ± 0.53	8.54 ± 3.59
			Nominal CO Concentration: 7500 ppm				
CO9B	532	7,060	35,310	Fail	4:45		
CO6B	527	7,140	35,680	Avoid		5.77 ± 1.01	8.12
CO10B	565	6,870	34,360	Fail	5:00		
CO13B	575	7,060	35,310	Fail	4:40		
CO5B	500	6,860	34,310	Escape		6.71 ± 5.07	12.93
CO11B	634	7,050	35,270	Avoid		7.36 ± 2.78	7.17
Mean ± S.D.		7010 ± 114	35,040 ± 566			6.61 ± 0.80	9.41 ± 3.09
			Nominal CO Concentration: 8000 ppm				
CO8B	532	7,590	37,940	Fail	4:20		
CO14B	527	7,590	37,940	Fail	4:45		
CO15B	565	7,520	37,580	Fail	4:50		
CO12B	575	7,450	37,250	Fail	4:55		
CO16B	500	7,570	37,870	Avoid		6.62 ± 2.80	4.18
CO7B	634	7,380	36,890	Avoid		9.12 ± 5.16	5.82
Mean ± S.D.		7520 ± 86	37,580 ± 431			7.87 ± 1.77	5.0 ± 1.16

* Obtained by dividing integrated CO dose by 5 minutes and rounding to nearest 10 ppm.

** Floor switch failed, time of avoidance not recorded.

*** Data not included because of leakage of CO into chamber prior to start of exposure.

TABLE 6. PRE-EXPOSURE AND TEST RESPONSES OF BABOONS IN ESCAPE PERFORMANCE TESTS WITH CARBON MONOXIDE

Test I.D. No.	Animal I.D. No.	Test Result	Pre-Exposure Mean Time ± S.D. to 1st Response (sec)	Test Time to First Response (sec)	Pre-Exposure Mean Time ± S.D. to 1st Correct Response (sec)	Test Time to 1st Correct Resp. (sec)	Pre-Exposure Mean No. ± S.D. of Correct Responses	Test No. of Correct Responses	Pre-Exposure Mean No. ± S.D. of Incorrect Responses	Test No. of Incorrect Responses	Pre-Exposure No. of Mean Responses/min ± S.D. During ITI*	Test No. of Responses/ minute During ITI*
						Nominal CO Concentration: 6400 ppm						
C031B	532	Failed	--	--	--	--	--	--	--	--	0.86 ± 1.11	3.8
C029B	527	Failed	--	--	--	--	--	--	--	--	0.95 ± 0.88	1.2
C030B	565	Avoid	2.68 ± 1.66	0.41	4.23 ± 3.10	0.41	1.17 ± 0.5	1	0.67 ± 0.98	0	1.08 ± 2.05	4.0
C032B	575	Avoid	3.77 ± 1.68	2.83	5.96 ± 3.31	2.83	1.25 ± 0.45	1	**	0	**	**
C028B	500	Avoid	**	**	**	**	**	1	0.09 ± 0.30	0	0.19 ± 0.26	0
C027B	634	Avoid	2.43 ± 1.02	2.33	3.54 ± 2.29	2.33	1.08 ± 0.28	1	0.67 ± 0.65	0	1.9 ± 2.49	0.8
	Mean ± S.D.		2.96 ± 0.71	1.86 ± 1.28	4.58 ± 1.25	1.86 ± 1.28	1.17 ± 0.09	1 ± 0	0.48 ± 0.33	0 ± 0	1.0 ± 0.61	2.45 ± 1.68
						Nominal CO Concentration: 6800 ppm						
C024B	527	Failed	--	--	--	--	--	--	--	--	1.59 ± 1.85	1
C026B	565	Failed	--	--	--	--	--	--	--	--	0.43 ± 0.96	3
C025B	575	Avoid	3.43 ± 1.84	2.25	7.30 ± 4.53	2.25	1.33 ± 0.49	1	2.92 ± 3.68	0	0.52 ± 1.10	1.4
	Mean ± S.D.										0.85 ± 0.64	1.8 ± 1.06
						Nominal CO Concentration: 7200 ppm						
C017B	532	Failed	--	--	--	--	--	--	--	--	2.11 ± 2.49	2.2
C020B	527	Failed	--	--	--	--	--	--	--	--	1.64 ± 1.16	0.8
C022B	565	Failed	--	--	--	--	--	--	--	--	0.35 ± 0.34	4.8
C021B	575	Escape	2.09 ± 0.98	0.59	2.75 ± 1.20	0.59	1.08 ± 0.29	1	0.5 ± 0.67	1	1.38 ± 1.52	3.2
C019B	500	Avoid	2.84 ± 1.33	3.36	3.26 ± 2.37	3.36	1.4 ± 1.26	1	0.1 ± 0.32	0	0 ± 0	0
C018B	634	Avoid	2.16 ± 0.87	3.88	3.22 ± 0.85	3.88	1.0 ± 0.0	1	0.68 ± 4.9	0	0.56 ± 0.68	0
	Mean ± S.D.		2.36 ± 0.41	2.61 ± 1.77	3.08 ± 0.28	2.61 ± 1.77	1.16 ± 0.21	1 ± 0	0.43 ± 0.30	0.33 ± 0.58	1.01 ± 0.82	1.83 ± 1.93
						Nominal CO Concentration: 7500 ppm						
C09B	532	Failed	--	--	--	--	--	--	--	--	1.74 ± 1.98	4.6
C06B	527	Avoid	1.68 ± 0.60	1.82	2.35 ± 1.27	1.82	1.17 ± 0.39	1	0.83 ± 1.03	2	0.91 ± 0.67	1.2
C010B	565	Failed	--	--	--	--	--	--	--	--	3.58 ± 3.72	2.6
C013B	575	Failed	--	--	--	--	--	--	--	--	1.29 ± 1.70	3.6
C05B	500	Escape	1.61 ± 0.28	3.71	4.35 ± 5.18	10.56	1.08 ± 0.29	1	1.17 ± 2.21	3	0.04 ± 0.15	0
C011B	634	Avoid	3.38 ± 2.31	1.74	3.66 ± 2.29	3.90	1.0 ± 0.0	1	0.58 ± 1.16	1	2.42 ± 2.07	2.2
	Mean ± S.D.		2.22 ± 1.00	2.42 ± 1.12	3.45 ± 1.02	5.43 ± 4.57	1.08 ± 0.09	1 ± 9	0.86 ± 0.30	2.0 ± 1.0	1.66 ± 1.23	2.37 ± 1.65
						Nominal CO Concentration: 8000 ppm						
C08B	532	Failed	--	--	--	--	--	--	--	--	1.16 ± 1.11	3.6
C014B	527	Failed	--	--	--	--	--	--	--	--	1.68 ± 1.08	0.8
C015B	565	Failed	--	--	--	--	--	--	--	--	0.73 ± 1.24	1.8
C012B	575	Failed	--	--	--	--	--	--	--	--	4.53 ± 1.13	4.6
C016B	500	Avoid	3.55 ± 2.94	1.36	3.75 ± 2.92	1.36	1.08 ± 0.29	1	0.1 ± 0.32	0	0 ± 0	0
C07B	634	Avoid	2.22 ± 1.07	1.93	3.46 ± 2.11	1.93	1.25 ± 0.62	1	1.0 ± 0.60	0	1.12 ± 2.21	2.0
	Mean ± S.D.		2.89 ± 0.94	1.65 ± 0.40	3.61 ± 0.21	1.65 ± 0.40	1.17 ± 0.12	1 ± 0	0.55 ± 0.64	0 ± 0	1.54 ± 1.57	2.13 ± 1.71

* ITI = Intertrial interval

** Data not available, equipment malfunction

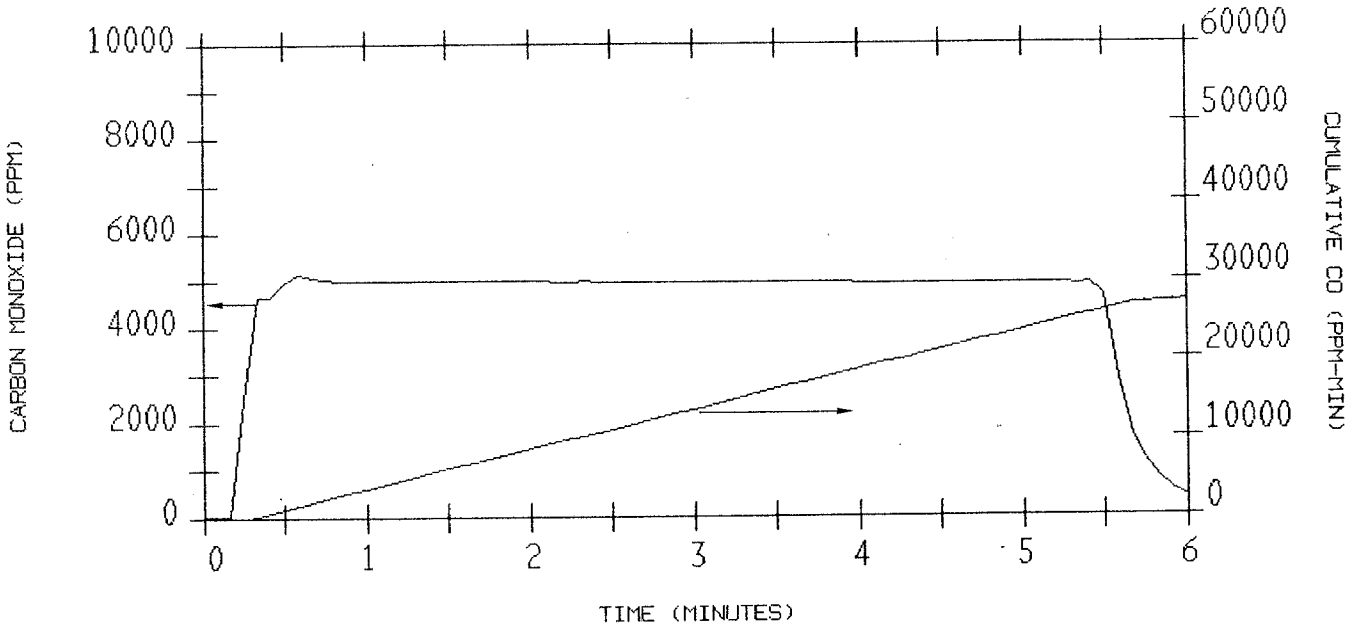

FIGURE 9. A TYPICAL CONCENTRATION VERSUS TIME CURVE FOR CO

Examination of the data in Table 5 reveals a decreasing percentage of escaping animals with increasing average concentrations of CO. At the lowest average concentration (Mean = 6120 ppm), 67 percent of the subjects escaped successfully but, at the highest average concentration (mean = 7520 ppm), only 33 percent of the subjects were able to complete the 5-minute exposure, press the correct lever and exit the chamber. The linear regression equation for the data is Y = .023X - 113, where Y = percent failing to escape and X = mean CO concentration (ppm). The equation is statistically significant ($r = .987$, $p < .009$, S.E. = 2.7, $r^2 = .975$). Using this equation, the estimated mean CO concentration for 50-percent failure is 6792 ppm (Figure 10). In Figure 11, the percentage of failures was plotted against the logarithm of the mean CO concentration and the best-fitting line through the points was derived by the probit method of Finney (reference 12). From this dose-response curve, the EC_{50} was determined to be 6850 ppm (95-percent confidence limits: 6043-7773) for the juvenile baboon. This corresponds to an integrated CO dose of 34,250 ppm-minutes.

Escape and Incapacitation Times. The mean escape times of the subjects (Table 5) which were not incapacitated were examined to determine if there was a relationship between the mean CO concentration and the escape time of the subjects. In this and all subsequent analyses of escape times, both the times to "avoidance" and the times to "escape" are included as escape times. Statistical analysis (linear regresson) of the data did not indicate a significant relationship. The equation for the data is Y = 0.001X - 2.01 with $r = 0.276$, df = 2 and $p < 0.68$ (Figure 12). Note that the square of the correlation coefficient indicates what percent of the variation in the data can be explained by the regression equation. In this case, the r-squared value is 0.076, meaning the line describes only about 8 percent of the variability in

