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The Effects of CO and HCN on Pole-Jump
Avoidance-Escape Behavior

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(NASA-CR-166512) THE EFFECTS OF CO AND HCN
ON POLE-JUMP AVOIDANCE-ESCAPE BEHAVIOR (San
Jose State Univ., Calif.) 120 p
HC A06/MF A01

N83-32301

CSC2 J6C

Unclass

G3/53 13327

GRANT NCC2-4
August 1981

NASA

**The Effects of CO and HCN on Pole-Jump
Avoidance-Escape Behavior**

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Prepared for
Ames Research Center
under Grant NCC2-4



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Chapter 1

INTRODUCTION

Each year fires cause thousands of deaths and millions of injuries in the United States (Autain 1974). Home owners, hotel guests, rail, bus and air travelers, military personnel and of course fire fighters are frequently effected.

Fires are a complex phenomenon that pose multiple hazards. Heat and flames are the most spectacular dangers encountered, and their consequences are fairly well understood. Unfortunately, over half of the deaths in fires are not caused by heat and flames but by exposure to toxic combustion products (Autain 1974).

Buildings, buses, trains and airplanes are constructed of or furnished with materials that produce dangerous gases, vapors and particulates when they burn. The gases commonly encountered have been classified by mode of toxic action into asphyxiants, including CO, HCN and H_2S ; and respiratory irritants, such as NO_2 , SO_2 , HCl, NH_3 , acrolein and other aldehydes (Hilado and Cumming 1977). Unfortunately, the vapors and particulates are extremely complex mixtures that have been difficult to separate.

During the past 15 years, the toxic threat of combustion products has been assessed by various agencies:

including NASA, the FAA, the armed forces, state governments and private industry. The toxicity of traditional building and decorating materials, such as wood, cotton and wool, have been compared to newly developed synthetics, like nylon and urethane foams. In most cases, assessment of toxicity involved exposing rodents to combustion products while monitoring changes in behavior or physiology. Researchers from each of the previously mentioned agencies have developed systems for measuring toxicity end points they feel are significant. Many of the behavioral test systems are described in the literature review.

The Toxicology Section of the Department of Biological Sciences, San Jose State University and NASA Ames Research Center have recently developed a toxicological assay, including both behavioral and physiological end points, to interface with the NASA Radiant Panel Test System. The test system, which is used by NASA to analyze the combustion products evolved from plastics, is a closed rectangular steel box with a quartz window in one wall. Samples of plastics are placed behind the window, exposed to radiant heat from an electric panel and the combustion products are identified. The plastic sample weight to test system volume is the same as exists in wide body jet aircraft.

In a fire, the critical change in the victim occurs when he is unable to recognize or respond to danger. As a result, it seemed logical that a behavior requiring integration of psychological and motor functions necessary for successful escape should be included in the new toxicological assay.

Robert Bolles (1970) described species specific defense reactions, including running and jumping, used by rodents to avoid or escape danger in their natural environments. The rodent defense reactions seemed to have the potential for being the basis of a pertinent and simple model to assess adverse behavioral changes caused by combustion products.

An automated pole-jump apparatus that uses a discrete trail avoidance-escape paradigm was developed to observe and quantify toxic symptomology in mice exposed to combustion products. Along with a continuous record of behavioral changes, two toxicity end points were determined, the first termed the initial behavioral change, and the second termed loss of escape. The animals were unencumbered in the apparatus to simulate a typical fire victim.

The pole-jump model assesses the initial and subsequent behavioral changes occurring in combustion product exposures. In contrast, previous models that used the

final stages of intoxication as their end points have provided little information about the early behavioral toxicity.

In order to determine the effects of combustion products on avoidance-escape behavior, this thesis considers behavioral changes caused by two gases, CO and HCN, commonly found in fires. The gases were tested first, instead of a burning polymer, because there is so little information on the effects of combustion product constituents. This approach is based on the belief that certain significant components are primarily responsible for combustion product toxicity.

As stated previously, the gases chosen to be tested were CO and HCN. Carbon monoxide, a product of incomplete combustion of carbon compounds, is found in all fires and is considered the primary cause of death due to smoke inhalation (Autain 1974). Hydrogen cyanide, produced by nitrogen containing materials, such as polymeric amines, is also a common component of combustion products; however, its contribution to toxicity is not known.

The CO study assessed the impact of exposure to increasing or dynamic and static CO concentrations. In contrast to a static condition, dynamic concentrations approximate CO production occurring in radiant panel tests. In order to illustrate that the mouse pole-jump model was

compatible with the radiant panel, the dynamic tests reproduced CO concentration changes previously recorded in burns of synthetic materials. The dose-response relationship between the average CO concentration in the dynamic tests and changes in avoidance-escape behavior was investigated. In addition, the possibility was considered that, during an exposure, CO would produce a typical pattern of behavioral changes comparable to the unique pattern produced by other toxic gases. Finally, the relative importance of CO concentration and exposure duration on loss of avoidance and escape, in the pole-jump test, was analyzed.

The static CO exposures were done at two CO concentrations. The intent of these experiments was to determine the variation in time to the behavioral end points among animals in the test population.

Carbon monoxide binds with hemoglobin interfering with oxygen transport in the blood (Stewart et al. 1970). The percent carboxyhemoglobin (COHb) is often associated with toxic symptomology. Thus, it was necessary to determine the percent COHb of test animals immediately after loss of escape. In order to discuss the equivalency of models used in combustion toxicology, the percent COHb at loss of escape was compared to similar values reported by other authors for their systems. In addition the percent COHbs at loss of escape and at death, in animals killed by

CO, were contrasted. Finally, the relationship of COHb concentration decay in mice after CO exposure and recovery of avoidance-escape behavior following CO intoxication was investigated.

A second series of experiments analyzed the toxicology of hydrogen cyanide. The first goal was to show a dose response for loss of escape in the pole-jump apparatus. The second was to compare the pattern of behavioral changes during an HCN exposure with the pattern caused by CO. The third goal was to subjectively observe behavioral symptomology characteristic of HCN intoxication. Finally, a method of using the CO and HCN data to interpret behavioral changes occurring in the radiant panel burns of synthetic materials was discussed.

Chapter 2

LITERATURE REVIEW

Behavioral End Points Currently Used in Acute Toxic Gas Studies

The behavioral test systems that are used to assess the effects of exposure to high concentrations of toxic gases in animals can be separated into three categories. The first category tests motor function. The second uses positive reinforcement to test for changes in motivated behavior. The final category includes tests of avoidance and escape behavior that use aversive control.

The rotating cage or wheel, the most common example of a motor function test, evaluates incapacitation. Animals are judged incapacitated when, instead of maintaining pace with the externally driven cage, they tumble. Birly et al. (1976), Crane et al. (1977), Saito (1977) and Russo and Kaplan (1978) used this device to determine how much CO and HCN were required to incapacitate rats.

In positive reinforcement test systems, rats are trained to obtain food pellets by performing on a behavioral schedule. Merigan and McIntire (1975) trained rats on a progressive ratio schedule and then exposed them to CO. In this paradigm, rats were rewarded with food pellets if they pressed a bar the correct number of times.

After each reward the number of bar presses required for the next pellet was increased. The animals were considered behaviorally affected when they would not press the bar enough times to complete the next ratio.

The majority of test systems use aversive control. The theory of aversive control or negative reinforcement is explained in the psychology literature (Hineline 1977). Fantino and Logan (1979), however, wrote an excellent book that discusses its use in biological experimentation. In aversively controlled test systems, shocks are used to shape desired behavior. Packham et al. (1976) developed a leg flexion apparatus that trained rats to keep their hind legs flexed above a shock grid. Inability of the rats to hold their legs above the grid was the behavioral change of interest during toxic gas exposures. Mitchell et al. (1978) and Russo and Kaplan (1978), to test for loss of coordination during toxic gas exposure, trained rats to walk on a rotating horizontal rod situated over a shock grid. The pole-jump response, used by Dilley et al. (1978) and discussed in this thesis, tests for changes in discrete trial avoidance-escape behavior.

The final system in the category of aversive control is Sidman or free operant avoidance. In Sidman avoidance, rats are trained to avoid a shock by pressing a bar. Each bar press delays the next shock for a specified

interval, for example 20 seconds. In time, the animal learns to press the bar often enough to avoid all shocks. Russo and Kaplan (1978) reported on the increase in shock rate of Sidman avoidance trained animals exposed to CO.

Carbon Monoxide

General Toxicology

The toxic effects of CO in animals and humans have previously been reviewed in a number of journals, and government publications. These include an Environmental Pollution Panel report in 1965, a comprehensive bibliography with abstracts (Cooper 1966), a National Research Council report in 1969 and a complete volume of the Annals of the New York Academy of Sciences in 1970 (Coburn 1970).

Most authors feel that carbon monoxide's adverse effects are due to its interference with normal oxygen transport in the blood (Bartlett 1968; Goldsmith and Landaw 1968). The affinity of CO with reduced hemoglobin is 200-250 times that of oxygen (Forbes et al. 1945; Forester 1970; Stewart et al. 1970), so that CO reduces the oxygen carrying capacity of blood producing symptomology similar to hypoxia. In addition, COHb shifts the oxygen-hemoglobin disassociation curve to the left causing available oxygen to be released in the tissues at lower than normal oxygen

partial pressures (Roughton and Darling 1944). Other authors, however, stress carbon monoxide's importance as an inhibitor of heme containing enzymes such as cytochrome c oxidase (Chance et al. 1970).

Whether CO works primarily to disrupt oxygen transport or acts in the mitochondria to inhibit cytochrome c oxidase, the net effect is that electron transport and oxidative phosphorylation are diminished. The reduced energy supply is particularly damaging to organs that have large energy requirements, such as the liver, the kidneys, the heart and the brain (Chance et al. 1970).

Behavioral Toxicology

The behavioral toxicology of carbon monoxide in both humans and animals was reviewed thoroughly by Latis and Merigan in 1979. The primary conclusion drawn was that CO affects animals and man by slowing the rate of any behavior tested: including eating, drinking, swimming, wheel running, active avoidance and free operant conditioning.

Although the literature covered in the Latis and Merigan review was extensive, most human and animal subjects were exposed to less than 700 ppm CO. Unfortunately, the combustion toxicologist is concerned about CO concentrations greater than 700 ppm occurring in the fire

environment. It is necessary, then, to consider the information available on exposure to high CO concentrations not included in the Latis and Merigan review.

Carter et al. (1973) reported that rats exposed to 1000 ppm CO for 1.5 hr had a reduction in their FR-15 response rate to 5% of base line. On a FR or fixed ratio schedule, positive reinforcement occurs after emission of a fixed number of responses (Fantino and Logan 1979). Rats exposed to 1947 ppm carbon monoxide fell off a rotating rod in 22 min and lost leg flexion behavior in 30 min (Mitchell et al. 1978). Russo and Kaplan (1978) measured the effects of increasing CO concentrations (0-3000 ppm) on 2 types of behavior. They found that rats trained in Sidman avoidance were incapacitated in 45 min when the CO concentration was 2900 ppm. Rats tested in rotating cages were unable to continue running after a 20 min exposure to a mean CO concentration of 1761 ppm.

Due to the obvious dangers, there is little information on the effects of exposure to high CO concentrations in humans. The primary studies in this field were done by Stewart and his colleagues (Stewart et al. 1970; Stewart et al. 1973). In the 1973 report, human volunteers were exposed to 1000-3000 ppm CO for 10 min at the low dose decreasing to 45 sec at the high dose. Parameters monitored were rate of COHb formation, changes in EKG and

respiration, spontaneous and evoked brain electrical activity and subjective responses. In 2 of the 19 subjects tested, mild frontal headaches were noted after 2 min exposure to 15000 ppm, and one other subject experienced precordial pounding in 45 sec at 35000 ppm. Stewart et al. (1970) exposed volunteers to CO concentrations of 100-1000 ppm. During the 1000 ppm test, the CO concentration was increased to 1000 ppm (2 hrs) and maintained for 30 min. Toxic symptomology included mild to incapacitatingly severe headaches, loss of coordination and changes in brain wave patterns.

Hydrogen Cyanide

The following is a general review of cyanide toxicology. Little attention has been paid to the behavioral toxicology of HCN gas. Although the few authors who have reported subjective observations of acutely exposed animals are included, a majority of the review covers a wider range of cyanide studies. The review, therefore, is intended as a starting point for the explanation of behavioral changes caused by HCN. It is also the only HCN review available and, hence, should be of value to others interested in the subject.

Information from the very diverse cyanide literature has been arranged into the following subject

headings: 1) the cyanide receptor, 2) acute dose-response and blood and tissue concentrations, 3) general symptomology of acute cyanide exposure, 4) bioenergetics, 5) cyanide and the central nervous system and 6) cyanide and the circulatory system.

The Cyanide Receptor

It is generally accepted that cyanide expresses its toxic effect by inhibiting cytochrome c oxidase, the final enzyme in the mitochondrial electron transport chain (Yonetani and Ray 1965; Nicholls et al. 1972). Hydrogen cyanide has a pK of 9.3 and so is undissociated at biological pH. The undissociated acid is considered an uncompetitive inhibitor of cytochrome c oxidase (Yonetani and Ray 1965; Yoshikawa and Orii 1973; Smith et al. 1977) that binds most readily to the $a^{2+}a^{3+}$ form of the enzyme (Nicholls et al. 1972; Nicholls et al. 1976). The 2+ and 3+ refer to the oxidation states of the enzyme's heme irons. The inhibited $a^{2+}a^{3+}$ -HCN can react with oxygen to form $a^{3+}a^{3+}$ -HCN, however the oxidized form can not be reduced. This terminates electron transport and, as a consequence, stops aerobic energy production in the mitochondria.

The inhibition constant of cyanide with cytochrome c oxidase had been determined by various authors. The

reported values include 1.5 μM (Schubert and Brill 1968), 0.1-1.0 μM (Nicholls et al. 1972), 0.5 μM (Yoshikawa and Orii 1973) and 0.5 μM (Auclair et al. 1976). Cyanide inhibited the catalytic activity of cytochrome c oxidase in both polarographic and spectrophotometric assay systems with an apparent velocity constant of $4.0 \times 10^3 \text{ M}^{-1}\text{s}^{-1}$ (Nicholls et al. 1972).

Acute Dose Response and Blood and Tissue Concentrations

In mammals, the cause of death in acute cyanide exposures by any route is respiratory arrest (Smith et al. 1977). The acute LD_{50} for 5 min exposures to HCN gas for rats and mice respectively were 503 and 323 ppm (Higgins et al. 1972). The LD_{50} for injected (im) HCN in male rabbits was 0.95 mg/kg (Ballantyne et al. 1972).

The blood and tissue cyanide concentrations of rabbits killed by HCN and KCN injections and sheep killed by KCN injections are summarized in Table 1 (Ballantyne et al. 1972; Ballantyne 1975). These authors drew the following conclusions. 1) The whole blood cyanide concentration is greater in animals killed by HCN injections than KCN injections, because undissociated HCN diffuses into the blood more rapidly. 2) Cyanide is found primarily in the blood, as most organ cyanide is lost in perfusion. An

TABLE I

Blood and Tissue Cyanide Concentrations
in Rabbits and Sheep Killed by Cyanide Injections
(Ballantyne et al. 1972; Ballantyne 1975)

Tissue	Rabbits		Sheep
	HCN	KCN	KCN
Whole Blood	685 ^b	453	331
Serum	275	161	157
Plasma	---	---	146
CSF ^a	---	---	124
Liver	148 ^c	82	---
Liver (perfused)	43	7	---
Brain	145	106	---
Brain (perfused)	289	98	---

^a CSF is cerebrospinal fluid

^b blood, serum, plasma and CSF
in $\mu\text{g CN}^-/100 \text{ ml}$

^c organs in $\mu\text{g CN}^-/100 \text{ gm wet}$
tissue

exception is the brain which may have a selective cyanide uptake. 3) In sheep, the plasma, serum and cerebrospinal fluid cyanide concentrations were similar indicating that cyanide moves across the blood brain barrier.

The reports of human tissue cyanide concentrations were accumulated from autopsies of suicide and fire victims. In humans the fatal whole blood concentration of cyanide from cyanide salts was estimated to be 300 $\mu\text{g}/100\text{ ml}$ (Curry 1963; Graham et al. 1977) and 500 $\mu\text{g}/100\text{ ml}$ (Sunshine and Finkle 1964). In one case a young man ingested a massive dose of cyanide salt. After 12 hours of hospitalization, his whole blood cyanide level was 200 $\mu\text{g}/100\text{ ml}$. Using the kinetic data on the rate of cyanide detoxification in humans, it was determined that his peak whole blood cyanide concentration was nearly 300 $\mu\text{g}/100\text{ ml}$ (Graham et al. 1977).

In a report summarizing autopsy findings from 26 victims of cyanide salt poisoning, Sunshine and Finkle (1964) found that the brain had the lowest cyanide concentration. Increasing concentrations were found in the liver, kidney, whole blood and spleen. The average whole blood concentration was 740 $\mu\text{g}/100\text{ ml}$ in the Sunshine and Finkle (1964) report, while Bonnichsen and Maehly (1966), in a similar study, reported an average value of 2100 $\mu\text{g}/100\text{ ml}$. The whole blood cyanide concentration in humans

after fatal gas exposure was 100 $\mu\text{g}/100\text{ ml}$ (femoral vein), 50 $\mu\text{g}/100\text{ ml}$ (carotid artery) (Curry 1963) and 265 $\mu\text{g}/100\text{ ml}$ (Sunshine and Finkle 1964).

These reports indicate that victims of fatal HCN gas exposure have lower whole blood cyanide concentrations than cyanide salt victims have. There are many problems, however, in estimating a fatal cyanide dose from human tissue or blood samples, because the samples are normally taken hours to days after death. For example, in one case whole blood removed from a HCN gas victim moments after death and analyzed 24 hours later had 350 $\mu\text{g}/100\text{ ml}$. Whole blood from the same victim removed and analyzed 24 hrs after death had 50-100 $\mu\text{g}/100\text{ ml}$ (Curry 1963). Curry (1963) has concluded that whole blood cyanide levels are labile and may increase or decrease depending on storage conditions. Bonnichsen and Maehly (1966) reported that cyanide in whole blood stored at room temperature declined in concentration with time, but cyanide in whole blood stored at 4°C increased in concentration for 1-2 weeks then declined. For this reason, it is difficult to equate whole blood and tissue cyanide concentrations with estimated fatal dose.

The efficacy of tissue and blood analyses for cyanide was also discussed by Pettigrew and Fell (1972 and 1973). These authors have shown free cyanide to be very

unstable in biological fluids. The time for one half the cyanide added to plasma to disappear was approximately 18 minutes. The analytical procedures used to determine cyanide that are based on the formation of a pyridine dye measure many forms of cyanide. So while it is the free cyanide in blood plasma that is toxicologically important, this form is rapidly lost and the analytical procedures measure other cyanide, including thiocyanate and cyanide bound to hemoglobin, to enzymes and to cofactors.

General Symptomology of Acute Cyanide Exposure

Ballantyne et al. (1972) described the symptoms of rabbits given im injections of HCN. In order they were:

- 1) increases in rate and depth of breathing,
- 2) vociferation,
- 3) uncoordinated movements of the head,
- 4) ataxia,
- 5) tremor and rectocolic spasm,
- 6) respiratory arrest, and
- 7) cardiac arrest.

Levine and Stypulkowski (1959) observed rats that were exposed to HCN gas and described 4 stages of intoxication. 1) The rat responded by restlessness and increasing activity leading to violent attempts to escape. 2) Voluntary muscular activity and postural tonus were greatly decreased, so that the rat lay on the exposure chamber floor. The animal, however, could respond to tapping on the exposure chamber walls. At this point,

respiration varied from very deep, slow and regular to irregular with short periods of apnea. 3) The animal was essentially moribund but could still respond to chamber tapping by ear twitches. Respiration was regular and of moderate depth and breathing varied from 40-80 per minute. 4) No response was obtained from chamber wall tapping. Respiration was slow and regular and diminished progressively in amplitude and frequency until death occurred. If cyanide was discontinued before irreversible damage was inflicted on vital centers, the stages were retraced but in much less regular fashion. The first sign of emergence from stage 4 was return of the ear reflex, movement of extremities or tail, or hyperpnea (Levine and Stypulkowski 1959).

According to Levine and Stypulkowski (1959) death occurred from respiratory paralysis in stage 4. If the cyanide was administered until respiration was shallow, death was almost certain. Sometimes the animal died even when it was removed from the chamber at the first sign of weakening respiration. They felt there was a level of intoxication which, if attained only very briefly, caused irreversible damage to the respiratory center. In many cases, the heart continued to beat after respiration ceased, but artificial respiration would not revive the rats (Levine and Stypulkowski 1959). Some rats recovered from

exposure slowly after several hours and were sluggish and weak. Other rats, however, recovered in less than an hour and showed no obvious abnormalities.

The symptoms of a human exposure to cyanide salt were described by Graham et al. (1977). The victim an adult male 24 years of age, was stuporous, cyanotic and emetic. His respiratory rate was 24 per min in gasps, his temperature was 36.5° C and his blood pressure was 168/112. Examination of the lungs revealed bilateral rales and ronchi and plumonary edema. The victim's ECG showed sinus arrhythmias with multi focal premature ventricular contractions.

Bioenergetics

According to Isom et al. (1975), lactic acid concentration increased in mice given KCN injections ip, as tissue switched from aerobic to anaerobic catabolism. Isom et al. (1975) also reported an increase in the pentose phosphate shunt to provide additional reducing power for glycolysis.