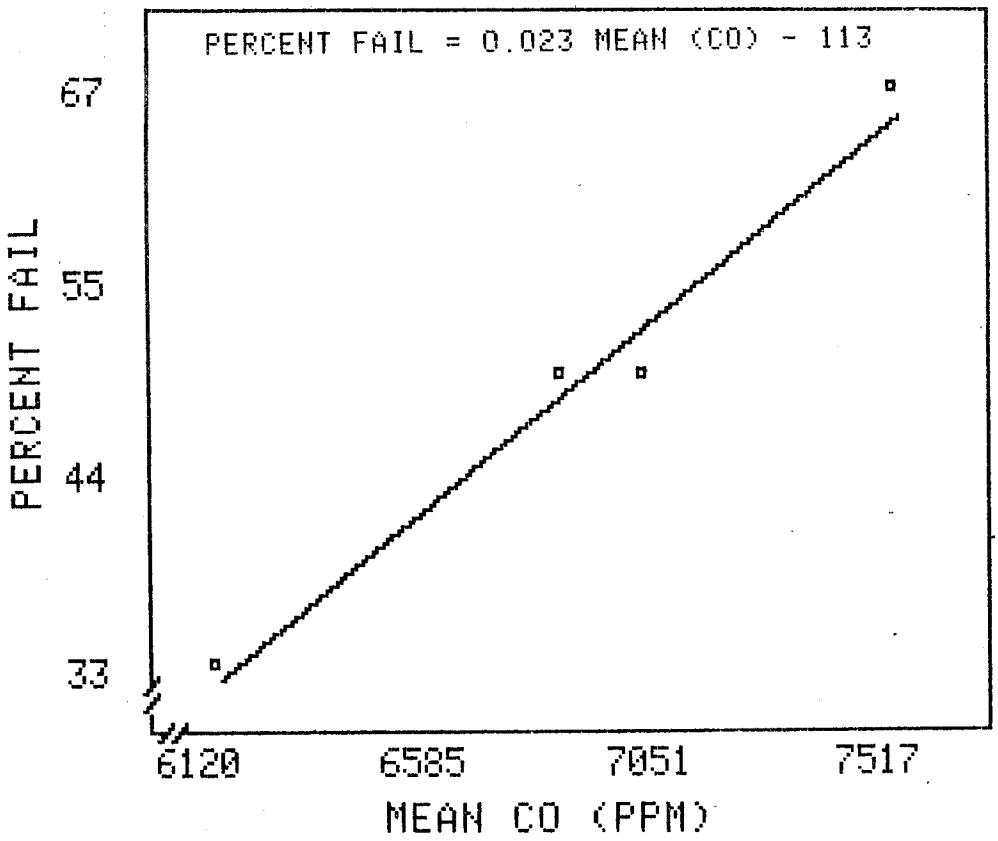

FIGURE 10. PERCENT OF BABOONS FAILING TO ESCAPE AS A FUNCTION OF MEAN CO CONCENTRATION

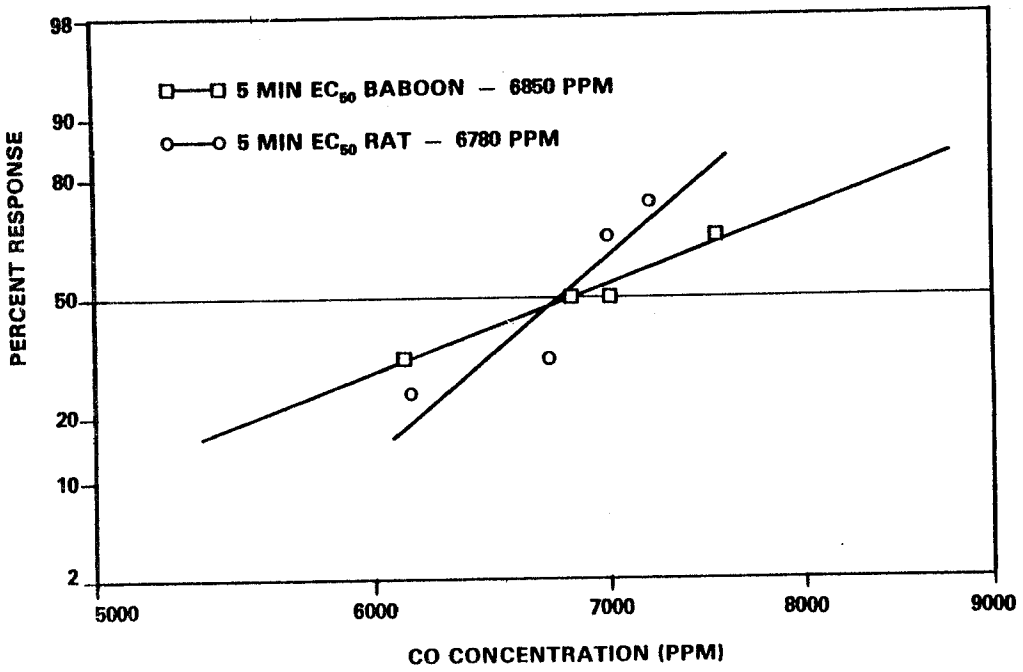

FIGURE 11. DOSE-RESPONSE CURVES AND EC_{50} VALUES FOR ESCAPE IMPAIRMENT OF THE BABOON AND THE RAT BY CARBON MONOXIDE

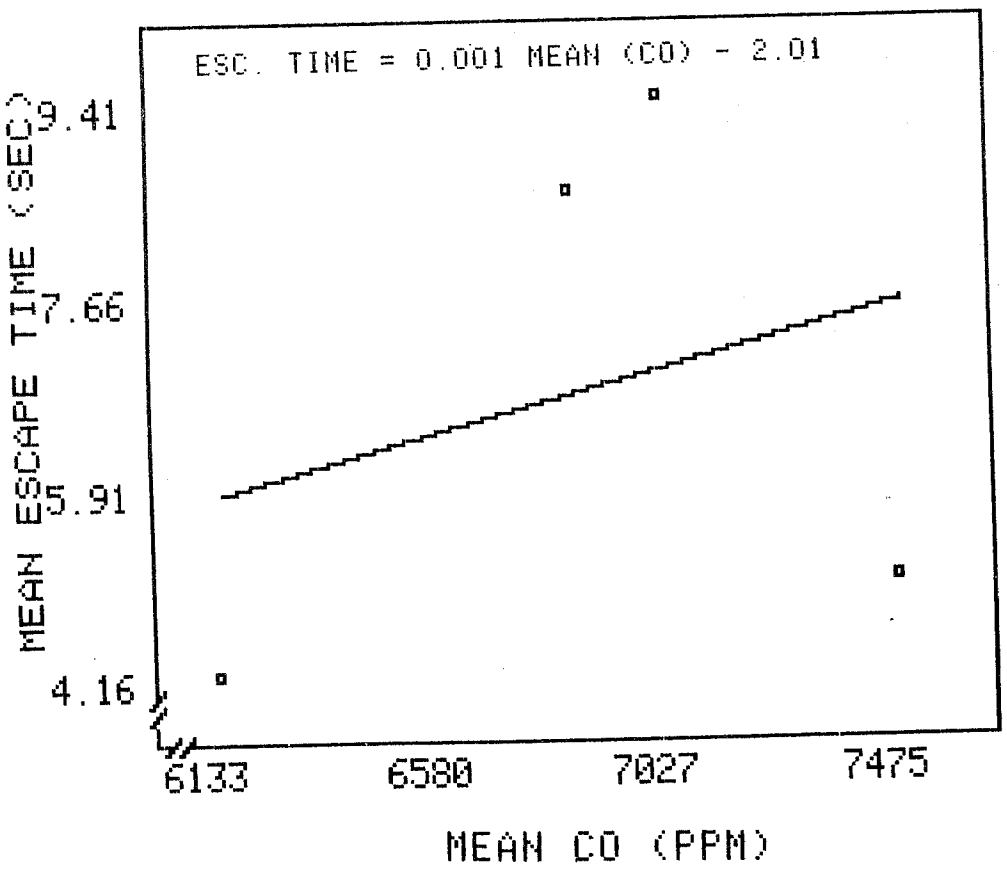

FIGURE 12. MEAN ESCAPE TIME OF BABOONS AS A FUNCTION OF MEAN CO CONCENTRATION

the data. (In contrast, 98 percent of the variability in the escape impairment dose-response data was fitted by the linear regression line).

In order to increase the degrees of freedom for this analysis, an alternative statistical approach was used; the escape times of the subjects were examined as if the times made a continuous distribution, rather than a discontinuous scale with four exposure concentrations. A statistically significant relationship between average exposure concentration of CO and escape time was not observed. The equation for the data is $Y = .001X - 5.81$ ($r = .279$, $p < 0.38$) meaning the data (Figure 13) were apparently random. Thus, the two analyses indicate that the CO concentration does not appear to affect the escape response time of a functioning subject, at least over the range of concentrations tested. Also, analysis of escape times using a z-score approach did not indicate the presence of statistically significant correlations with CO exposure for either individual scores or grouped means.

The same two statistical approaches were used to examine the relationship between the average CO concentration and the times to incapacitation of those subjects which did not escape and were visibly incapacitated by the exposure. With the data grouped according to four mean average CO concentrations (Figure 14), a highly statistically significant relationship ($Y = -.010X + 361.64$, $r = -.999$, $p < .005$) was apparent, indicating that time to incapacitation decreased as the mean of the average CO concentrations increased. When incapacitation times and average CO concentrations from indi-

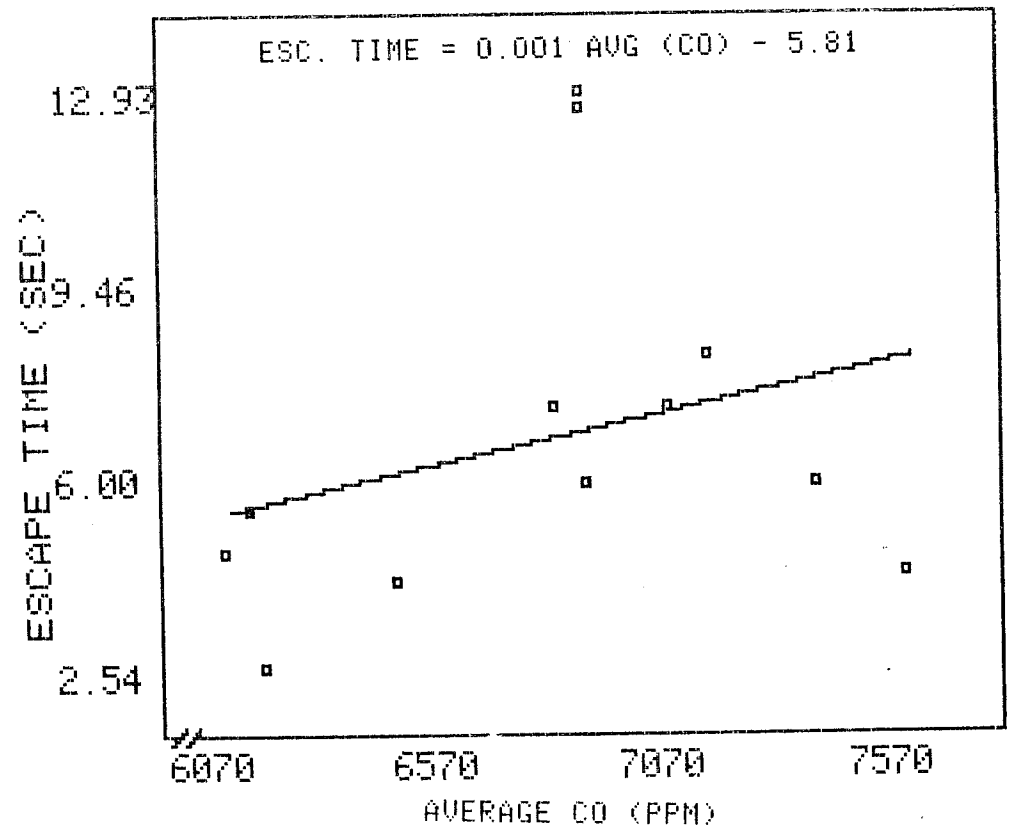

FIGURE 13. ESCAPE TIME OF BABOONS AS A FUNCTION OF AVERAGE CO CONCENTRATION

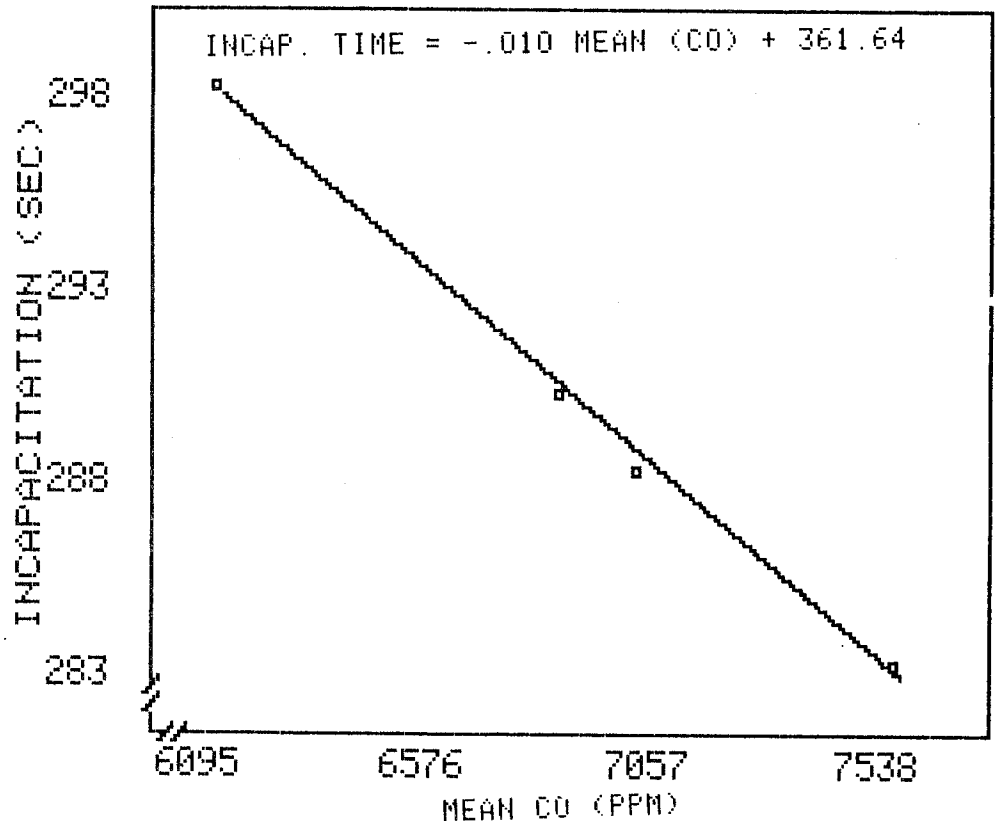

FIGURE 14. MEAN INCAPACITATION TIME OF BABOONS AS A FUNCTION OF MEAN CO CONCENTRATION

vidual tests were analyzed, the relationship between average CO concentration and time to incapacitation was statistically significant, but there was considerable variability among subjects (Figure 15). The equation for the data is $Y = -.011X + 368.78$ ($r = -.56$, $p < .04$, $r^2 = .31$).

Other Performance Measures. Table 6 provides additional data on the performance of baboons as a function of average CO concentration. Data for each of the five dependent variables (time to first response, time to first correct response, number of correct responses, number of incorrect responses, and mean number of responses per minute during the intertrial interval [ITI]) were carefully examined. Whenever it appeared as though there might be a relationship, linear regression analyses, using CO data as X and behavioral data as Y, were completed. Data were examined both grouped by the four average CO concentrations and as individual exposures. In no case were any remotely significant relationships apparent.

Using another approach, test performance data for each of the four mean average CO concentrations were compared statistically with the pre-exposure performance data by the use of z scores. The pre-exposure and test performance data expressed as mean z scores are shown in Table 7. The mean z scores are not large in magnitude and do not follow the mean average CO concentrations in rank order, except for the number of responses per minute during the

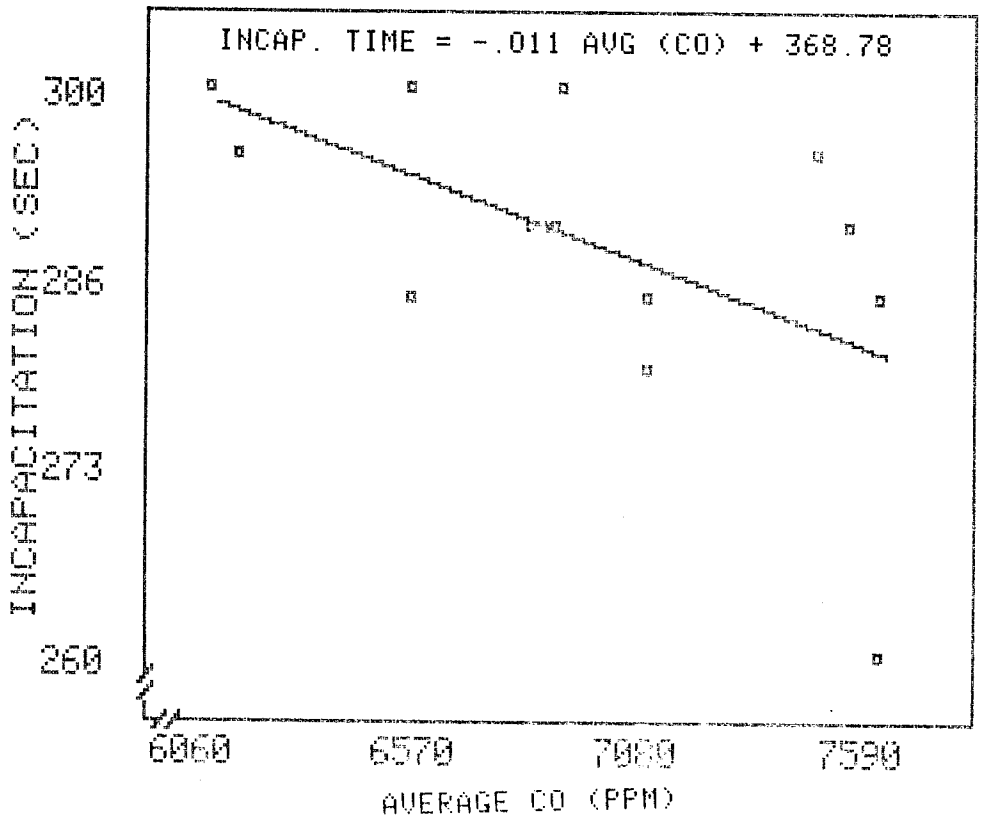

FIGURE 15. INCAPACITATION TIMES OF INDIVIDUAL BABOONS AS A FUNCTION OF AVERAGE CO CONCENTRATION

TABLE 7. PRE-EXPOSURE AND TEST PERFORMANCE DATA OF BABOONS EXPRESSED AS MEAN Z SCORES (CO EXPOSURES)

Mean of Average CO Concentration	Time to First Response	Time to Correct Response	Number Correct Responses	Number Incorrect Responses	Number of Mean Responses/ min During ITI*
6120	0.68	0.90	0.40	0.67	-2.38
6837	-0.28	0.33	0.30	0.84	-1.00
7007	-2.34	-0.29	0.36	-0.78	-0.58
7517	0.51	0.78	0.48	0.99	-0.38

* Intertrial interval.