Cyanide and the Central Nervous System

The immediate effect of cyanide on the central nervous system of dogs and man was stimulation of the carotid and aortic chemoreceptors that produced a deep gasp

lesions could be predetermined, to a certain extent, by varying the depth and duration of intoxication (Levine and Stypulkowski 1959). The first lesions, which were observed within 2-4 hours of exposure, were in the corpus callosum (Levine and Stypulkowski 1959). There was fenestration of the callosum due to the appearance of spaces among the fibers. The callosum went through various structural changes, and the fenestrations became progressively less prominent and were inconspicuous after one week (Levine and Stypulkowski 1959). One month after exposure, the callosum was grossly shrunken and narrower than normal with demyelination detectable to the naked eye.

Hirano et al. (1967) reported that administration of cyanide by inhalation for 30 min yielded corpus callosum lesions in nearly all rats tested and that swelling in myelinated axons was evident after only 2 hours. In contrast to earlier findings by Levine (1960), Hirano et al. (1967) showed that axonal swelling occurred with no apparent initial damage to the myelin, however damage to the myelin did occur later in the necrotic process. This later stage, called the reactive process by Hirano et al. (1967), included disruption of all cellular elements.

Hirano and Zimmerman (1971) have done a more recent electronmicrographic study of rats exposed to cyanide gas. They found that the swollen areas, located

between the nodes, were filled with cell organelles and debris.

in the region of the corpus striatum, lesions involved both grey and white matter. Necrotic neurons showed extreme shrinkage, and the necrotic white matter was swollen and extremely vacuolated (Levine and Stypulkowski 1959).

Levine and Stypulkowski (1959) reported that while lesions of the corpus callosum were the most common type in large rats of 200-300 gm, 100-200 gm rats had more lesions in the corpus striatum. According to Levine (1960), the location and number of brain lesions was related to the size and species of rodents tested. There were more animals with lesions and more lesions per animal among large rats and hamsters than among small rats, hamsters and mice. Larger animals' lesions were selective for the white matter while small animals exhibited predominant involvement of the grey matter (Levine 1960).

The difference in numbers of lesions between large and small animals may be due to a greater body temperature drop in smaller animals during cyanide exposure (Levine 1960). Small animals exposed in artificially heated exposure chambers had increased numbers of brain lesions (Levine 1960). The different location of the lesions in small and large animals of the same or different species

could not, however, be explained by differences in body temperature (Levine 1960).

The cause of the white and grey matter lesions was discussed by Levine and Stypulkowski (1950). Cyanide inhibits cytochrome c oxidase and, as a result, aerobic energy production. CNS cells have a large energy requirement to maintain the ionic flux across their membranes. Certain brain cells, particularly in the white matter, have relatively few mitochondria and so are more sensitive to cyanide inhibition. When inhibition occurs there is not enough ATP to maintain the ion pumps. The ionic concentration on the inside of the cell increases causing movement of fluid into the cell. This process may account for swollen cells and the resulting fenestrations. A second explanation for the lesions is also based on a metabolic deficit, but it involves a localized difference in the vascular bed that limits the amount of oxygen available for aerobic energy production (Lessel 1971).

Burrows et al. (1973) studied the effect of cyanide on brain electrical activity in the dog using bipolar encephalographic leads attached to the frontal area. According to these authors, after 15 sec, NaCN injections produced an abrupt loss of electrical activity, lasting for as long as 6 min, followed by a period of depressed wave amplitude.

Cyanide and the Circulatory System

Electrocardiograms of human volunteers given 0.11-0.20 mg of NaCN per kg of body weight revealed a sinus pause (0.88-4.20 sec) followed by sinus irregularity and slowing of heart rate for a few seconds. Finally, heart rate climbed above control for one minute before returning to pre-exposure levels (Wexler et al. 1947).

Continuous electrocardiograms of men executed by HCN gas revealed the following characteristics:

1. Initial slowing of the heart rate which reached the slowest point between the first and third minutes. The slowing was from highly agitated control values of 106-160 beats per minute.
2. Irregularity and disappearance of P waves.
3. Irregular reappearance of P waves, some of which were not conducted, and a slight increase in heart rate.
4. A-V dissociation with a secondary decrease in heart rate during the fifth minute. During the sixth and seventh minutes, the heart rate showed a slight increase and a return to normal sinus rhythm.
5. Heart rate slowed.
6. A-V conduction disrupted.
7. Heart block or ventricular fibrillation.

Burrows et al. (1973) reported that the electrocardiograms of cyanide treated anesthetized dogs showed sinus pause (stage one heart block), bradycardia, elevated and diphasic T waves and other associated arrhythmias. These changes were accompanied by a slight decrease, then immediate increase in both systolic and diastolic blood pressures (Burrows et al. 1973).

The effect of cyanide on cerebral hemodynamics was discussed by Pitt and Traystman (1976). Cyanide was given to anesthetized dogs by intracarotid bolus injection or iv infusion. After cyanide injection, cerebral venous blood flow increased depending on dose from 162-300% of control. These responses also occurred in chemoreceptor denervated animals.

Auclair and his co-workers treated heart muscle cells with 1×10^{-3} molar KCN and showed a complete clearing of the mitochondrial matrix, disorganized cristae and lack of an outer mitochondrial membrane (Auclair et al. 1976). After 24 hours, beating rhythm was reduced 50% and action potentials had no overshoot. Auclair et al. (1976) was able to correlate mitochondrial ultrastructure damage with reduction in depolarization during the action potential. These effects were not evident when KCN concentration was 1×10^{-4} M. Ganote et al. (1976) reported that

in perfused dog hearts cyanide required 30-40 minutes for maximal inhibition of cellular respiration.

Conclusion and Summary

It is apparent that not much is known about the behavioral effects of cyanide exposure. Most reports concern either cyanide's inhibition of cytochrome oxidase, or its ability to cause CNS lesions and changes in the circulatory system. Hence, the importance of examining cyanide's effects on avoidance-escape behavior is evident.

A brief summary of major points covered in the cyanide review includes the following:

1. Cyanide is an uncompetitive inhibitor of cytochrome c oxidase and does not compete directly with O_2 or CO for their receptor site. The in vitro inhibition constant, k_i , (Lehninger 1977) of cyanide with cytochrome c oxidase is approximately 1.0 μM .
2. The cause of death due to cyanide exposure in any form and by any route is respiratory arrest.
3. Because the fatal blood concentration of cyanide is lower in HCN gas exposed animals and man than in those exposed to cyanide salts, HCN gas is considered to be more toxic.

4. Cyanide in dead animal tissue and blood is labile which makes it difficult to estimate the lethal concentration.
5. It is cyanide dissolved in the blood plasma that is toxicologically important.
6. An easily recognized symptom of cyanide exposure is a deep gasp followed by hyperpnea.
7. Single HCN exposures of 20-30 min produce lesions in white and grey matter of rodents. The location and number of the lesions depends on body size; smaller animals have more grey matter lesions.
8. Cyanide exposure causes oxygen conservation phenomena to occur, such as vaso-constriction in non-critical muscles, increases in cerebral blood flow and reduction in cerebral vascular resistance.

Chapter 3

METHODS AND MATERIALS

General Introduction of Methodology and Equipment

Introduction to Terminology

The behavioral assay apparatus, also called a response chamber or pole-jump apparatus, tests for changes in performance of a discrete trial avoidance task (Fantino and Logan 1978). The avoidance task used allows test subjects to escape an aversive environment, a shock grid, by climbing to a safe place. Rats and mice learn this behavior rapidly; because, as Bolles (1970) theorizes, it is one of the species specific defense reactions already available in their response repertoire to meet aversive or damaging situations in the natural environment.

The response chamber is designed to quantify changes in avoidance-escape behavior. To understand the apparatus and methodology, it is necessary to provide a brief introduction to behavioral toxicology. Behavioral toxicology, founded in experimental psychology and pharmacology, receives its terminology from the psychology literature. Thus, these experiments test for changes in what is termed operant or motivated behavior. The test animals are exposed to a conditional stimulus (CS) that is either a tone or a light. Following the CS, an electrical shock or

unconditional stimulus (UCS) begins. The subject learns to avoid the UCS by climbing an aluminum pole. After the UCS there is a pause or inter-trial interval (ITI) before the next CS begins. One cycle through the CS-UCS-ITI sequence is termed a trial. During testing the trial sequence repeats a specified number of times to complete a session. This type of conditioning is termed operant as opposed to Pavlovian or reflex, because the animal's response directly affects the test sequence or paradigm by eliminating the shock (Honig and Staddon 1977).

Pole-Jump Apparatus

The response chamber is a plexiglass box 12 in long, 8½ in wide, and 11 in deep (Figure 1). The chamber is divided into 4 cubicles (4½ in by 6 in) by stainless steel partitions (Figure 2). The floor of each cubicle is made of corrosion resistant steel rods in a plexiglass frame (Figure 3). The floor grids are designed, so that contact with adjacent rods completes an electrical circuit. Each floor grid is independently supported by 4 springs, one in each corner (Figure 3). The springs are held in place by mounting pegs that are attached to the stainless steel chamber bottom (Figure 4). The floor grids are supported on springs, so that horizontal and vertical

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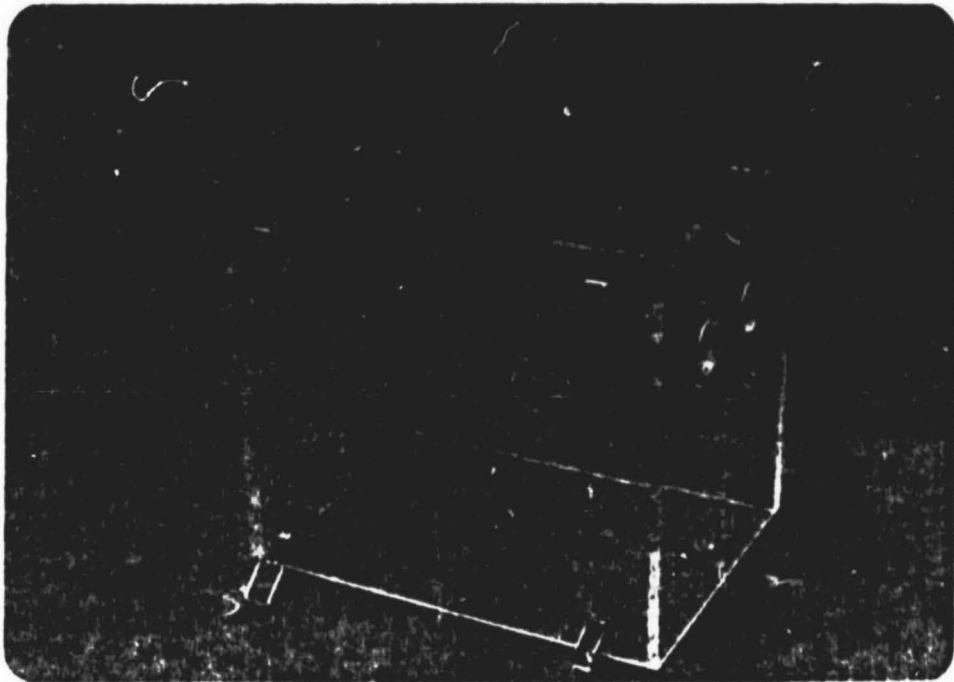
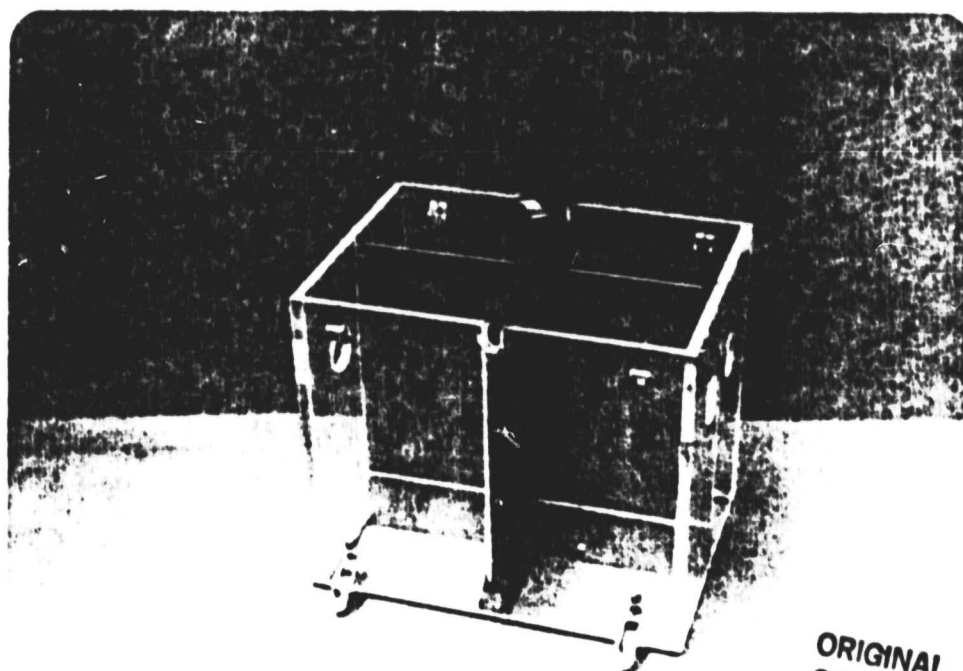
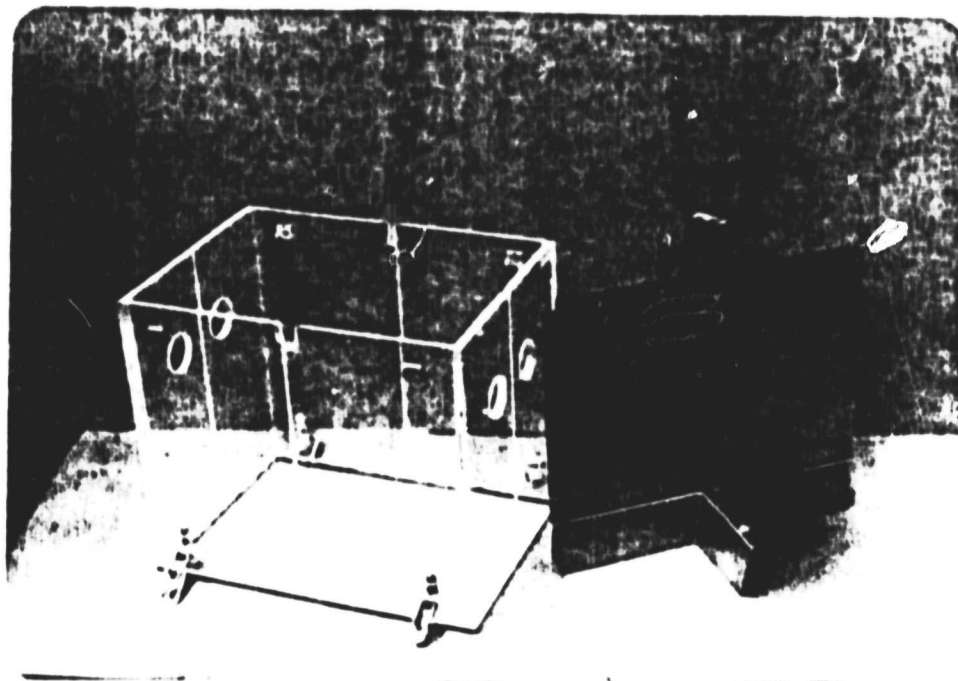


Figure 1
Plexiglass Box



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Figure 2
Stainless Steel Partitions

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Spring

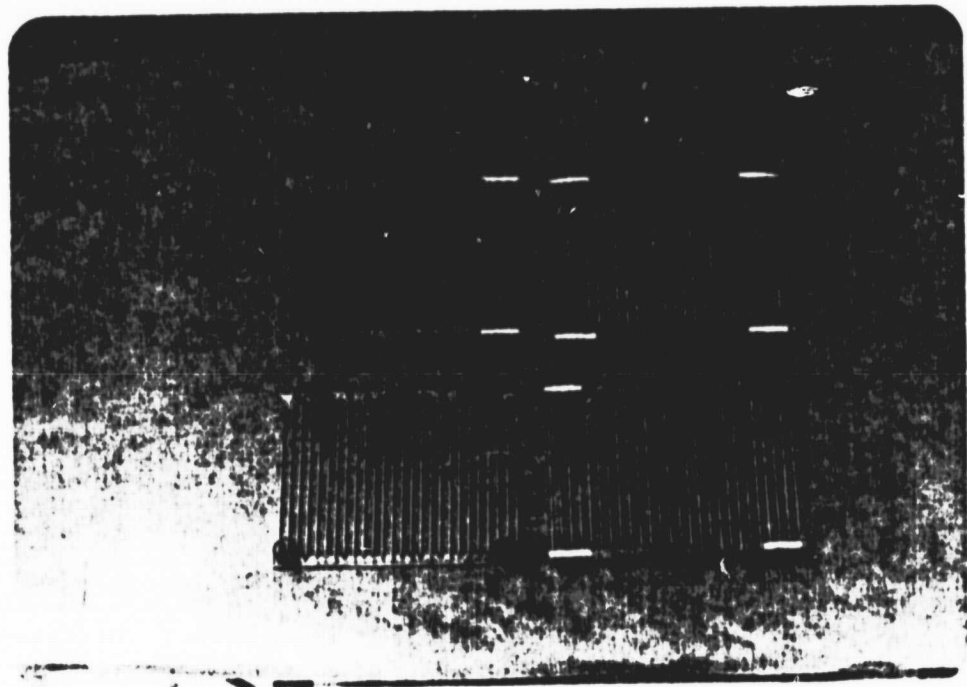


Figure 3
Stainless Steel Shock Grids

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Mounting
Pegs

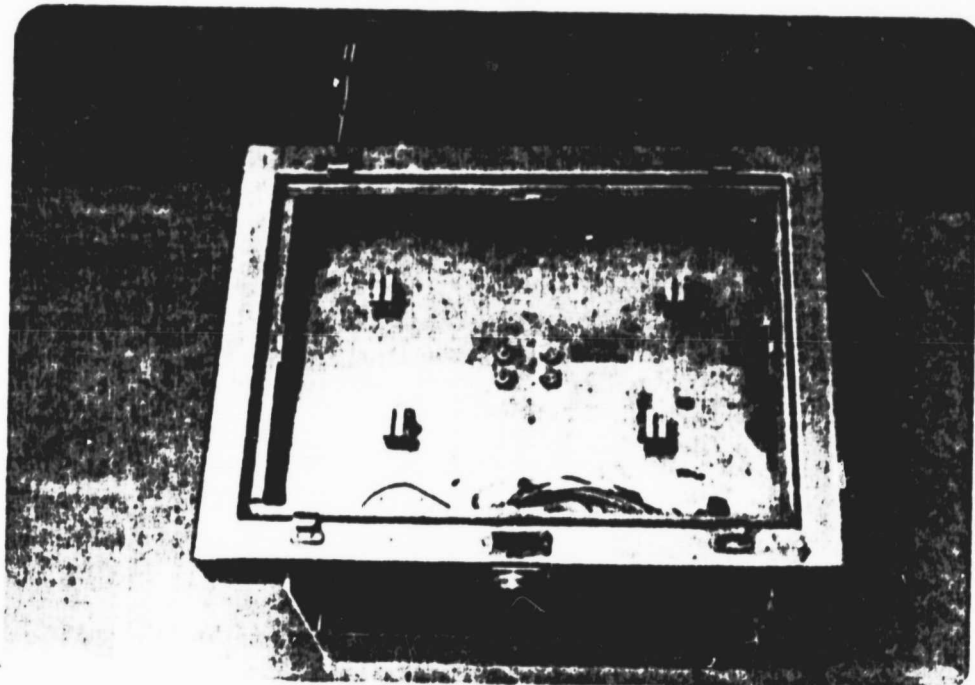


Figure 4
Chamber Bottom

displacements of the grids can be translated by flexure beams (Figure 5) into strip chart records of mouse activity.

A one half inch aluminum pole is suspended in the center of each cubicle by a lever system (Figure 6) attached to the stainless steel partition. The poles are spring controlled and are adjustable to individual animal weights. When a subject jumps to the pole (Figure 7), the pole drops about 1 mm and closes a microswitch connected to a strip chart recorder. During testing, the poles are lightly greased at the top to prevent the mice from climbing up onto the lever system.

The conditional stimuli (CS) are four 6 watt lights or an 80 db tone (Figure 8). Each light is mounted on the plexiglass box, so that it is observable only from the appropriate cubicle (Figure 8). The unconditional stimulus (UCS) is provided by a Grass SD9 stimulator. The UCS and CS are controlled by a special module (Figure 9) that provides the following features:

1. a constant voltage to each grid
2. a basic trial paradigm of CS-UCS-IMI
3. a deactivation or adjustment of the CS from 1-15 sec in 1 sec increments
4. a pause between the CS and UCS from 0-15 sec adjustable in 1 sec increments