ITI. With the data grouped according to the four means of the average CO concentrations (Figure 16), a statistically significant ($p < .04$) relationship ($Y=.002X - 11.41$, $r=.955$) was apparent, indicating that the number of ITI responses decreased as CO concentration increased. No other apparent relationships exist between the performance parameters and the CO concentration to which the subjects were exposed.

Clinical Observations. The onset and severity of symptoms exhibited by the subjects varied with the exposure concentration as well as with individual animals. Typical symptoms at lower concentrations, and initial symptoms at the higher concentrations, were yawning, redness of the mucous membranes of the mouth and penile sheath, disorientation, weakness in the legs, and ataxia. Some animals also appeared nauseous and vomited. Depending on the concentration and the test subject, these symptoms progressed to loss of normal posture, staggering, prostration, loss of righting ability, semicomatose state and unconsciousness. Generally, once prostration occurred, the animal rapidly became semicomatose or unconscious and unable to right itself, even with shock administered. In the 11 unconscious animals from which blood samples were obtained, carboxyhemoglobin (COHb) levels ranged from 60.1 to 71.3 percent. Unconscious animals typically returned to a conscious state within 10 to 12 minutes after being placed in the oxygen box.

There was considerable variability among animals in their sensitivity to CO. Certain animals were consistently affected more severely than others and failed to perform the escape task at all or almost all concentrations tested, whereas others successfully performed the task at all concentrations. For example, Baboon No. 532 failed the test at each of four concentrations and Baboon No. 527 failed at all concentrations except one. In contrast, two animals (Nos. 500 and 634) performed the task at all concentrations tested. Differences in activity among different animals were observed during the baseline trials, with some animals continually pacing, others climbing and others engaged in more sedate activities. These differences in activity would

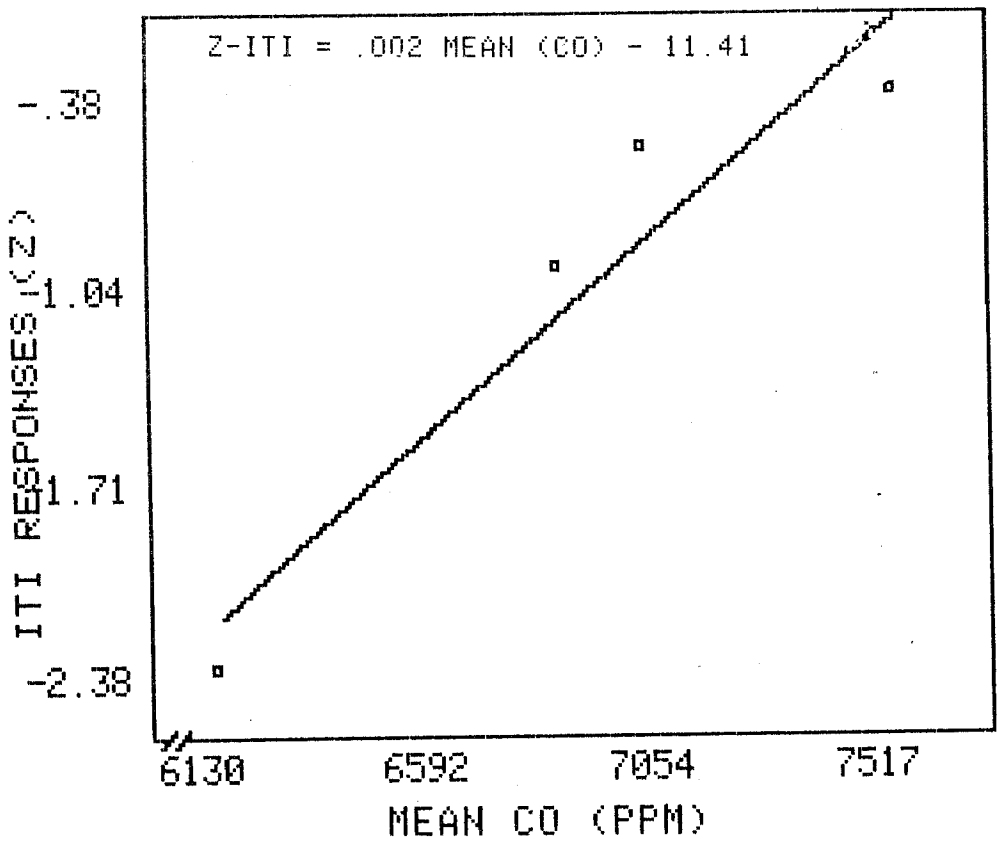

FIGURE 16. MEAN ITI Z SCORE AS A FUNCTION OF MEAN CO CONCENTRATION

affect respiratory minute volume and the rate of uptake of CO. However, it was not possible from the subjective observational data to establish a relationship between the type or degree of activity exhibited by an animal and its apparent sensitivity to CO.

EFFECTS ON ESCAPE PERFORMANCE OF RATS

Escape Impairment: Dose-Response Relationship and EC_{50} Value. To determine the dose-response relationship and EC_{50} value for escape impairment by CO in the rat, four or six animals were exposed for 5 minutes to each of four concentrations of CO. The concentrations selected for these experiments are designated in Table 8 as "nominal concentrations". The average concentrations obtained in these experiments were, with few exceptions, within 5 percent of the nominal values.

Examination of the dose-response data in Table 8 reveals a decreasing percentage of escaping animals with increasing average CO concentrations. At the lowest concentration (mean = 6147 ppm), 75 percent of the subjects were able to escape, while only 25 percent performed the escape task successfully

TABLE 8. SUMMARY OF CARBON MONOXIDE DATA ON ESCAPE PERFORMANCE OF THE RAT

Test I.D. No.	Animal I.D. No.	Average CO Concentration (ppm)*	Integrated CO Dose (ppm·min)	Test Result	Pre-Expo. Mean Avoid/Escape Time ± S.D. (sec)	Test Avoid/ Escape Time ± S.D. (sec)	Pre-Expo. Mean Time ± S.D. to 1st Correct Response (sec)	Test Time to 1st Correct Resp. (sec)	Pre-Exposure Mean No. ± S.D. of Correct Responses	Test No. of Correct Responses	Pre-Exposure Mean No. of Responses/min ± S.D. During ITI**	Test No. of Responses/ minute During ITI**
Nominal CO Concentration: 6400 ppm												
CO-2R	5	6170	30,870	Escape	2.76 ± 2.32	11.95	***	***	1.15 ± 0.37	1	0.56 ± 1.0	0.2
CO-4R	7	6130	30,640	Escape	11.18 ± 4.04	11.15	***	***	1.30 ± 0.48	3	0.0 ± 0.0	0.6
CO-1R	9	6200	30,980	Fail	--	--	***	***	1.0 ± 0	3	0.07 ± 0.20	1.4
CO-3R	2	6090	30,430	Escape	5.47 ± 5.53	13.50	***	***	1.25 ± 0.45	1	0.0 ± 0.0	0
Mean ± S.D.		6147 ± 47.9	30,730 ± 245		6.47 ± 4.3	12.2 ± 1.19			1.18 ± 0.13	2 ± 1.15	0.175 ± 0.27	0.55 ± 0.62
Nominal CO Concentration: 7000 ppm												
CO-10R	5	6700	33,490	Escape	4.26 ± 3.97	22.39	3.34 ± 4.08	13.13	1.08 ± 0.29	1	0.22 ± 0.35	0.2
CO-11R	7	6740	33,690	Escape	6.95 ± 6.26	14.85	4.82 ± 4.79	13.34	1.0 ± 0	1	0.26 ± 0.67	0.8
CO-12R	9	6730	33,640	Escape	8.53 ± 7.56	24.12	7.36 ± 7.71	19.49	1.08 ± 0.29	1	0.09 ± 0.30	1.6
CO-9R	2	6650	33,270	Escape	5.58 ± 5.18	12.98	3.50 ± 4.43	11.53	1.0 ± 0	1	0.09 ± 0.20	0
CO-14R	6	6760	33,790	Fail	--	--	--	--	--	--	0.0 ± 0.0	1.6
CO-13R	3	6780	33,890	Fail	--	--	--	--	--	--	0.17 ± 0.34	3.2
Mean ± S.D.		6727 ± 46.3	33,628 ± 222		6.33 ± 1.83	18.59 ± 5.49	4.78 ± 1.86	14.37 ± 3.5	1.04 ± 0.05	1 ± 0	0.14 ± 0.1	1.23 ± 1.18
Nominal CO Concentration: 7250 ppm												
CO-16R	5	6920	34,580	Fail	--	--	--	--	--	--	1.44 ± 2.22	1.4
CO-17R	7	6950	34,770	Avoid	2.17 ± 0.54	4.17	1.12 ± 0.54	2.50	1.0 ± 0	2	0.26 ± 0.72	1.8
CO-18R	9	7010	35,050	Escape	4.49 ± 3.08	13.22	3.29 ± 3.23	10.07	1.0 ± 0	1	0.05 ± 0.16	0
CO-15R	2	7040	35,220	Fail	--	--	--	--	--	--	0.0 ± 0.0	1
CO-19R	6	7030	35,140	Fail	--	--	--	--	--	--	0.08 ± 0.15	0.8
CO-20R	10	7000	35,020	Fail	--	--	--	--	--	--	0.09 ± 0.3	0.4
Mean ± S.D.		6992 ± 47.1	34,963 ± 242		3.33 ± 1.64	8.7 ± 6.4	2.21 ± 1.53	6.29 ± 5.35	1.0 ± 0	1.5 ± 0.71	0.31 ± 0.56	0.9 ± 0.65
Nominal CO Concentration: 7500 ppm												
CO-6R	5	7480	37,380	Fail	--	--	--	--	--	--	0.04 ± 0.15	2
CO-7R	7	7010	35,030	Fail	--	--	--	--	--	--	0.22 ± 0.35	2.8
CO-8R	9	7200	35,990	Fail	--	--	--	--	--	--	0.04 ± 0.15	1.2
CO-5R	2	7180	35,910	Escape	11.31 ± 5.41	18.92	***	***	1.18 ± 0.40	1	0.0 ± 0.0	0.4
Mean ± S.D.		7219 ± 195	36,078 ± 971								0.075 ± 0.1	1.6 ± 1.03

* Obtained by dividing the CO integrated dose by 5 minutes and rounding to nearest 10 ppm.

** ITI = Intertrial interval

*** Data not available, equipment malfunction

at the highest concentration (mean = 7219 ppm). A linear fit was made to the data (Figure 17); the equation is Y = -.048X + 380.95, with r = -.92, but p <.08. Although the p value is slightly greater than the conventional p <.05, it is quite reasonable to accept this as a significant finding. Estimation of the "50-percent effective dose" for rats using the derived equation suggests that half of the rat population would not escape after a 5-minute exposure to approximately 6660 ppm CO. This value is quite comparable to the EC_{50} value for the rat of 6780 ppm (95-percent confidence limits: 6367-7231) obtained by plotting the percentage of failures against the logarithm of the average CO concentration (Figure 11) and deriving the best-fitting line by the probit method of Finney (reference 12).

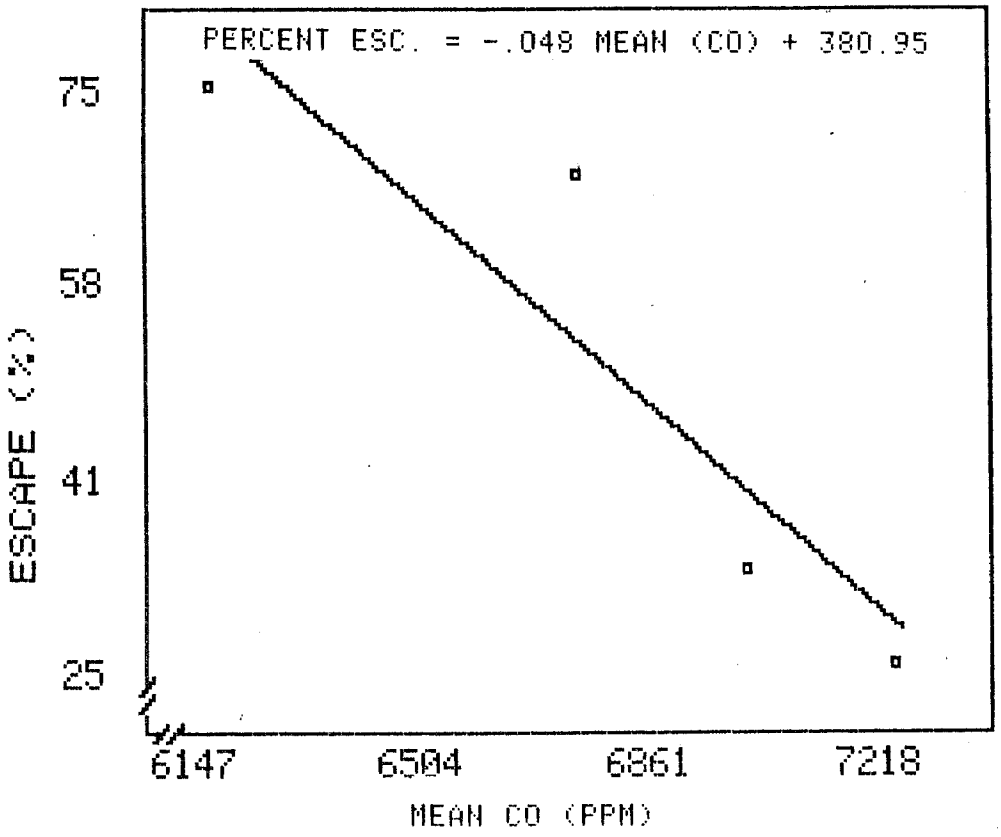

FIGURE 17. PERCENT OF RATS ESCAPING AS A FUNCTION OF MEAN CO CONCENTRATION

Escape Time. The escape times of those subjects (Table 8) which performed the task successfully were examined to determine if there was a relationship between the average CO concentration and the escape time of the animal. For this analysis, the rat escape time data were grouped into three CO concentration clusters by combining data from the nominal 7250 and 7500 ppm groups in order to increase the number of observations per point; this procedure resulted in sample sizes of 3, 4 and 3 for the three mean average CO exposure concentrations. A statistically significant relationship (Y = -.001X + 3.87; r = -1.00; p <.006) was found using z transformed scores (Fig-

ure 18), indicating that escape time increased as the average CO concentration increased.

Other Performance Measures. Rats were observed for incapacitation, but it was difficult to obtain precise time data because of limited visibility into the shuttlebox within the primate exposure chamber. Therefore, statistical analysis of time to incapacitation was not performed. Other test performance measures, such as time to first correct response, mean number of correct responses and number of responses per minute during the intertrial interval, were recorded by the behavioral control system and were analyzed statistically. Comparison of pre-exposure values with test values did not show any statistically significant differences.

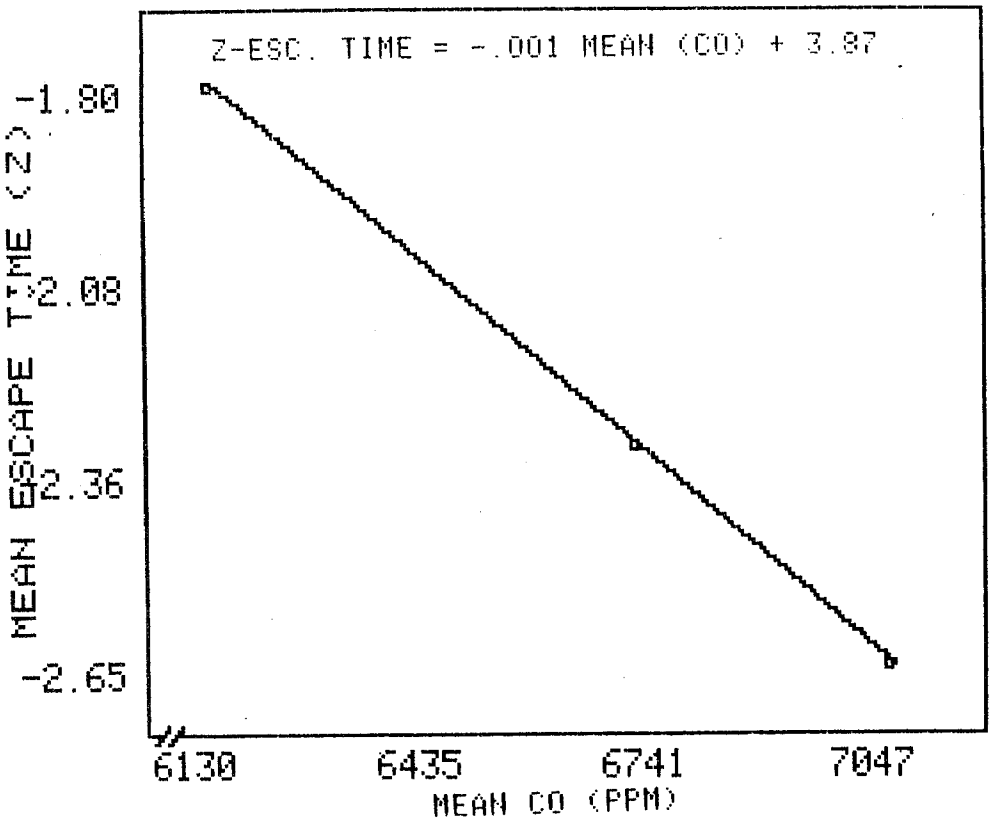

FIGURE 18. MEAN ESCAPE TIME (Z SCORES) OF RATS AS A FUNCTION OF MEAN CO CONCENTRATION

ACROLEIN

EFFECTS ON ESCAPE PERFORMANCE OF PRIMATES

Escape Impairment. Nine baboon tests, in the range of average acrolein concentrations of 12 to 2780 ppm (integrated doses of 60 to 13,900 ppm-min) were conducted (Table 9). A typical acrolein concentration versus time curve is shown in Figure 19. In all exposures except one, the animals pressed the

TABLE 9. EFFECT OF ACROLEIN ON ESCAPE PERFORMANCE OF THE BABOON

Test I.D. No.	Animal I.D. No.	Average Acrolein Conc. (ppm)*	Integrated Acrolein Dose (ppm·min)	Test Result	Pre-Exposure Mean Avoid/ Escape Time ± S.D. (sec)	Test Avoid/ Escape Time (sec)
AC-1B	565	12	60	Avoid	6.32 ± 2.41	4.94
AC-2B	500	25	120	Avoid	5.03 ± 0.41	6.50
AC-3B	634	95	480	Fail**	--	--
AC-4B	532	100	510	Avoid	5.88 ± 1.23	5.83
AC-5B	500	250	1240	Avoid	5.79 ± 1.36	5.17
AC-6B	565	505	2530	Avoid	6.84 ± 1.84	5.06
AC-8B	527	505	2530	Avoid	6.55 ± 1.68	4.72
AC-7B	575	1025	5120	Avoid	5.94 ± 1.69	3.95
AC-9B	532	2780	13,900	Avoid	6.59 ± 1.72	4.34
				Mean ± S.D.	6.12 ± 0.58	5.06 ± 0.81

* Obtained by dividing acrolein integrated dose by 5 minutes and rounding to nearest 5 ppm.

** Animal not shocked.

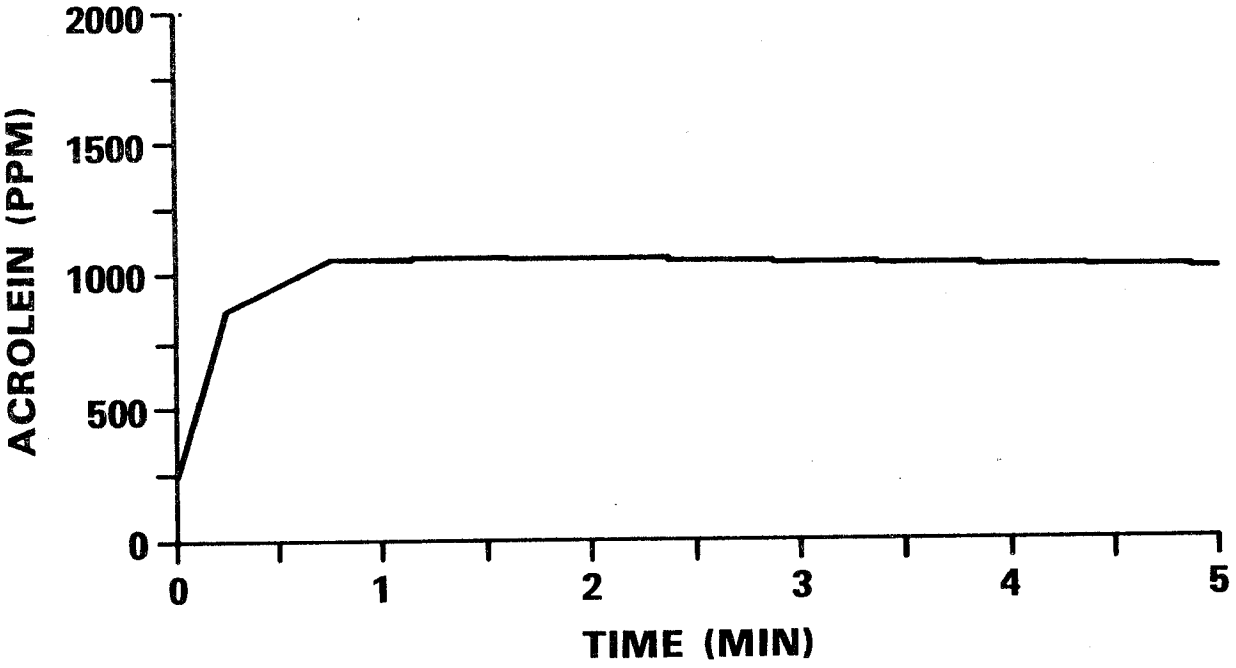

FIGURE 19. A TYPICAL CONCENTRATION VERSUS TIME CURVE FOR ACROLEIN

correct lever and exited the chamber within 10 seconds and "avoided" shock. In the one exception (95 ppm), the animal moved to the incorrect lever, clutched the lever and placed both feet on one bar of the floor grid, thereby avoiding the shock. The subject was mobile and did not exhibit any apparent signs of incapacitation, although it did appear somewhat confused. Although the performance is designated a "failure," the test result may be invalid because of the absence of the shock stimulus. However, the fact that, in all eight other tests, the animals made their correct lever press prior to initiation of shock argues against the significance of the animal's avoidance of shock. Thus, in the nine tests, one animal failed to perform the escape task (perhaps due to the absence of shock), while eight others successfully escaped prior to shock ("avoidances"). It is significant that most of those animals that escaped were exposed to much higher concentrations of acrolein than the animal that failed.

Escape Time. The escape times of the eight animals (Table 9) which performed the task successfully were examined to determine if there was a relationship between the escape time of the subject and the average acrolein concentration. The escape times do not appear to be related strongly to the acrolein concentration (Figure 20): the fitted equation is $Y = -.001067X + 5.41$; $r = -.61$; $df = 6$, $p < .11$). The visual impression given by the data is not impressive, and the data do not allow an impressive statistical analysis because linear regressions are sensitive to the ends, and the values tested here are not evenly distributed across the range of observations.

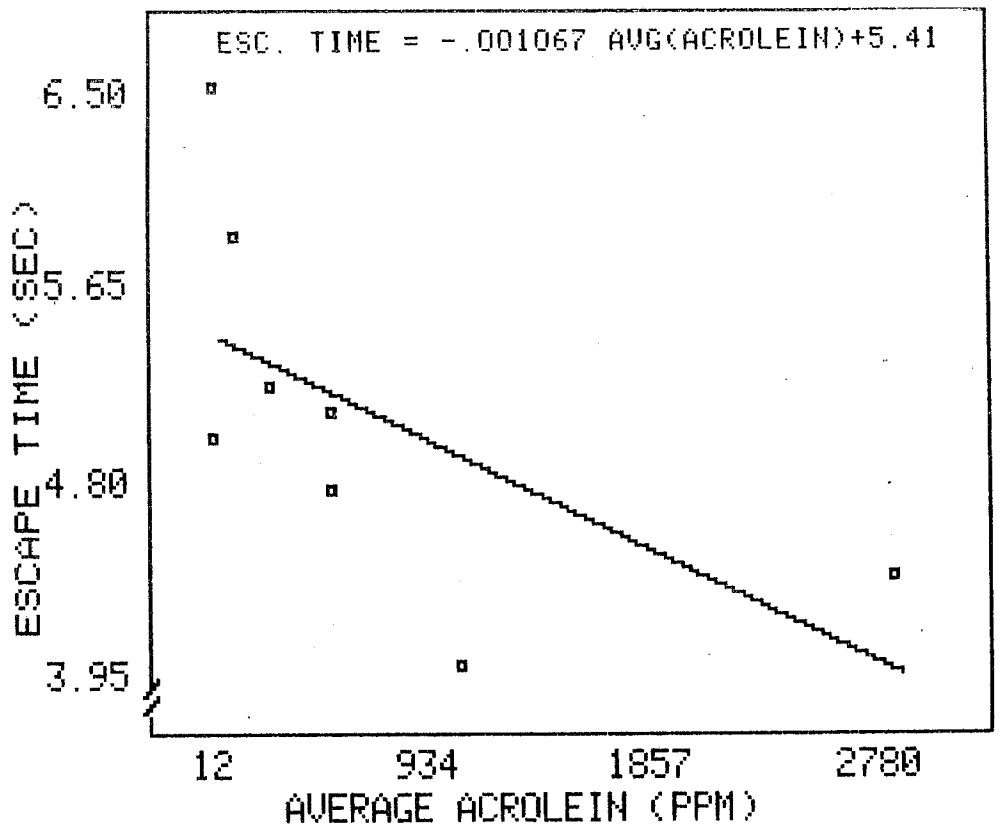

FIGURE 20. ESCAPE TIMES OF BABOONS AS A FUNCTION OF AVERAGE ACROLEIN CONCENTRATION

Escape times were also examined to determine if there was a difference between pre-exposure and test values. The mean of the average escape times of the eight animals in pre-tests was 6.12 ± 0.58 seconds, and the mean escape time following acrolein exposure was 5.06 ± 0.81 seconds, only slightly less. In addition, pre-exposure and test escape times of individual subjects were compared using z scores. There was no evidence that exposure to acrolein affects escape time. Only one of the eight z-scores was greater than 1.65, and the mean of the eight scores was 0.25. Animal No. 500, when exposed to 25 ppm acrolein, was almost 1.5 seconds slower than before exposure; the small standard deviation of 0.41 resulted in a z score of -3.59. When No. 500 was tested again, with an acrolein concentration of 250 ppm, the animal was more than 0.5 seconds faster than pre-exposure, with a z-score of +0.46.

Other Performance Measures. The other performance measures in Table 10 were also examined to determine if there were any significant differences between pre-exposure and test values. No statistically significant correlations were observed between acrolein exposure and any of the behavioral measures. Although acrolein is a potent irritant, it does not appear to have acute effects on the performance of the baboon.

TABLE 10. COMPARISON OF PRE-EXPOSURE AND TEST RESPONSES OF BABOONS IN ESCAPE PERFORMANCE TESTS WITH ACROLEIN

Test I.D. No.	Animal I.D. No.	Test Result	Pre-Exposure Mean Time ± S.D. to First Response (sec)	Test Time to First Response (sec)	Pre-Exposure Mean Time ± S.D. to 1st Correct Response (sec)	Test Time to 1st Correct Response (sec)	Pre-Exposure Mean No. ± S.D. Correct Responses	Test No. of Correct Responses	Pre-Exposure Mean No. ± S.D. of Incorrect Responses	Test No. of Incorrect Responses	Pre-Exposure Mean No. of Responses/min ± S.D. During ITI*	Test No. of Responses/ minute During ITI*
AC-1B	565	Avoid	1.74 ± 0.91	1.08	3.00 ± 2.42	1.45	1.67 ± 0.98	3	1.33 ± 1.67	1	1.42 ± 1.38	2.6
AC-2B	500	Avoid	1.78 ± 0.37	3.16	1.78 ± 0.37	3.16	1.0 ± 0	1	0	0	0.0 ± 0.0	0
AC-3B	634	Fail	--	--	--	--	--	--	--	--	0.61 ± 0.82	0
AC-4B	532	Avoid	2.65 ± 1.28	3.09	2.65 ± 1.28	3.09	1.09 ± 0.3	1	0	0	0.75 ± 1.07	0.6
AC-5B	500	Avoid	2.27 ± 1.41	2.05	2.29 ± 1.41	2.05	1.09 ± 0.3	1	0	0	0.0 ± 0.0	0
AC-6B	565	Avoid	2.57 ± 1.46	1.71	3.66 ± 1.81	1.71	1.25 ± 0.45	1	1.25 ± 1.66	0	0.04 ± 0.15	0.4
AC-8B	527	Avoid	1.16 ± 0.55	0.85	1.67 ± 1.38	0.85	1.33 ± 0.65	1	1 ± 0.85	0	1.85 ± 1.66	3.6
AC-7B	575	Avoid	1.70 ± 0.87	1.10	2.55 ± 1.31	1.10	1.17 ± 0.39	1	0.92 ± 0.9	0	2.84 ± 0.81	4
AC-9B	532	Avoid	2.41 ± 1.82	1.76	2.41 ± 1.82	1.80	1.09 ± 0.3	1	0	1	1.51 ± 2.30	1.8
	Mean ± S.D.		2.04 ± 0.52	1.85 ± 0.88	2.5 ± 0.64	1.9 ± 0.85	1.21 ± 0.21	1.25 ± 0.71	0.56 ± 0.61	0.25 ± 0.46	1.00 ± 0.98	1.44 ± 1.61

* ITI = Intertrial interval

Clinical Observations. The clinical observations of baboons exposed to acrolein are summarized in Table 11. At lower concentrations, symptoms exhibited by animals included blinking of eyes, closure of eyes, twitching of nose and rubbing of eyes and nose. As concentrations increased, these symptoms became more pronounced, and violent shaking of the head, salivation, nasal exudate and signs of nausea were evident. No post-exposure symptoms were evident in the subjects except for one of the two animals exposed to 505 ppm (depressed appetite for two days) and the two animals exposed to the highest concentrations of acrolein. The animal exposed to 1025 ppm died the day after exposure and the animal exposed to 2780 ppm died approximately one and one-half hours following the exposure.