Flexure
Beams

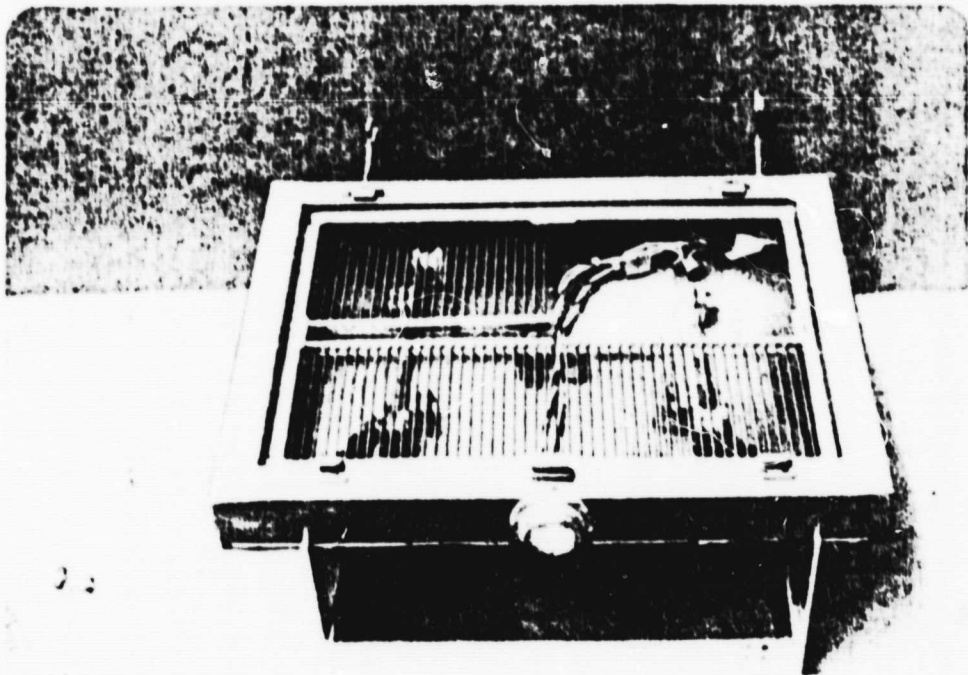
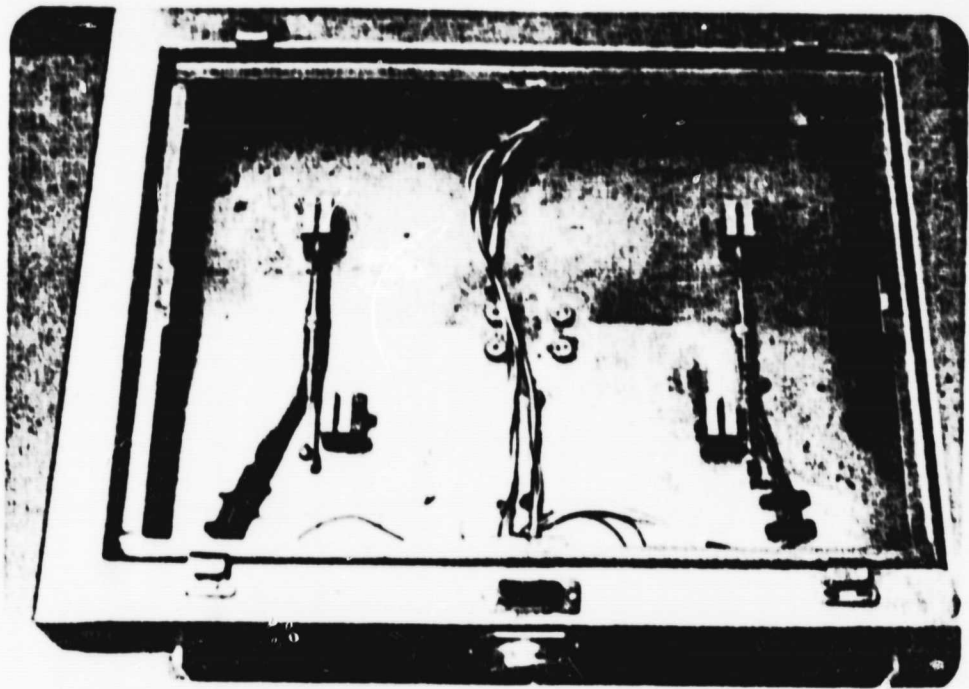


Figure 5

Flexure Beams

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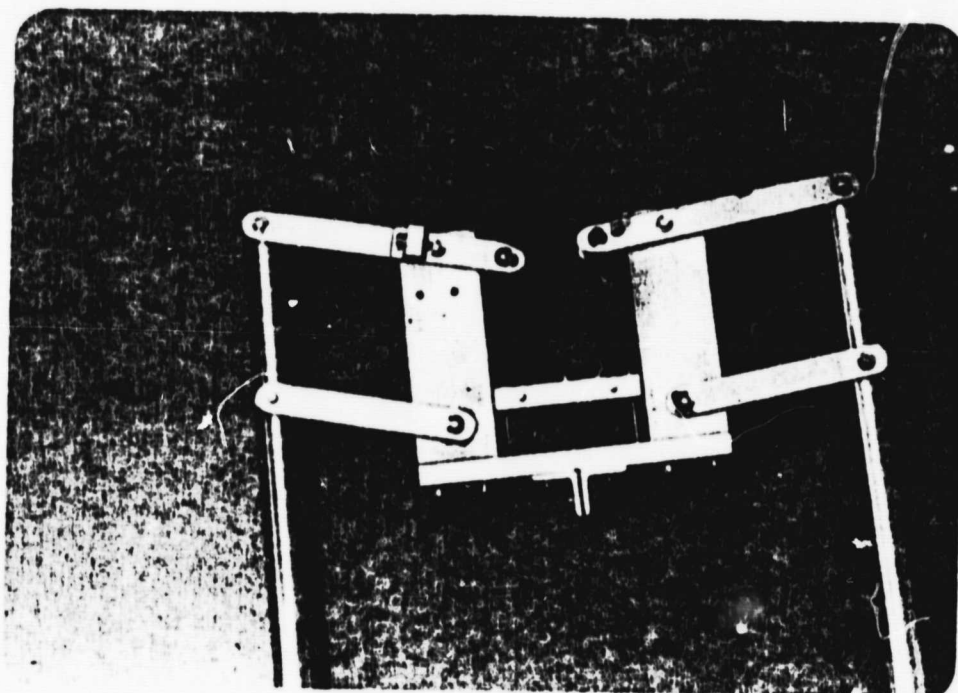


Figure 6
Pole Lever System

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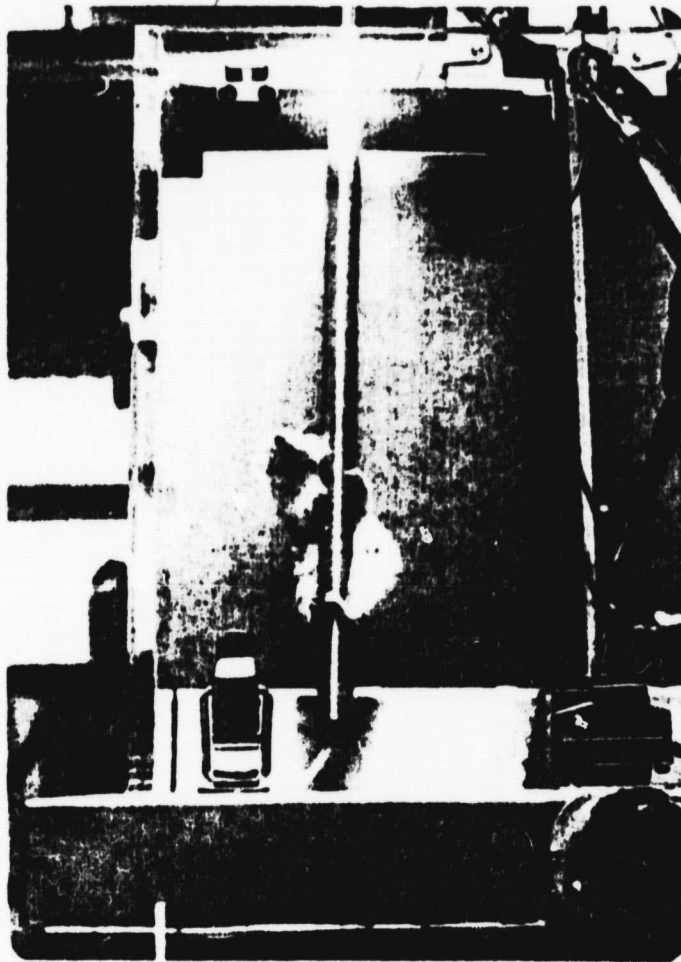
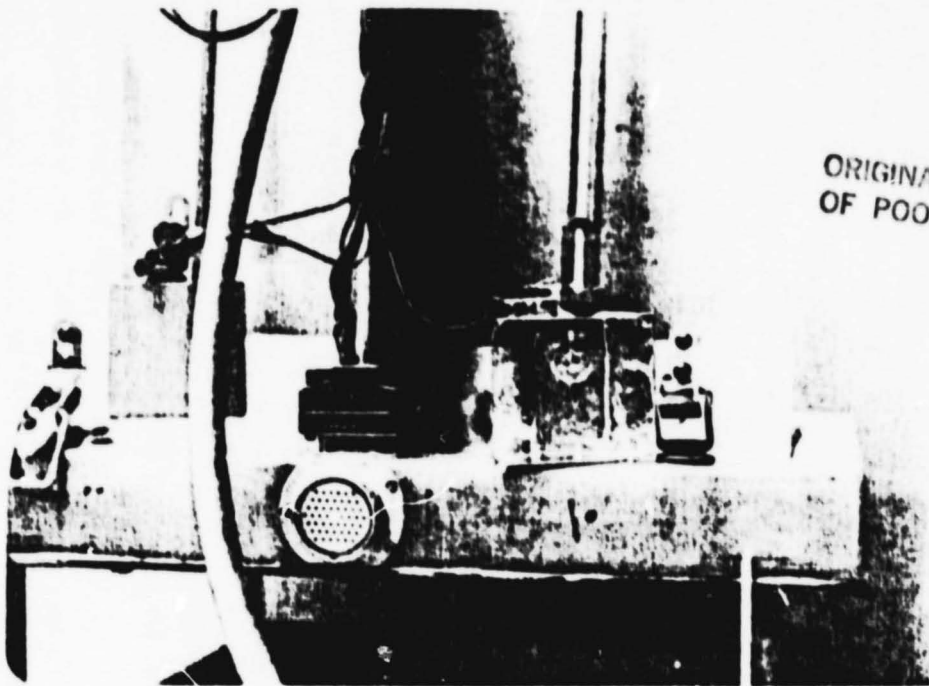


Figure 7
Mouse Pole-Jump

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Light



Tone

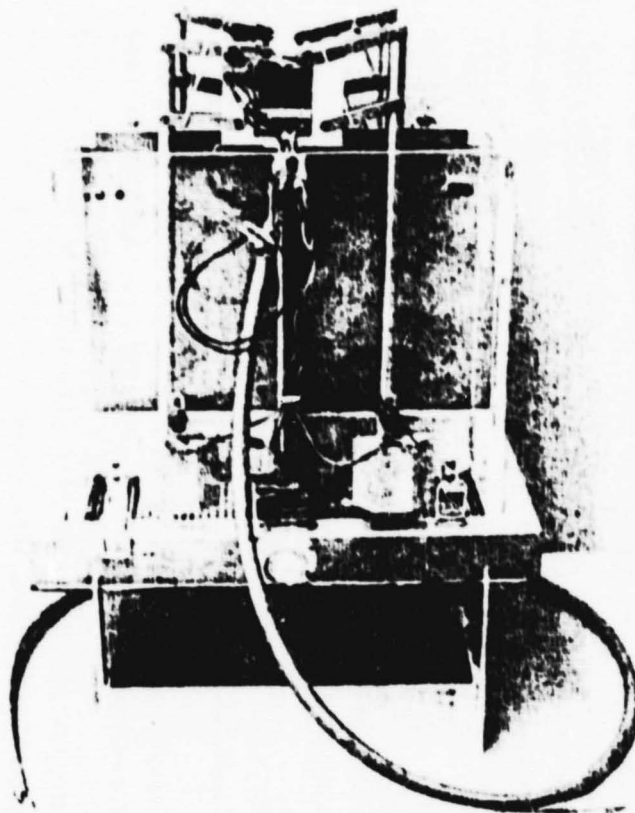


Figure 8
Lights and Tone (CS)