Pathology/Histopathology. There were two fatalities among the baboons exposed to acrolein. Baboon No. 575 was exposed in Test No. AC-7B to an average concentration of 1025 ppm acrolein at 1400, April 27, 1983. The animal did not exhibit immediate clinical problems after exposure except for vomiting, salivation and nasal exudate. No signs of respiratory difficulty were apparent. Treatment following exposure included oxygen, Flocillin and Metacorten. At approximately 1600, April 28, 1983 the animal collapsed and died. The necropsy was performed by the project veterinarian; his findings were pulmonary edema, gastric dilatation and toxic nephritis. Histopathologic examination of major organs was performed by a board-certified veterinary pathologist. The results of his examination are summarized as: lung, severe diffuse edema; liver, severe congestion; spleen, severe lymphoid depletion; kidney, severe nephrosis; and trachea, necrosis and sloughing of epithelial lining.

The other baboon lethality resulting from exposure to acrolein was Baboon No. 432. This animal was exposed to an average concentration of 2780 ppm on May 13, 1983. During the 5-minute exposure, the animal appeared to be in severe respiratory distress, with profuse mucoid discharge and froth from the nose, coughing and mouth breathing evident. After exposure, the animal was in severe dyspnea, with froth coming from the nose and mouth. Treatment consisted of oxygen and Azium. The animal expired approximately 1.5 hours after exposure and was necropsied. The most significant finding was the severe edematous and hemorrhagic condition of the lungs. Histopathologic examination of the major organs was performed by the veterinary pathologist. His findings were consistent with the histopathology observed with Baboon No. 575, except that the lesions were generally more severe in this baboon and lesions were not observed in the kidneys.

HYDROGEN CHLORIDE

EFFECTS ON ESCAPE PERFORMANCE OF PRIMATES

Escape Impairment. Eight exposure tests were conducted, using seven different baboons, with average HCl concentrations ranging from 190 to 17,290 ppm (integrated doses of 930 to 86,470 ppm-min) (Table 12). A typical concentration versus time curve for HCl is shown in Figure 21. All animals were able to exit the test chamber following 5 minutes of exposure. In six tests, the animals made a correct lever response and exited within 10 seconds after presentation of the visual and audio cues, thus avoiding the shock. In the other two tests, the animals received a shock stimulus but did escape. Thus,

TABLE 11. CLINICAL OBSERVATIONS OF BABOONS DURING EXPOSURE TO ACROLEIN AND POST EXPOSURE

Test I.D. No.	Animal I.D. No.	Average Acrolein Concentration, ppm	Clinical Observations During and Post Exposure
AC-1B	565	12	Rapid blinking of eyes initially, which decreased during exposure; nose twitching. No post-exposure symptoms.
AC-2B	500	25	Rapid blinking of eyes initially, then closure of eyes during most of exposure; rubbing of eyes and nose. No post-exposure symptoms.
AC-3B	634	95	Initial blinking and rubbing of eyes, then closed eyes; at tone, fumbled with and hung on to incorrect lever; appeared disoriented and sight appeared to be impaired; no post-exposure symptoms.
AC-4B	532	100	Immediate response of blinking and rubbing of eyes, shaking of head and curling of lips; partial closure of eyelids during exposure. No post-exposure symptoms.
AC-5B	500	250	Signs of severe irritation (rubbing nose, eyes, moving rapidly and crawling) immediately and during test; eyes closed during exposure. No post-exposure symptoms.
AC-6B	565	505	Signs of severe irritation (blinking and rubbing of eyes, shaking head, moving rapidly) immediately and during test; appeared slightly sedate and nauseated. Reddened conjunctiva post exposure. Depressed appetite for 2 days following exposure.
AC-8B	527	505	Signs of severe irritation (closing eyes, rubbing nose, shaking head, moving rapidly), immediately and during exposure; signs of mouth breathing, salivation, gagging and attempts to vomit. No post-exposure symptoms.
AC-7B	575	1025	Signs of severe irritation (closing eyes, rubbing nose, moving rapidly) immediately and during exposure; salivation and signs of nausea. Post-exposure signs of salivation and nasal exudate, no dyspnea or coughing. Following morning, animal slightly depressed but ate fruit. No immediate signs of respiratory difficulty. Died approximately 4:00 PM the next day.
AC-9B	532	2780	Signs of severe irritation, rubbing eyes, coughing, mouth breathing and salivation, moving rapidly, nausea. Animal was in severe respiratory distress throughout test. Animal in critical condition following exposure. Immediately post exposure, profuse mucoid salivation, coughing and mouth breathing. Died (still in O_2 box) approximately 1.5 hours post-exposure.

TABLE 12. EFFECT OF HCl ON ESCAPE PERFORMANCE OF THE BABOON

Test I.D. No.	Animal I.D. No.	Average HCl Concentration (ppm)*	Integrated HCl Dose (ppm·min)	Test Result	Pre-Exposure Mean Avoid/ Escape Time ± S.D.	Test Avoid/ Escape Time (sec)
HCl-1B	527	190	930	Avoid	5.39 ± 1.38	4.46
HCl-3B	500	810	4,030	Avoid	5.08 ± 0.96	4.40
HCl-4B	527	890	4,450	Avoid	7.74 ± 1.74	5.20
HCl-2B	565	940	4,690	Avoid	4.98 ± 2.42	4.21
HCl-5B	844	2,780	13,880	Avoid	6.04 ± 1.25	7.75
HCl-6B	833	11,400**	57,020	Escape	6.66 ± 2.14	16.31
HCl-8B	634	16,570	82,850	Avoid	8.26 ± 3.57	5.86
HCl-7B	861	17,290	86,470	Escape	8.15 ± 3.46	10.93
				Mean ± S.D.	6.54 ± 1.37	7.39 ± 4.26

* Obtained by dividing integrated HCl dose by 5 minutes and rounding to nearest 10 ppm.

** HCl data based on analysis of soda lime absorption tubes, except Test No. 6B which is based on syringe/IC method.

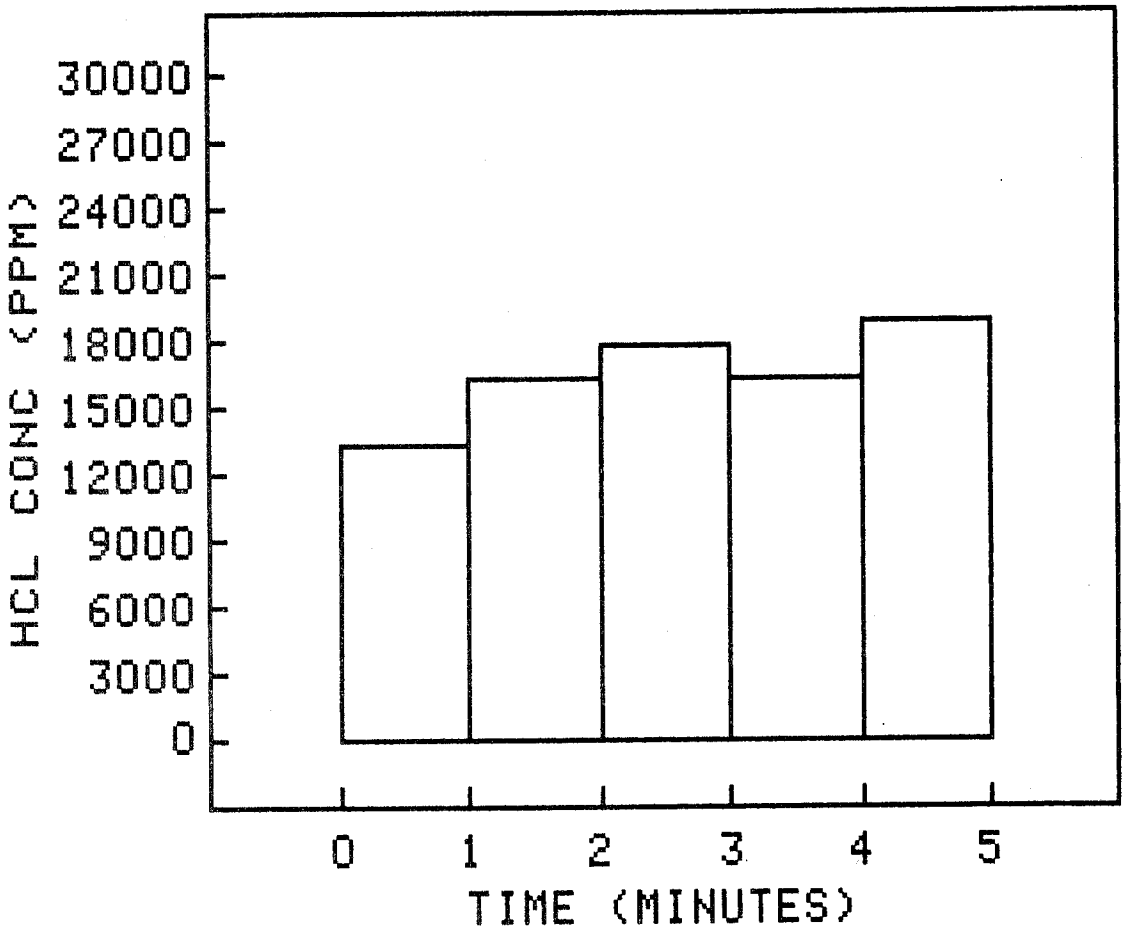

FIGURE 21. A TYPICAL CONCENTRATION VERSUS TIME CURVE FOR HYDROGEN CHLORIDE

escape impairment, defined as failure to exit the exposure cage within 30 seconds, did not occur in any of the animals, even though average concentrations of HCl exceeded 1.6 and 1.7 percent in two tests.

Escape Time. The escape times of the eight animals (Table 12) were examined to determine if there was a relationship between escape time and average HCl concentration. The escape times of the eight subjects appeared to increase as the average concentration of HCl increased (Figure 22). The regression equation is $Y = -0.0000697X + 5.26$, and $r = .59$; with only 6 df, the probability that the slope is non-zero is $p < .12$, a value usually not considered to be statistically significant. The pre-exposure and test escape times were also examined to determine whether exposure to HCl affected escape time. The mean of the pre-test average escape times of the eight subjects was 6.54 ± 1.37 seconds, while the mean of the test escape times was 7.39 ± 4.26 seconds. The difference between these escape times is not statistically significant.

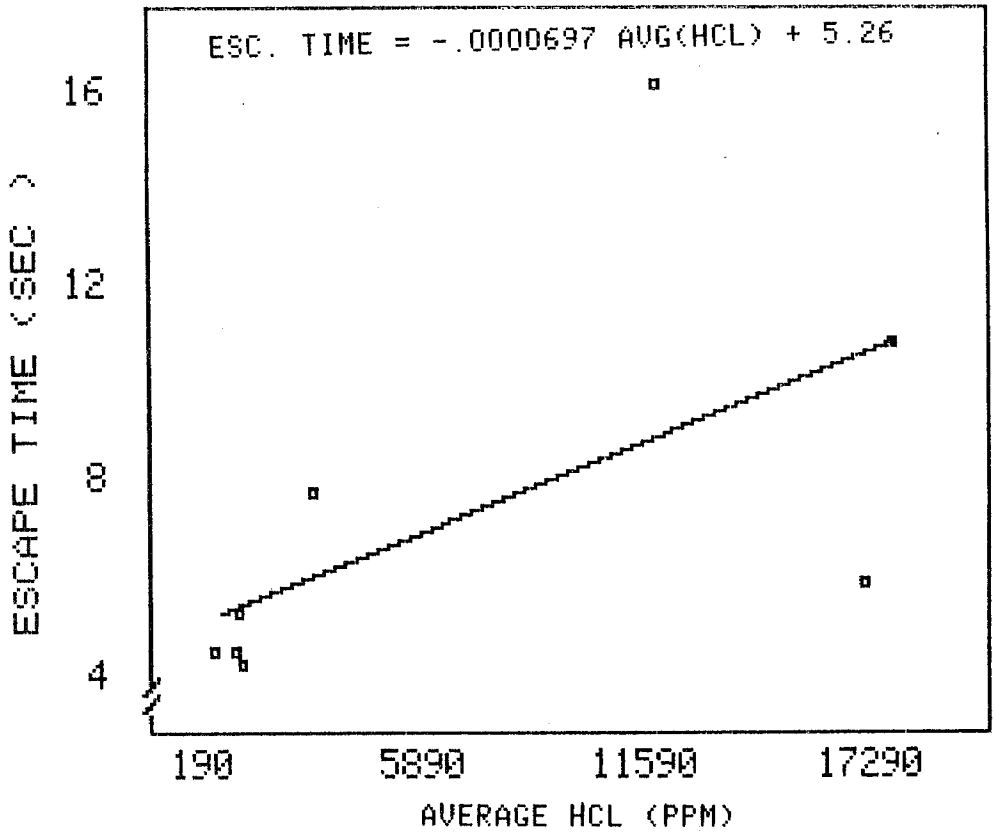

FIGURE 22. ESCAPE TIMES OF BABOONS AS A FUNCTION OF AVERAGE HCl CONCENTRATION

Other Performance Measures. The other performance measures in Table 13 were examined to determine if a relationship existed between any measure and the HCl concentration. The eight individual animal values for time to first response, time to first correct response, number of correct and incorrect responses, and response rate during the ITI were tested for correlations with

TABLE 13. COMPARISON OF PRE-EXPOSURE AND TEST RESPONSES OF BABOONS IN ESCAPE PERFORMANCE TESTS WITH HYDROGEN CHLORIDE

Test I.D. No.	Animal I.D. No.	Test Result	Pre-Exposure Mean Time ± S.D. to First Response (sec)	Test Time to First Response (sec)	Pre-Exposure Mean Time ± S.D. to 1st Correct Response (sec)	Test Time to 1st Correct Response (sec)	Pre-Exposure Mean No. ± S.D. of Correct Responses	Test No. of Correct Responses	Pre-Exposure Mean No. ± S.D. of Incorrect Responses	Test No. of Incorrect Responses	Pre-Exposure Mean No. of Responses/min ± S.D. During ITI*	Test No. of Responses/ minute During ITI*
HCl-1B	527	Avoid	1.47 ± 0.91	0.87	1.76 ± 1.16	0.87	1.17 ± 0.34	1	0.83 ± 0.72	1	2.97 ± 3.03	2.8
HCl-3B	500	Avoid	1.54 ± 0.24	1.48	1.54 ± 0.24	1.48	1.0	2	0	0	0.0 ± 0.0	1.8
HCl-4B	527	Avoid	2.61 ± 1.05	1.78	3.50 ± 1.39	1.78	1.12 ± 0.35	1	0.87 ± 1.23	2	0.97 ± 2.13	2.6
HCl-2B	565	Avoid	1.38 ± 1.43	1.42	2.22 ± 1.92	1.42	1.55 ± 0.70	1	0.94 ± 0.94	1	2.56 ± 2.22	1.2
HCl-5B	844	Avoid	2.04 ± 1.26	3.46	2.83 ± 1.24	3.67	1.15 ± 0.37	3	1.38 ± 0.96	1	2.78 ± 1.29	9.2
HCl-6B	833	Escape	2.14 ± 0.99	10.00	3.85 ± 2.05	13.43	1.15 ± 0.37	1	1.31 ± 1.25	6	8.20 ± 5.44	11
HCl-8B	634	Avoid	1.42 ± 0.38	2.38	3.93 ± 3.77	2.38	1.43 ± 0.51	1	2.43 ± 2.38	0	0.04 ± 0.14	1.4
HCl-7B	861	Escape	4.20 ± 1.95	1.93	5.44 ± 3.43	7.07	1.21 ± 0.43	1	2.21 ± 2.61	1	0.33 ± 0.66	9.6
	Mean ± S.D.		2.1 ± 0.95	2.92 ± 2.96	3.13 ± 1.31	4.01 ± 4.29	1.22 ± 0.18	1.38 ± 0.74	1.25 ± 0.79	1.5 ± 1.93	2.24 ± 2.71	4.95 ± 4.19

* ITI = Intertrial interval

average HCl concentration. No statistically significant dose-related effects on performance were detected. In addition, the pre-exposure values of these performance measures and the test values were evaluated using z scores. The data indicated the presence of one statistically significant relationship. As the concentration of HCl increased, the z score for ITI responses became increasingly negative (Figure 23), and linear regression analysis indicated a significant relationship of an increasing number of ITI responses with increasing concentration ($Y = -.000602 + .11$, $r = .81$, $p < .03$). Figure 23 contains only seven data points because a z score could not be computed for animal Test I.D. No. HCl-3B; this animal never made any responses in the intertrial intervals during the baseline trials (Table 13).

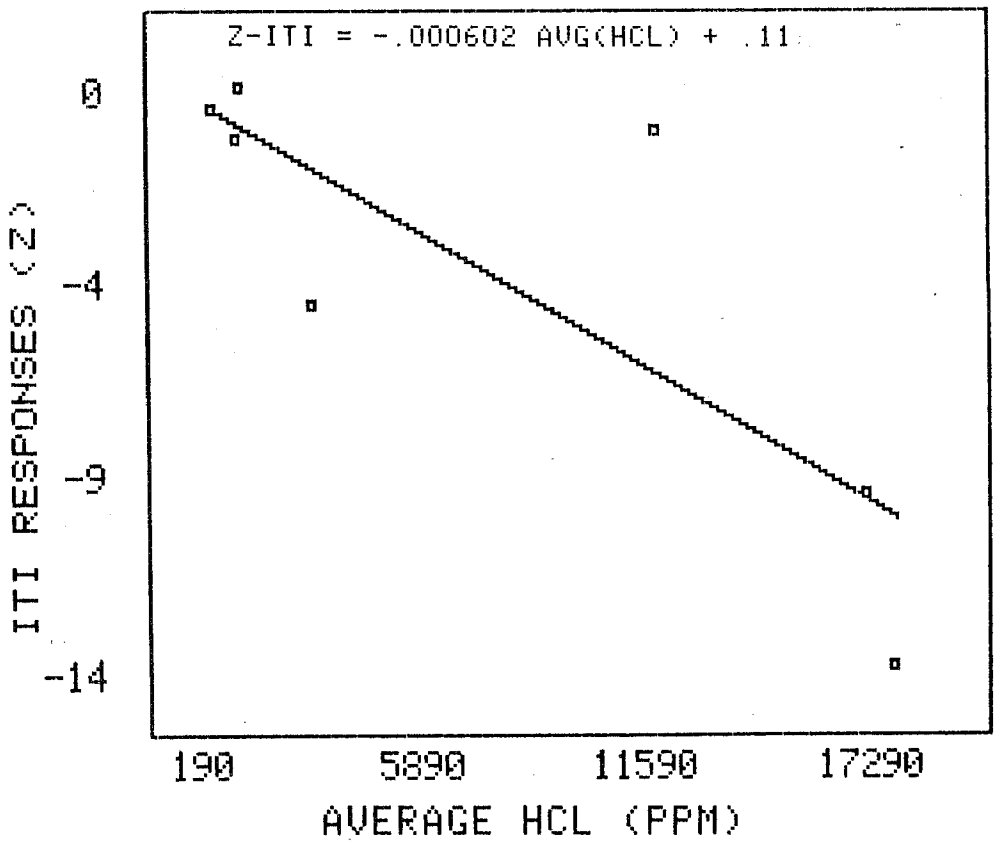

FIGURE 23. ITI RESPONSES (Z SCORES) OF BABOONS AS A FUNCTION OF AVERAGE HCl CONCENTRATION

Clinical Observations. The clinical observations of the baboons exposed to HCl are summarized in Table 14. At the lowest concentration (190 ppm), the animal did not exhibit any symptoms or post-exposure effects. At higher concentrations, immediate agitation of the animal was observed, followed by frothing and salivation from the mouth and coughing. At the highest concentrations, these symptoms were more pronounced, and shaking of the head, frequent coughing and blinking and rubbing of the eyes were observed. Post-exposure effects were not observed in animals exposed to concentrations from 190 to 940 ppm; in the animal exposed to 2780 ppm, a dry cough persisted for a

TABLE 14. CLINICAL OBSERVATIONS OF BABOONS DURING EXPOSURE TO HCl AND POST EXPOSURE

Test I.D. No.	Animal I.D. No.	Avg. HCl Concentration (ppm)	Clinical Observations During and Post Exposure
HCl-1B	527	190	No reaction to HCl observed during exposure. No post-exposure symptoms.
HCl-2B	565	940	Immediate agitation. Froth appeared at mouth approximately 40 seconds, followed by gagging motions and coughing which continued for remainder of exposure. No significant post-exposure symptoms.
HCl-3B	500	810	Immediate agitation; froth appeared at mouth approximately 45 seconds, followed by slight coughing. These signs continued for the remainder of the exposure. No significant post-exposure symptoms.
HCl-4B	527	890	Slight increase in activity level. Salivation observed at 1 minute, but did not persist for the exposure duration; no significant post-exposure symptoms.
HCl-5B	844	2,780	Animal immediately began to cough and choke. By 30 seconds, mouth is open and copious salivation began. Continued to cough and salivate for remainder of the exposure. In addition, animal blinked eyes and occasionally vocalized. No significant post-exposure symptoms except for dry cough immediately after exposure.
HCl-6B	833	11,400	Animal immediately began to cough, blink and rub eyes, and salivate. Frequent, periodic coughing, sneezing and head shaking continued for the duration of exposure. Cough and froth from nose/mouth after exposure, responded to treatment over 3 to 4 days. Slightly raspy breathing when animal is excited has persisted.
HCl-7B	861	17,290	Animal immediately began to salivate, blink eyes, rub his muzzle and shake his head. Animal also displayed an initial increase in activity. The animal continued to cough, shake head and salivate for the duration of exposure. The animal's eyes were closed for majority of the exposure. First day post exposure, the animal had respiratory difficulties, mucous discharge from nose, and cyanosis. Then symptoms later faded to slightly raspy breathing. Dyspnea continued, receded and appeared periodically for the next 2-1/2 months. Died 76 days after exposure.
HCl-8B	634	16,570	Animal immediately began to salivate and violently shake head. Animal continued to salivate heavily, keep mouth open, close eyes and shake head throughout the exposure. Salivation and eye irritation were still evident in the immediate post exposure period. First day post exposure, the animal suffering from extreme dyspnea. The dyspnea, accompanied by mucous discharge, continued for approximately two weeks, then worsened and the animal died 18-days post exposure.

few days after exposure. In the animal exposed to 11,400 ppm, the post-exposure cough and froth from the nose and mouth responded to treatment in 3 to 4 days, but slightly raspy breathing persisted for several months after exposure. In the two animals exposed to the highest concentrations of HCl, dyspnea was evident following exposure and continued until death at 76 days and 18 days following exposure.

Pathology/Histopatholgy. There were two fatalities among the baboons exposed to HCl. These occurred in animals exposed to the two highest concentrations. Both animals exhibited dyspnea immediately after exposure. In Baboon No. 634 (16,570 ppm), severe dyspnea, which worsened despite treatment (Longicil, Azium and Flocillin), was observed for approximately two weeks, and the animal died 18-days post exposure. Necropsy and histopathologic examination of the animal were performed by a board-certified veterinary pathologist; the major findings were acute severe pneumonia, severe tracheitis with virtual obliteration of the normal mucosa, focal hemorrhage of the lymph nodes and epicarditis. Bacteriologic examination of the lungs revealed the presence of Staphylococcus aureus and Corynebacteria which are not considered significant and of Diplococcus which is a pneumonia-causing bacterium.

The other fatality was Baboon No. 861 which was exposed to 17,290 ppm HCl. Dyspnea, mucous discharge from the nose and severe eye irritation were present for several days. The animal's condition improved gradually until only the dyspnea remained. Treatment during the first two weeks consisted of Azium and Longicil. The dyspnea continued, improving and worsening periodically for the next 2-1/2 months. The animal died 76 days following exposure. A gross pathological examination was not performed because the animal died on the weekend and was placed in a freezer for two days. The results of the histopathologic examination of the major organs by the veterinary pathologist may be summarized as pulmonary edema and hemorrhage with patchy bronchopneumonia, subacute tracheitis with partial erosion of epithelium and hepatocellular degeneration.

EFFECTS ON ESCAPE PERFORMANCE OF RATS

Escape Impairment. Fourteen rat tests, in the range of average HCl concentrations of 11,800 to 87,660 ppm (integrated doses of 59,200 to 438,280 ppm-min) were conducted (Table 15); two of these tests were aborted because of equipment malfunction. At all concentrations except the highest, the subjects were able to perform the escape task. In two tests, the rat met the criterion for avoidance; in nine tests, the rat exited the cage only after shock was administered. This overall performance was consistent with pre-exposure baseline levels. At the highest HCl concentration, the subject expired during the 5-minute exposure period.

Although the subjects were able to perform the escape task at all concentrations except the highest, the animals died at various times following exposure, with two exceptions. These were the animals exposed to the two lowest concentrations (11,800 and 14,410 ppm). The times at which post-exposure deaths occurred ranged from 3 minutes to 13 days following exposure. Although there was considerable variability, statistical analysis of the data revealed a significant correlation of the time of post-exposure death with HCl concentration (Figure 24). The regression equation is $Y = -.000151X + 10.85$, with $r = -.737$ and $p < .02$.

TABLE 15. EFFECTS OF HYDROGEN CHLORIDE ON ESCAPE PERFORMANCE OF THE RAT

Test I.D. No.	Animal I.D. No.	Average HCl Concentration (ppm)*	Integrated HCl Dose (ppm-min)	Test Result	Pre-Exposure Mean Avoid/ Escape Time (sec)	Test Avoid/ Escape Time (sec)	Post-Exposure Effects
HCl-1R	9	11,800	59,200	Escape	15.52 ± 5.05	25.68	Respiratory difficulty for several days, corneas clouded for 14 days. Initial weight drop with apparent recovery.
HCl-14R	60	14,410	72,070	Escape	13.29 ± 1.24	13.88	Respiratory difficulties for several days, weight after 14-days lower than test weight.
HCl-13R	57	15,250	76,250	Escape	13.54 ± 1.68	15.27	Persistent respiratory difficulties and clouded corneas. Dead 13-days post exposure. Severe weight loss.
HCl-6R	45	18,430	92,120	Avoid	9.97 ± 3.67	7.04	Persistent respiratory difficulties and clouded corneas. Severe weight loss, dead 13 days.
HCl-5R	44	22,260	111,280	Escape	13.47 ± 5.26	21.47	Severe respiratory difficulties post exposure. Moribund by next morning. Dead by afternoon.
HCl-7R	46	25,300	126,500	Escape	13.59 ± 4.49	16.09	Persistent respiratory difficulties and clouded corneas. Severe weight loss. Dead 6 days post exposure.
HCl-2R	2	25,850	129,200	Escape	15.07 ± 5.00	14.04	Persistent respiratory difficulties, clouded corneas, severe weight loss. Dead 7-days post exposure.
HCl-12R	52	27,690	138,430	Escape	16.81 ± 4.55	16.59	Persistent respiratory difficulties, severe weight loss. Dead 7-days post exposure.
HCl-10R	54	50,910	254,550	Escape	12.17 ± 2.52	14.66	Animal was in severe respiratory difficulty immediately following exposure. Animal had open mouth gasping, collapsed with a series of jerks and spasms. Died 7:40 after escape.
HCl-4R	5	53,900	269,610	Avoid	13.27 ± 2.25	2.91	Animal in severe respiratory distress following exposure, open mouth gasping accompanied by spasms. Dead 3 minutes post exposure.
HCl-8R	51	76,730	383,650	Escape	12.08 ± 3.88	21.44	Animal in severe respiratory distress following exposure, open mouth gasping accompanied by spasms. Dead next morning.
HCl-11R	50	87,660	438,280	Died	--	--	At 3:30 during exposure, the animal was gasping heavily. By 3:50, the animal was undergoing breathing spasms resembling those seen in other animals post exposure at lower concentrations 4:13 animal collapsed. Dead approx. 5:00 during exposure.
				Mean ± S.D.	13.52 ± 1.84	15.37 ± 6.41	

* Obtained by dividing integrated HCl dose by 5 minutes and rounding to nearest 10 ppm.

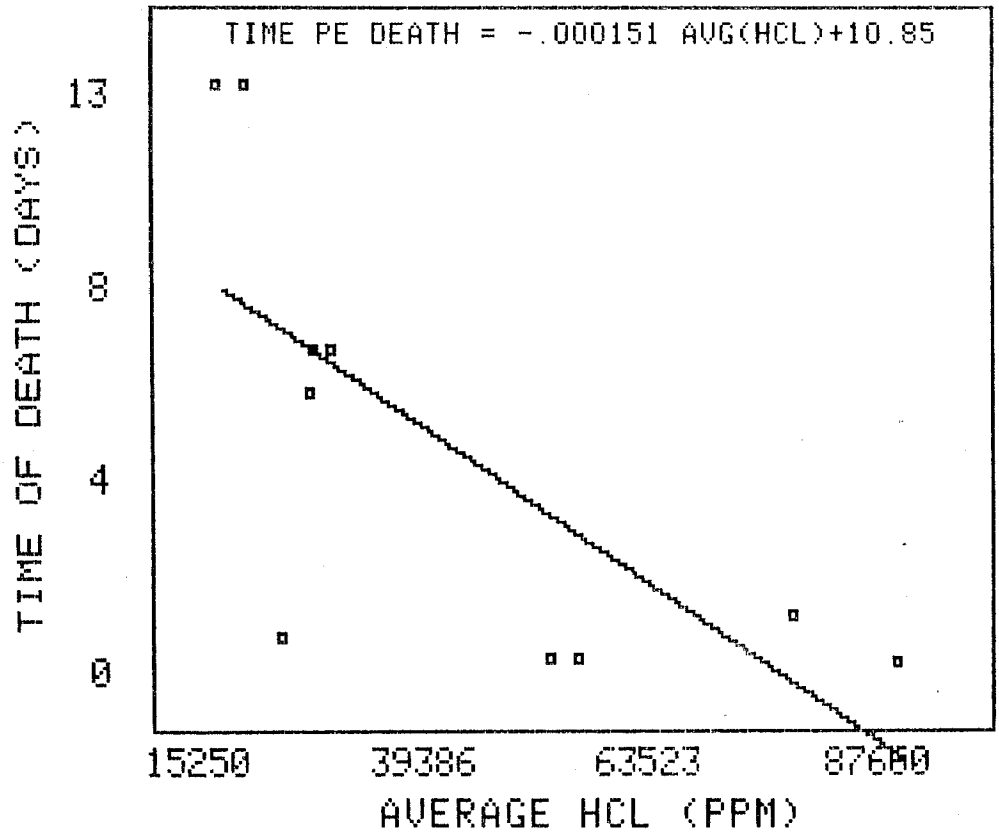

FIGURE 24. TIME OF POST-EXPOSURE DEATH OF RATS AS A FUNCTION OF AVERAGE HCl CONCENTRATION

Escape Time. Linear regression analysis was used to evaluate whether escape time was correlated with average HCl concentration (Figure 25). The equation is $Y = -.00000681X + 16.43$, but $r = -.11$. Therefore, there is not a statistically significant correlation between mean escape time and average HCl concentration. Examination of these data expressed as z scores indicated the same result.