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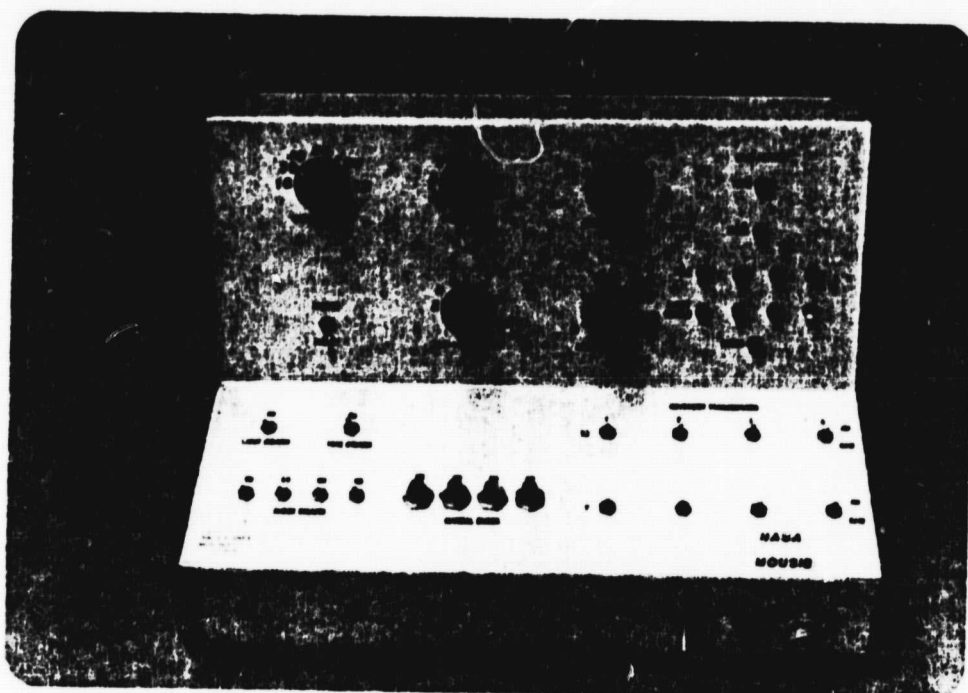


Figure 9
Control Module

5. an adjustable UCS to a maximum of 60 sec in 5 sec increments
6. an adjustable ITI from 5-60 sec in 5 sec increments
7. a random ITI of 30-90 sec

Other modules provide the following adjustments (Figure 10):

1. a tone and light intensity adjustment
2. flexure beam sensitivity adjustments

The CS, UCS and ITI adjustments are common for the four cubicles. The cubicles are independent in the respect that if a subject climbs on a pole after initiation of the light, the light is terminated and the UCS eliminated in that cubicle for that trial. The UCS and the floor grid movements, the CS and the pole deflections for each cubicle are monitored on a Beckman Offner Type 9 Dynograph with 8 channels (Figure 10).

General Training Methodology

Mice were trained in the pole-jump apparatus until their performance level became asymptotic, termed base line (see Figure 11 and page 47). Training was done during the same time period that toxic gas exposure would occur.

The paradigm used for training and exposure in the CO and HCN studies is described in Figure 12. Each trial lasted 60 seconds (Figure 12, Line 1). The paradigm, which

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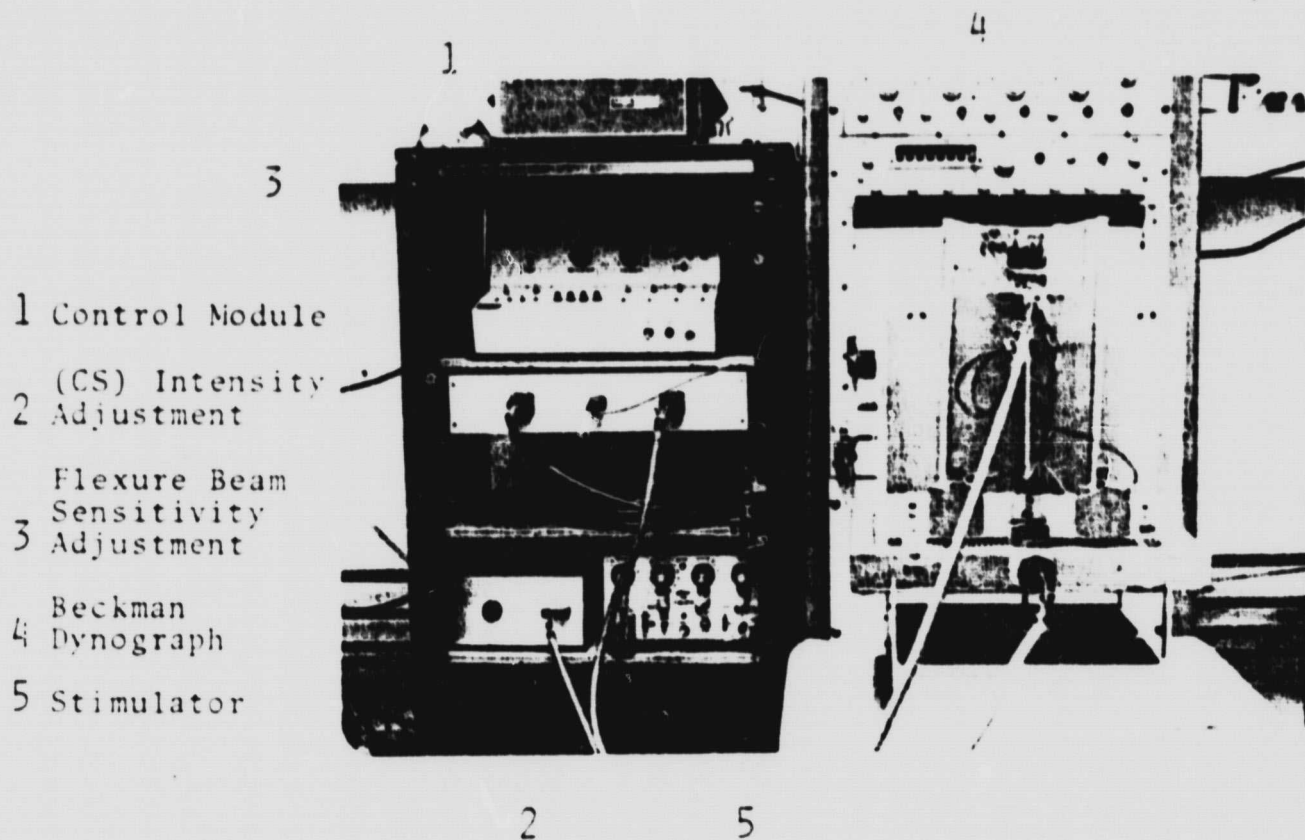


Figure 10

All Elements of the Pole-Jump Apparatus

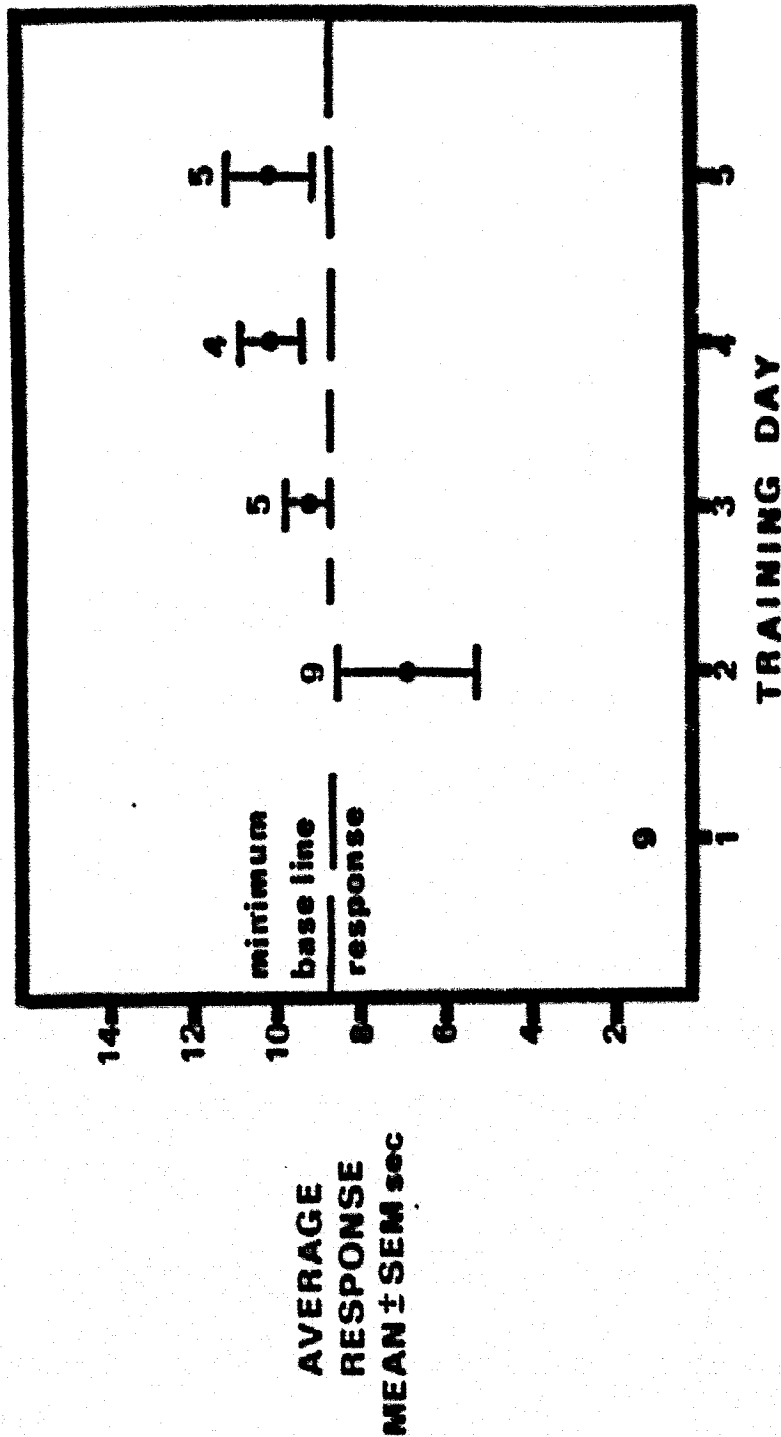


Figure 11

Typical Mouse Training. The numbers within the box are the number of mice, out of a group of 9, trained each day. Response, which is defined on page 46, was not recorded on Day 1.

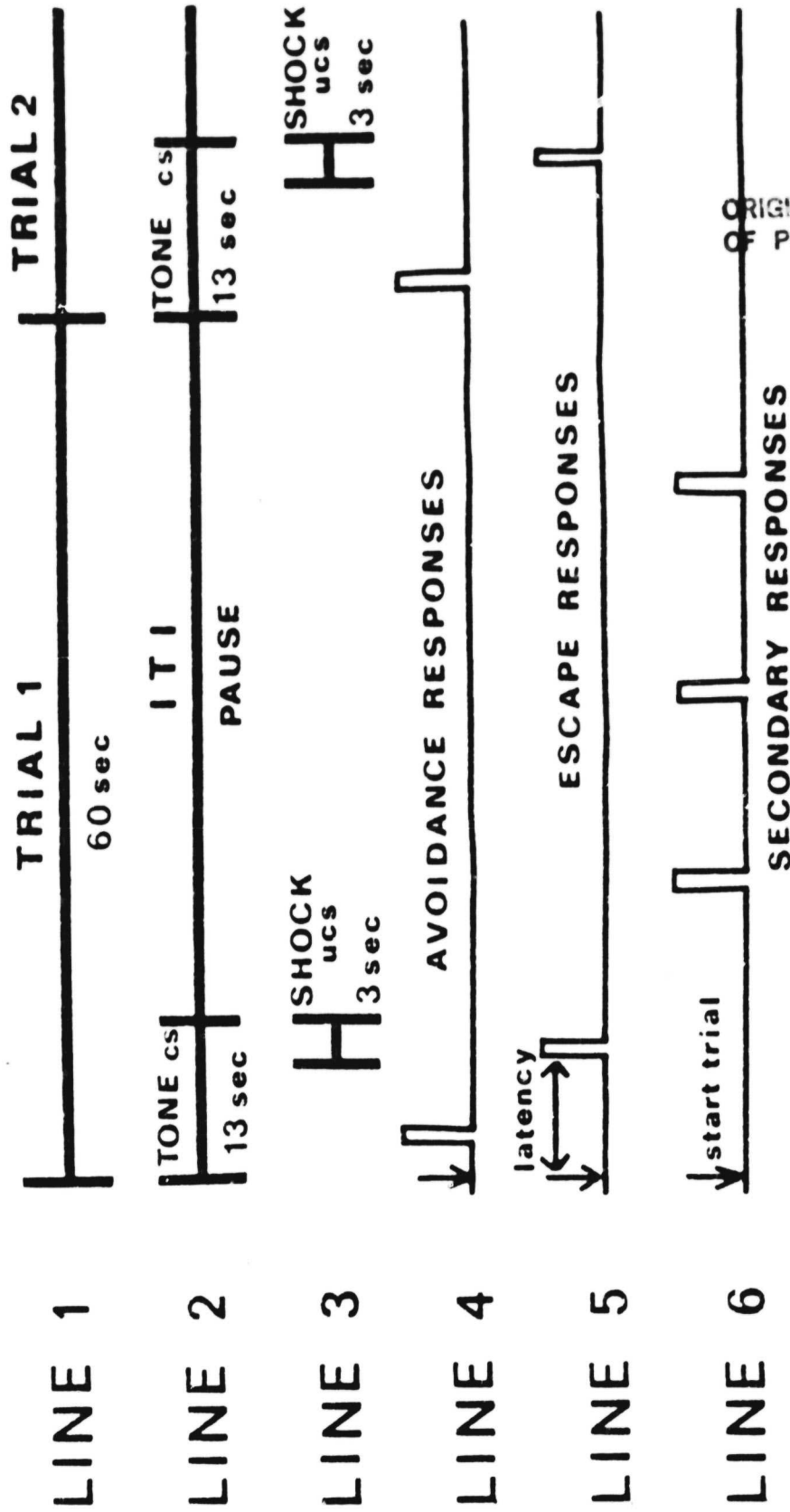


Figure 12

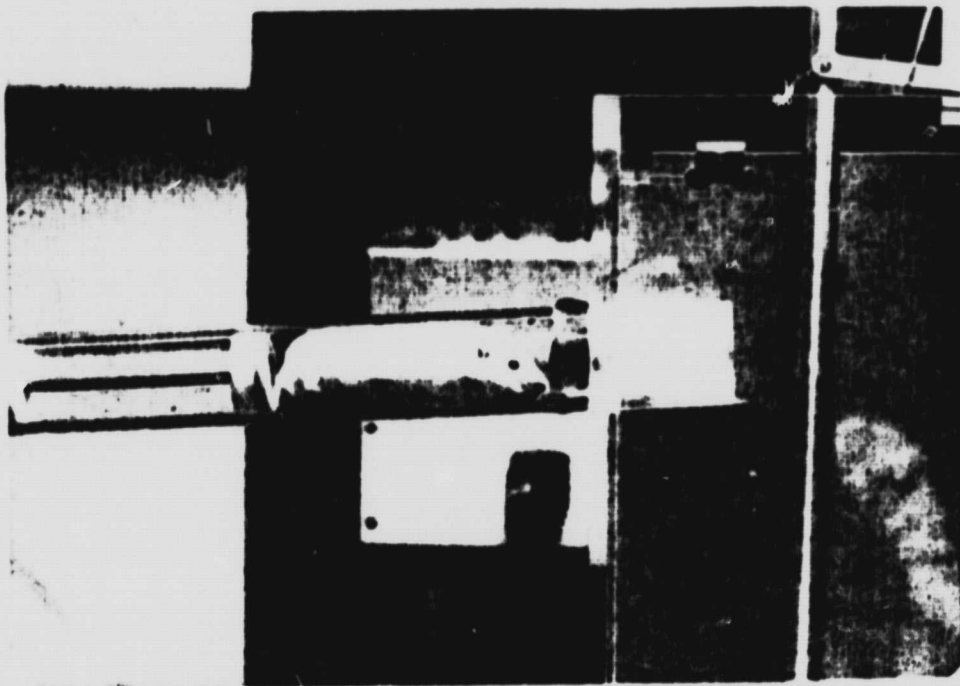
Training Paradigm-Sequence of Events. The times represented by line lengths in Lines 1, 2 and 3 are only figurative.

included a tone (Line 2), a shock (Line 3) and a pause (Line 2), allowed the animal 13 sec to jump to the pole (Figure 7). A pole-jump the first 10 sec shut off the tone and eliminated the shock (Figure 12, Line 4). A pole-jump the final 3 sec shut off the tone and the shock (Figure 12, Line 5). Jumps to the pole during the ITI (Line 6) were infrequent and not considered in the analysis of behavior.

The following methodology was used to condition a naive animal.

Day 1

1. The animal was placed in the pole-jump apparatus by injecting it through the chamber wall hole (Figure 13).
2. The animal was allowed 10 min to become accustomed to its new environment.
3. The paradigm was begun.
4. The animal was observed for its reaction to the shock. The most effective training shock, a current commonly between 0.3-0.5 ma, caused the animal to jump. Shocks of less intensity, which caused the animal to run but not jump, weren't as effective.
5. After 15 min the paradigm was turned off, and the animal was allowed 10 min to recover.
6. The paradigm was then restarted, and it continued for 30 minutes.



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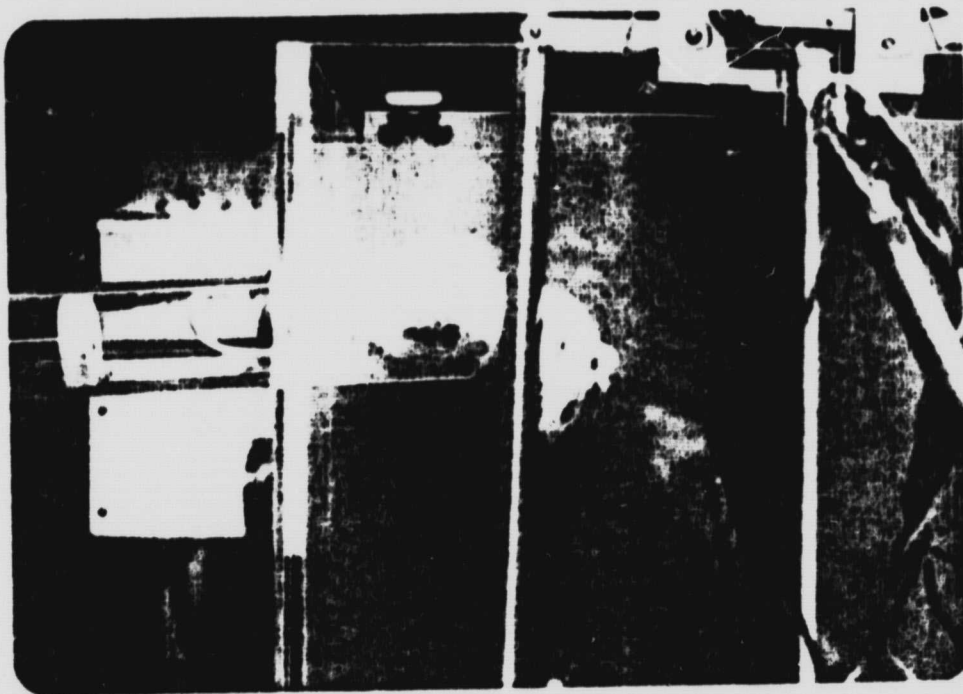


Figure 13
Injecting a Mouse Into the Chamber

7. Finally, the paradigm was turned off, and the animal was removed from the chamber.

from Day 2 until the end of training

1. The animal was injected into the pole-jump apparatus and given 5 min to become oriented before the paradigm was begun.
2. The mixing fan was started (see page 48 and Figure 16).
3. The paradigm continued for 30 min.
4. The animal was removed and placed back in its cage.

The animal's progress in learning the pole-jump behavior was assessed each day. The paradigm allowed 13 sec to jump to the pole (Figure 12, Line 2). The time from the beginning of the CS until the pole-jump, called the latency (Figure 12, Line 5), was subtracted from 13 sec to yield a number termed the response. A response of less than 3 sec meant the animal was shocked before jumping to the pole, and a response greater than 3 sec meant the animal avoided the shock. In other words, the larger the response the faster the animal jumped to the pole. The paradigm repeated 30 times in a session, and the responses of 30 pole-jumps were calculated. Daily training continued until the average response was at least 8.8 seconds (Figure 11, minimum base line response), a point at which the response was becoming asymptotic.

Gas Delivery Apparatus and Gas Analysis Procedures

Carbon monoxide and hydrogen cyanide of known concentration (Matheson Company certified standard) were used. The CO included one bottle of 3011 ppm CO in air and one bottle of 99.5% CO. The HCN was 2.13% in nitrogen.

The bottled gases were connected to a plumbing system mounted under a walk-in hood (Figure 14). The plumbing system included 3 gas flow controllers, control valves and rubber tubing (Figure 15). Gases from the 3 controllers were homogenized by passing them through 2 glass tubes filled with glass beads (Figure 15). The valves and homogenizers were connected through an isolation valve (Figure 15), so that HCN could not contaminate the CO tubing. The mixture then flowed from the homogenizers to the top of the animal response chamber (Figure 16).