Other Performance Measures. The other performance measures in Table 16 were also examined to determine whether any of these measures was affected by HCl exposure and whether any effect was concentrated-related. The only significant effect is the large increase in response rate during the ITI intervals. In pre-tests, the mean response rate per minute was 0.185 ± .24; the mean of the test values was 4.20 ± 3.26 responses per minute during exposure. The large difference between pre-test and test values is a very unlikely "random draw" based on the expected distribution of scores from pre-test trials and suggests that HCl increases the response rate of the animal during exposure. Because of the considerable difference between pre-exposure and test means, linear regression analysis was used to evaluate whether the increase in ITI responses correlated with average HCl concentration (Figure 26). The number of responses per minute during the ITI was significantly related ($Y = .000095X + .80$; $r = .74$; $p < .005$) to HCl concentration.

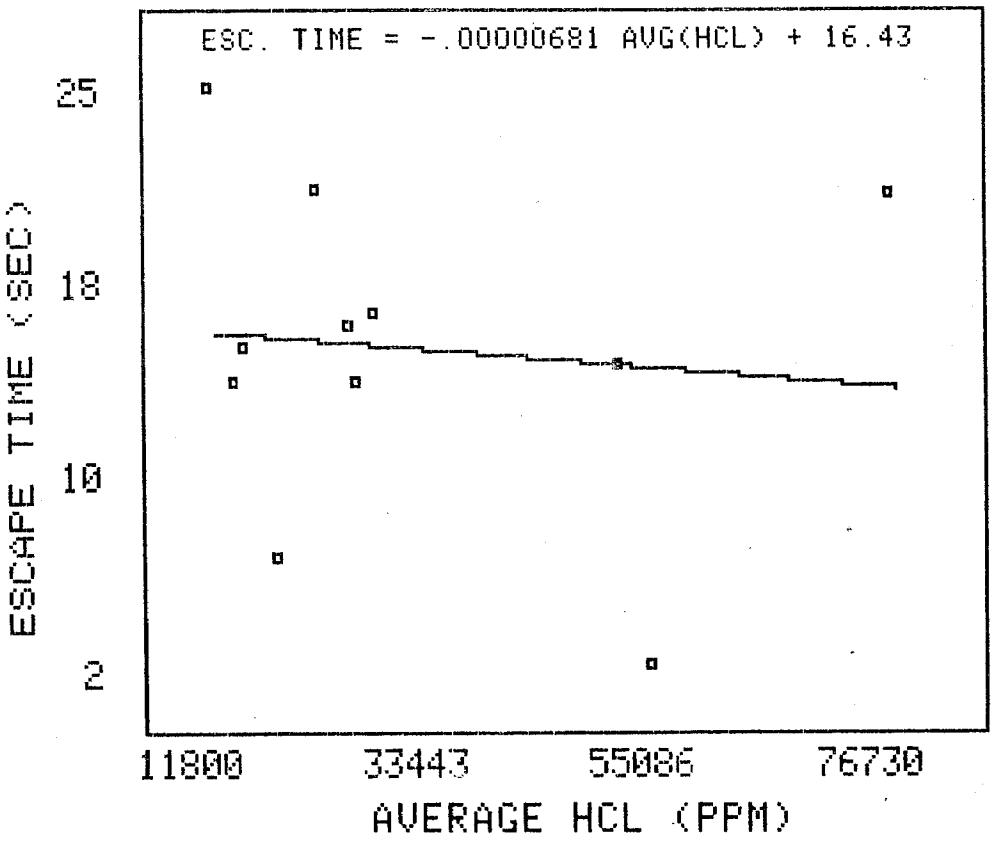

FIGURE 25. ESCAPE TIME OF RATS AS A FUNCTION OF AVERAGE HCl CONCENTRATION

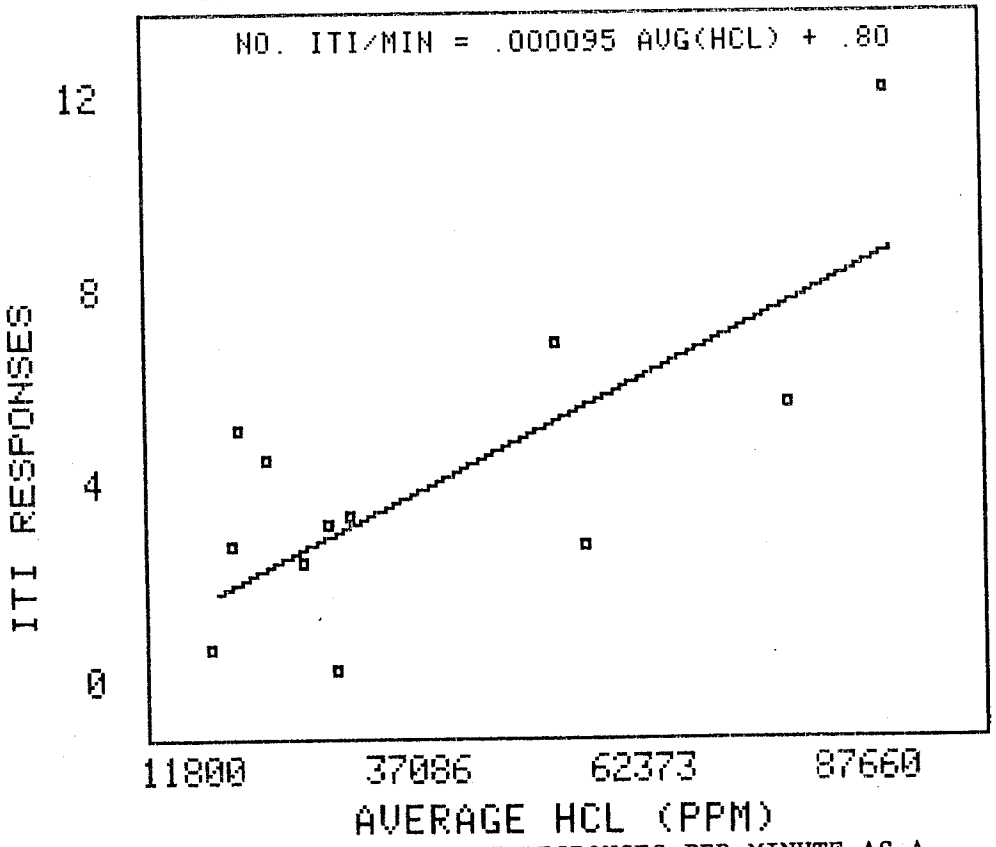

FIGURE 26. NUMBER OF ITI RESPONSES PER MINUTE AS A FUNCTION OF AVERAGE HCl CONCENTRATION

TABLE 16. COMPARISON OF PRE-EXPOSURE AND TEST RESPONSES OF RATS IN ESCAPE PERFORMANCE TESTS WITH HYDROGEN CHLORIDE

Test I.D. No.	Animal I.D. No.	Test Result	Pre-Exposure Mean Time ± S.D. to First Response (sec)	Test Time to First Response (sec)	Pre-Exposure Mean No. ± S.D. of Responses	Test Number of Responses	Pre-Exposure Mean No. of Responses/min During ITI*	Test No. of Responses/ minute During ITI*	Average HCl Concentration (ppm)	Integrated HCl (ppm-min)	Observed Time to Incapacitation	Pre-Exposure Mean Avoid/ Escape Time (sec)	Test Avoid/Escape Time (sec)
HCl-1R	9	Escape	11.58 ± 1.26	14.43	1.12 ± 0.35	1	0.0 ± 0.0	0.6	11,800	59,200	--	15.52 ± 5.05	25.68
HCl-14R	60	Escape	11.32 ± 1.60	13.08	1.2 ± 0.42	2	0.8 ± 1.03	2.8	14,410	72,070	--	13.29 ± 1.24	13.88
HCl-13R	57	Escape	10.77 ± 1.20	10.03	1.4 ± 0.52	6	0.0 ± 0.0	5.2	15,250	76,250	--	13.54 ± 1.68	15.27
HCl-6R	45	Avoid	9.53 ± 3.84	6.32	1.0	1	0.45 ± 1.03	4.6	18,430	92,120	--	9.97 ± 3.67	7.04
HCl-5R	44	Escape	13.70 ± 5.49	12.97	1.1 ± 0.32	2	0.0 ± 0.0	2.4	22,260	111,280	--	13.47 ± 5.26	21.47
HCl-7R	46	Escape	13.10 ± 4.46	7.92	1.0	1	0.17 ± 0.58	3.2	25,300	126,500	--	13.59 ± 4.49	16.09
HCl-2R	2	Escape	4.52 ± 5.22	13.44	1.5 ± 0.92	1	0.06 ± 0.26	0.2	25,850	129,200	--	15.07 ± 5.00	14.04
HCl-12R	52	Escape	12.45 ± 3.80	10.21	1.5 ± 0.84	13	0.33 ± 0.82	3.4	27,690	138,430	--	16.81 ± 4.55	16.59
HCl-10R	54	Escape	10.88 ± 1.06	13.96	1.5 ± 0.71	1	0.1 ± 0.32	7.0	50,910	254,550	--	12.17 ± 2.52	14.66
HCl-4R	5	Avoid	12.68 ± 2.21	2.42	1.07 ± 0.27	1	0.0 ± 0.0	2.8	53,900	269,610	--	13.27 ± 2.25	2.91
HCL-8R	51	Escape	10.60 ± 2.91	10.24	1.0	11	0.17 ± 0.39	5.8	76,730	383,650	--	12.08 ± 3.88	21.44
HCl-11R	50	Died During Exposure					0.08 ± 0.29	12.4	87,660	438,280	--	--	--
	Mean ± S.D.		11.01 ± 2.49	10.45 ± 3.72	1.22 ± 0.21	3.64 ± 4.41	0.185 ± 0.24	4.2 ± 3.26				13.52 ± 1.84	15.37 ± 6.41

* ITI - Intertrial Interval

With one exception, examination of the other performance data, even when expressed as z scores, did not reveal instances where a systematic relationship between a measure and HCl exposure appeared even remotely likely. Responses during the ITI were significantly correlated ($Y = -.00045X + 6.66$; $r = -.88$; $p < .005$) with HCl; as HCl concentration increased, animals made more responses during the ITI (Figure 27), producing increasingly large, negative z scores.

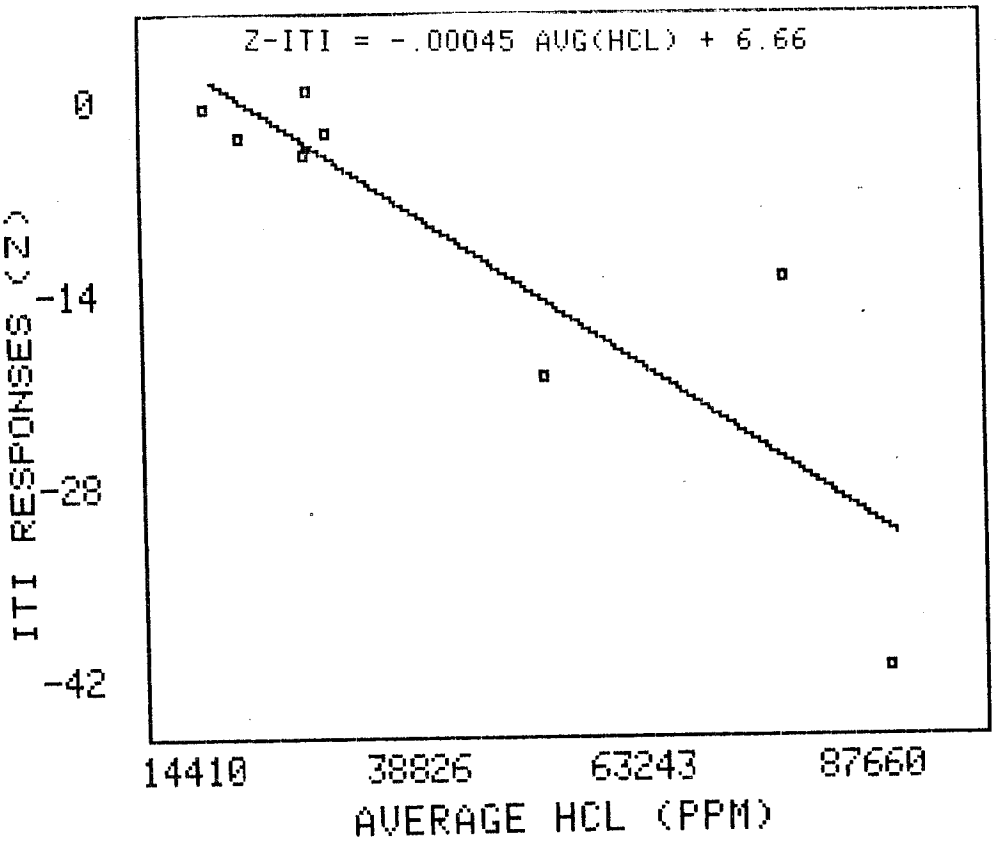

FIGURE 27. ITI RESPONSES (Z SCORES) OF RATS AS A FUNCTION OF AVERAGE HCl CONCENTRATION

DISCUSSION

CARBON MONOXIDE

COMPARISON OF EFFECTS ON ESCAPE PERFORMANCE OF THE BABOON AND THE RAT. The slopes of the concentration response curves for escape impairment of the baboon and the rat were not significantly different, although they differed slightly. The EC_{50} values for the two species were remarkably similar. The values for escape impairment by a 5-minute exposure to CO were determined to be 6850 ppm (34,250 ppm-min) for the baboon and 6,870 ppm (33,900 ppm-min) for the rat. In the baboon, the data suggest that incapacitation time, but not escape time, may be concentration-related, whereas, in the rat, escape time may be directly related to CO concentration. However, considerably less data were available for assessing these performance parameters than for determining

the EC_{50} values for escape impairment of the two species. The minor difference in these values provides substantial evidence that escape of the baboon and the rat is prevented by a 5-minute exposure to approximately the same concentration of CO. The baboon is recognized as a surrogate of man; the operant behavioral task used to measure escape performance of this animal required mental and motor functions considered by the investigators to be typical of those required for human escape. The escape tasks used in the rat and baboon experiments were similar, except that light cues were provided to the baboon to indicate which lever would open the escape door. Therefore, the data obtained in these studies indicate that the rat can be used as a valid model for predicting incapacitation or escape impairment of humans from 5-minute exposures to CO.

Additional insight into the predictive capability of the rat model may be obtained by a review of human CO toxicity data. In Table 17, symptoms observed in humans at various COHb blood saturation percentages are shown. These data indicate that, at COHb levels below 30 percent, effects are normally not sufficiently severe as to impair human escape capability and, at

TABLE 17. HUMAN RESPONSES AT VARIOUS CONCENTRATIONS OF CARBOXYHEMOGLOBIN (REFERENCE 13)

Percent COHb Concentration	Symptoms in Humans
0-10	None
10-20	Tension in forehead, dilation of skin vessels
20-30	Headache, pulsation in sides of head
30-40	Severe headache, ennui, dizziness, weakening of eyesight, nausea, vomiting, prostration
40-50	Same as above, increase in breathing rate and pulse, asphyxiation and prostration
50-60	Same as above, coma, convulsions, Cheyne-Stokes respiration
60-70	Coma, convulsions, weak respiration and pulse, death possible
70-80	Slowing and stopping of respiration, death within hours
80-90	Death in less than an hour
90-100	Death within a few minutes

levels above 40 percent, escape may be severely impaired or even not possible. Thus, the threshold COHb blood level for escape impairment of human subjects appears to be in the range of 30 to 40 percent. Concentration-time curves for COHb loading, derived from the Stewart-Peterson equation, are shown in Figure 28 (reference 14). From the curves corresponding to 30 to 40 percent COHb, human escape impairment would likely be anticipated after inhalation of an accumulated Ct dose of CO in the range of approximately 30,000 to 40,000 ppm-minute (RMV = 20 L/min). The EC_{50} Ct values obtained for the baboon (34,250 ppm-min) and the rat (33,900 ppm-min) are within this range. Thus, these human CO data provide support for the validity of the rat (and the baboon) as a model for predicting human escape impairment by CO.

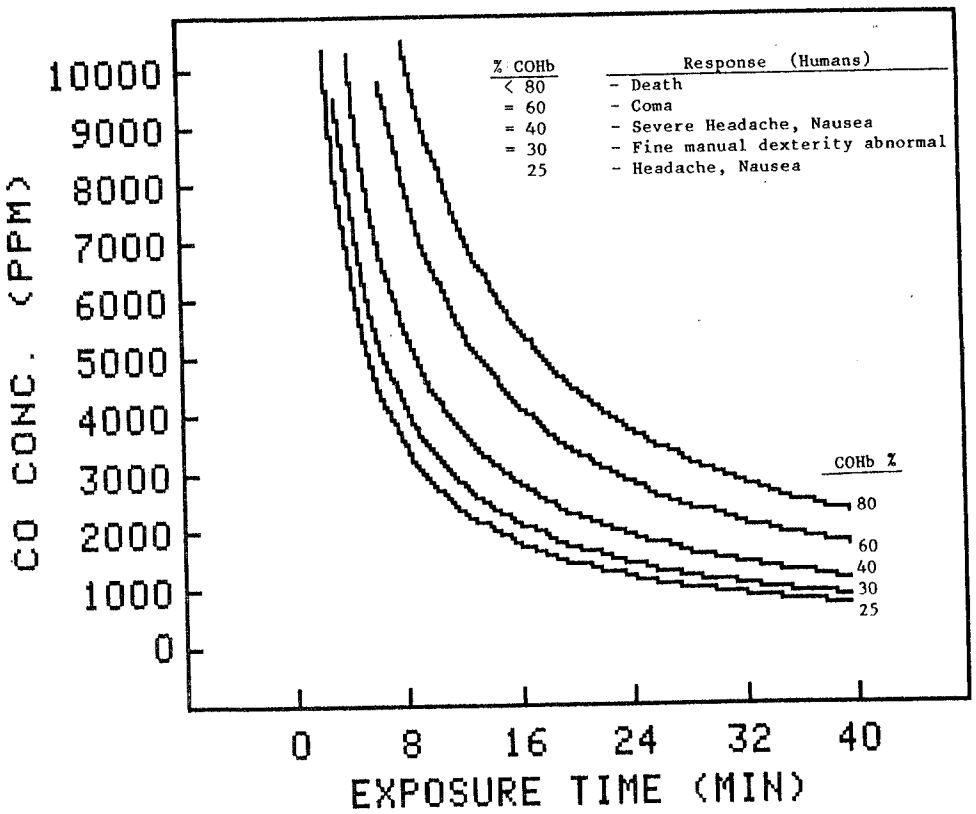

FIGURE 28. PERCENT COHb AS A FUNCTION OF AMBIENT CO CONCENTRATION AND EXPOSURE TIME FOR HUMANS WITH A RMV OF 20 L. BASED ON STEWART-PETERSON EQUATION: LOG (Δ% COHb/L) = 1.036 LOG (CO CONCENTRATION) - 4.4793 (REFERENCE 14)

EVALUATION OF VALIDITY AND RELEVANCE OF LABORATORY TEST METHODS WITH RODENTS. Several laboratory behavioral methods have been used to measure the incapacitating effects of CO in rodents. These include leg-flexion shock avoidance (reference 15), the motor-driven exercise wheel (references 16, 17), the rotarod (reference 18), the pole-climb conditioned avoidance/escape response (references 19, 20) and the shuttlebox (reference 18). Data obtained with these methods have been reported in the literature. However, experimental protocols used by the various investigators differ and, consequently, the forms of data reported vary considerably. Therefore, the data were converted

to Ct products in order to obtain a common denominator to allow comparisons and are listed in this form in Table 18. This treatment of data is based on Haber's rule, the principle of which is that, within certain time and concentration ranges, the product of the concentration and the time to effect is a constant, characteristic of the toxicant (reference 21). From examination of the table, it is evident that most of the CO Ct products (or ranges) for incapacitation of rodents are very close to those obtained for escape impairment of the baboon and the rat in these studies and to the range considered incapacitating to humans. Thus, it would appear that, with respect to CO, most of these test methods with rodents can be used to reliably predict human escape impairment as a result of exposure to CO.

ACROLEIN

EFFECTS ON ESCAPE PERFORMANCE OF THE BABOON. The results of the tests with baboons demonstrate that a 5-minute exposure to acrolein does not prevent these animals from performing the escape task, at least at concentrations that will not cause post exposure lethality. Even at the two highest concentrations (1025 and 2780 ppm), which did cause severe pulmonary damage and post-

TABLE 18. ACCUMULATED DOSES (Ct PRODUCTS) OF CARBON MONOXIDE (CO) ASSOCIATED WITH INCAPACITATION*

Subject/Method	CO Ct ppm-min	Reference No.
Humans	30,000-40,000	13, 14
Baboons (SwRI)	34,000	22
Monkeys (Huntingdon)	30,000-60,000	23
Rats		
Leg Flexion (Utah)	30,000-40,000	24, 25
(SwRI)	35,000-40,000	24, 25
Activity Wheel (FAA)	37,000	16
(Michigan)	30,750	18
(McDonnell Douglas)	22,000-36,000	17
Pole-Climb Avoidance (SRI)	48,000	20
Rotarod (Michigan)	31,500	18
(SwRI)	36,000-44,000	22
Shuttlebox (Michigan)	41,000-53,000	18
(SwRI)	33,900	22

* Ct products are presented as single values, rather than ranges, when data for only one concentration or time were available or an EC_{50} was reported.

exposure deaths, the animals were not incapacitated. In fact, with one exception, all subjects appeared to be mobile, alert and capable of reacting quickly, as evidenced by their lever pressing and exit within 10 seconds ("avoidance"). The one exception was the animal exposed to an average concentration of 95 ppm and this is considered an anomaly.

Although the escape performance of the baboons did not appear to be affected by exposures from 12 to 2780 acrolein, the severe irritant effects of the gas were evident. At the lower concentrations, blinking of the eyes, rubbing of the eyes and muzzles, coughing and salivating were typical symptoms. These become more pronounced, with frequent or continuous mouth breathing (and even gasping) at the highest concentrations. The animal exposed to 1025 ppm died the day following exposure and the animal exposed to 2780 ppm expired approximately one and one-half hours after exposure. Pulmonary damage was evident in both animals, but was particularly severe in the latter animal. From these results, it would appear that a 5-minute exposure to a very high concentration (such as 5000 ppm or higher) of acrolein might cause such severe damage to the lungs and impair oxygen intake to a degree that incapacitation of the animal would result during the exposure.

These results are consistent with literature reports that acrolein is a potent sensory and pulmonary irritant (reference 9). However, statements that a 5-minute exposure to 1.2 ppm is "only just tolerable" (reference 26) or that an atmosphere of 20 ppm is "at once insufferable" (reference 27) may be accurate but they are subjective in nature. Such statements should not be interpreted as meaning that these exposures would incapacitate humans or prevent their escape, at least on the basis of the results obtained with baboons. Furthermore, these results are not in agreement with claims by certain investigators that the RD_{50} concentration of an irritant gas will incapacitate man within a few minutes (references 10, 11). The RD_{50} concentration of 1.7 ppm reported for acrolein (reference 28) is, without any doubt, not incapacitating to the baboon. In fact, based on the symptoms exhibited by the baboons, a 5-minute exposure to the RD_{50} concentration would not constitute a severe exposure for this animal.

EVALUATION OF RELEVANCE AND VALIDITY OF LABORATORY TEST METHODS WITH RODENTS. It is not possible to compare the effects of acrolein on the escape performance of the baboon and the rat because tests were not conducted in the shuttlebox with rats. Nor are experimental data for acrolein using other methods for measuring incapacitation of rodents available in the literature. However, unpublished data indicate that 5-minute exposures to approximately 5000 to 10,000 ppm of acrolein are required to incapacitate the rat in the motor-driven exercise wheel (reference 29).

Exposures at comparable concentrations were not attempted with baboons because of post-exposure lethalities that occurred at 1025 and 2780 ppm acrolein. However, it is possible that sufficient pulmonary damage would result from exposure to 5,000 to 10,000 ppm so that the baboons would not be able to perform the escape task at the end of 5 minutes of exposure. Although this is speculative, nevertheless, the results with baboons are not inconsistent with the rat data and suggest that laboratory tests with rodents (at least the motor-driven exercise wheel) may be used to predict escape impairment in humans exposed to acrolein.

HYDROGEN CHLORIDE

COMPARISON OF EFFECTS ON ESCAPE PERFORMANCE OF THE BABOON AND THE RAT. The results obtained with HCl in baboons were similar to those with acrolein. It was not possible to prevent baboons from performing the escape task at concentrations that did not cause post-exposure lethality and even at concentrations that did cause severe post-exposure respiratory effects and lethality of two animals. In five tests, with average concentrations of HCl ranging from 190 ppm to 2780 ppm, as well as in one test at 16,570 ppm, the animals pressed the correct lever and exited the exposure chamber within 10 seconds ("avoidance"). At 11,400 ppm and 17,290 ppm, the subjects performed the escape task within 30 seconds ("escape").

The potent sensory irritant effects of HCl were evident in the symptoms exhibited by the baboons, with symptoms during exposure becoming more pronounced with increasing concentration. The two highest concentrations, 16,570 and 17,290 ppm, caused severe post-exposure dyspnea in two baboons and these subjects subsequently expired at 18 and 76 days post exposure, respectively. Since these animals were treated periodically during the post-exposure period with antibiotics and steroids, it is likely that death would have occurred sooner without treatment.

Thus, the data demonstrate that a 5-minute exposure to HCl does not prevent escape performance in a baboon, at least at concentrations in the order of 1.6 to 1.7 percent. It is possible that concentrations severalfold greater could cause sufficient respiratory tract damage to incapacitate the animals during the 5-minute exposure. Although experiments at the higher concentrations were limited in number in order to minimize lethalities, the threshold concentration at which severe post-exposure effects and post-exposure lethality result appears to be in the range between 11,400 and 16,570 ppm.

The results obtained in these studies with baboons are not in disagreement with a frequently referenced source of HCl toxicity data, Henderson and Haggard (reference 30). According to these authors, 1000 to 2000 ppm of HCl is dangerous for even short exposures. Unfortunately "dangerous" is not defined and has been interpreted by some as meaning "incapacitating." The effects observed in baboons during the 5-minute exposure to approximately 1000 ppm and, certainly, those at 2780 ppm could be described as dangerous but not incapacitating. However, the results are not in agreement with claims by some investigators that the RD_{50} concentration of an irritant gas is incapacitating to man within a few minutes (references 10, 11). Based on the data with baboons, a short exposure to 309 ppm (RD_{50} concentration in mice) of HCl is likely to be irritating and cause discomfort and even pain, but is unlikely to incapacitate man or even cause severe post-exposure effects.

Rats, also, tolerated high concentrations of HCl (11,800 to 76,730 ppm) and performed the escape task. At the highest concentration, 87,660 ppm, the animal died during exposure. Post-exposure effects were caused by all concentrations; however, the respiratory effects were not persistent and death did not result at the two lowest concentrations (11,800 and 14,410 ppm). Beginning at the next higher concentration (15,250 ppm), post-exposure respiratory effects became more severe with increasing concentrations, with subsequent lethality of the animals. Thus, in rats, the threshold HCl concentration at which severe respiratory effects and post-exposure lethality resulted appears

to be approximately 15,000 ppm. This is in agreement with the 5-minute LC_{50} value (14-day observation) of 15,900 ppm for HCl in the rat as determined in this laboratory. Although there were exceptions, the time of post-exposure lethality appeared to be concentration-related.

The results obtained with the baboon and the rat suggest that there are similarities between the two species and that the rat may have predictive capability for human escape impairment during exposure to HCl. Both species are able to tolerate 5-minute exposures to high concentrations of HCl without loss of escape capability; in fact, even concentrations which resulted in post-exposure lethality did not prevent escape performance. Although high concentrations of HCl did not prevent escape, both species exhibited an increased response to increasing concentrations of HCl, as evidenced by the concentration-related increase in intertrial repsonses. Also, the threshold concentration which caused severe post-exposure respiratory effects and lethality appears to be fairly close in the two species, although it is noted that baboons received drug therapy whereas the rats were not treated.

EVALUATION OF RELEVANCE AND VALIDITY OF LABORATORY TEST METHODS WITH RODENTS. There are no published reports of the measurement of incapacitation of rodents by HCl using laboratory methods other than respiratory rate depression. However, unpublished experimental data have been provided by Crane (reference 29) who used the motor-driven exercise wheel in determining the time-to-incapacitation (Ti) of rats during exposure to various concentrations of HCl. Using linear regression analysis, he derived the following formula for Ti (minutes) as a function of HCl concentration: $(Ti-3)\ (HCl-0.5) = 345$, where HCl is the concentration in parts per thousand. Although this equation predicts a concentration of approximately 175,000 ppm for incapacitation of a rat after 5 minutes of exposure, incapacitation and deaths of some animals were observed during exposures of approximately 5 to 7 minutes to much lower concentrations (65,000 to 100,000 ppm). With the shuttlebox, one subject was not incapacitated after a 5-minute exposure to 76,730 ppm, but, at 87,660 ppm, another animal collapsed and died near the end of the exposure. Thus, these results appear to be consistent with the data provided by Crane and suggest that laboratory tests with rodents may be used to predict escape impairment of humans exposed to HCl.

CONCLUSIONS

With regard to the narcotic gas, CO, the results of these studies, as well as experimental data from other methods and human data, indicate that the rat, the baboon and man are incapacitated after exposure to approximately the same dose. This might be anticipated in view of the fact that the mechanism of action of CO (anemic hypoxia) is the same in all species. Therefore, when insignificant concentrations of other gases are present in combustion atmospheres, the rat should be a reliable model for predicting the effects of CO atmospheres on human escape capability.

In the case of the irritant gases, acrolein and HCl, the results of these studies are less definitive but suggest that the rat may have utility in predicting escape impairment of humans exposed to these gases. These gases do not incapacitate the rat or the baboon, at least without causing severe respiratory tract damage and possible lethality. In fact, the threshold concentration for incapacitation by these gases is very near or even within the lethal

range. The results of these studies indicate that humans may be able to tolerate these gases at considerably higher concentrations than have been anticipated, without being prevented from escaping a postcrash aircraft fire environment.

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