The response chamber lid contained a 4 inch fan to insure rapid mixing of inflowing gases with the chamber atmosphere (Figure 16). The fan's efficiency was checked with a stannic chloride smoke generator placed at the chamber inlet which showed that the fan mixed the smoke quickly. The increased chamber pressure caused by the inflowing gases was vented out the bottom. The outlet fitting was a stainless steel tee with one arm serving as an exhaust and the other containing a septum (Figure 17). To insure the chamber was sealed, a tube connected to the

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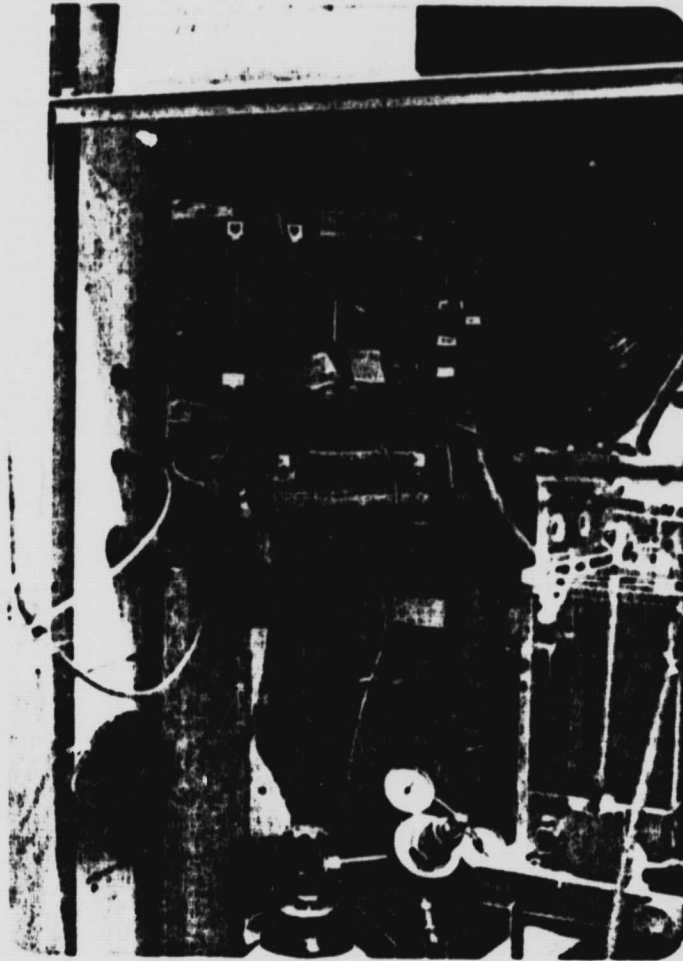
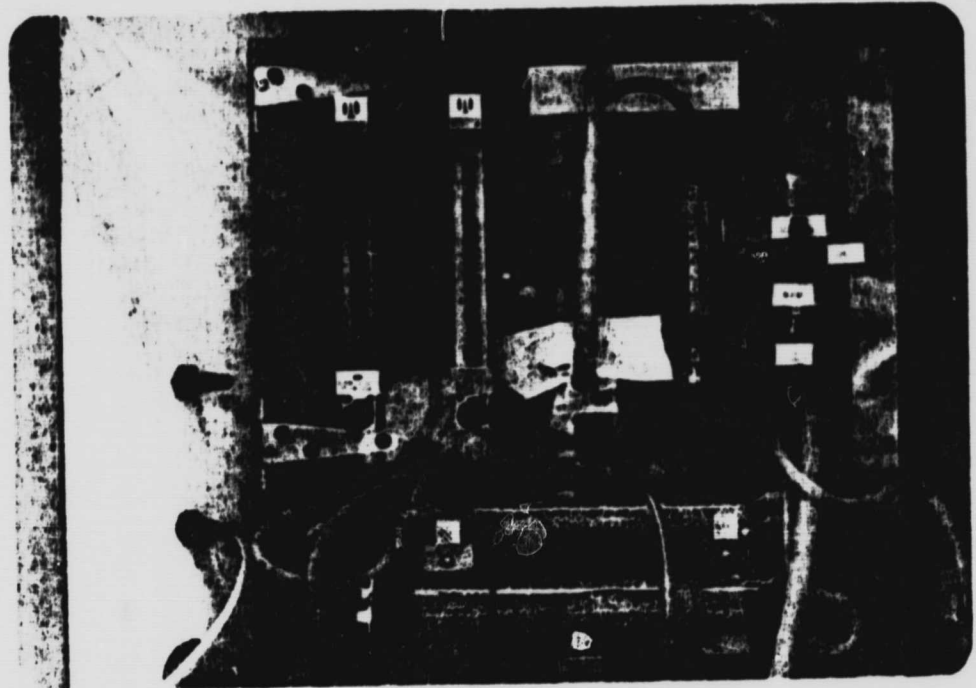


Figure 14
Gas Mixing Apparatus Under the Walk-In Hood

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Flow
Controllers
Homogenizers

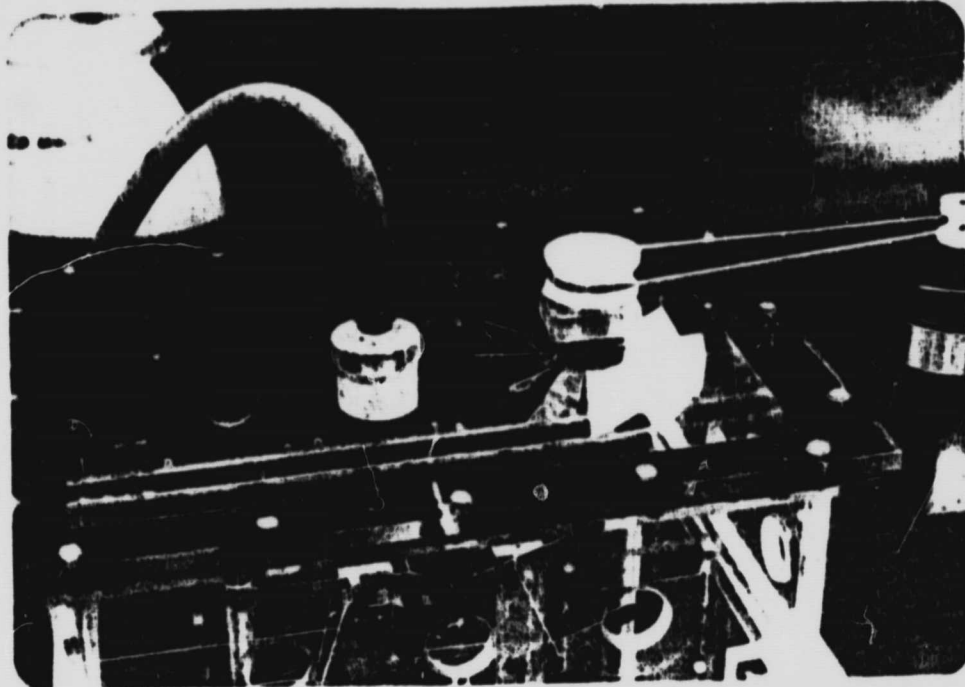


CO and HCN
Isolation Valve

Figure 15
Homogenizers and Flow Controllers

Inlet Fitting

Four Inch
Fan



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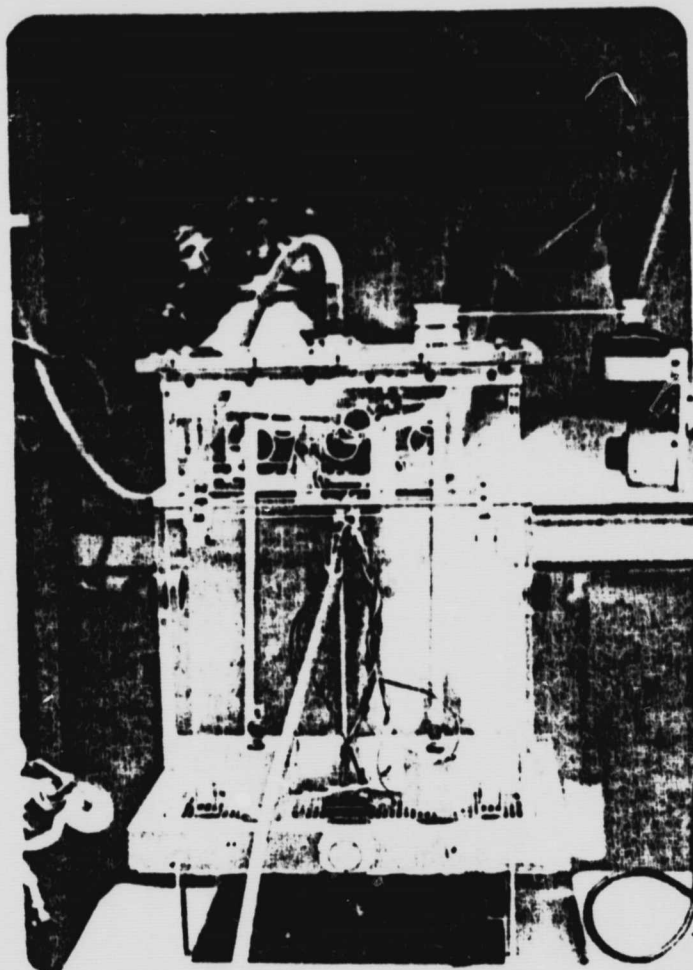


Figure 16
Gas Inlet Fitting Into the
Response Chamber Lid and
the Four Inch Mixing Fan

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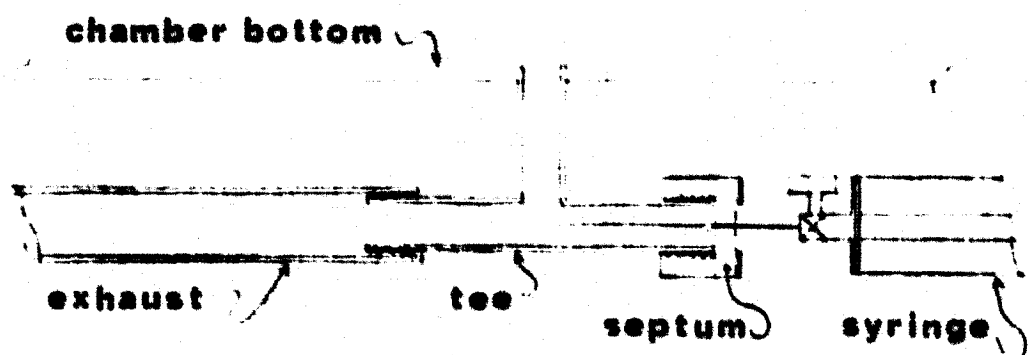


Figure 17
CO Sampling Septum

exhaust was placed in a beaker of soap solution to observe the bubbles.

Gas samples were drawn from the septum into a 50 ml plastic syringe (Figure 17), with a shut off valve, for CO analysis by molecular sieve gas chromatography (Varian Instruments). CO concentration was sampled at 2 min, at 5 min and then sampled at 5 min intervals until the end of an experiment. The HCN concentration was determined at 2 min, at 5 min and then at 10 min intervals using a NaOH scrubber (Figure 18) and an Orion cyanide specific ion electrode.

The cyanide analysis was as follows:

1. Each day, solutions of 10^{-3} , 10^{-4} and 10^{-5} M CN^- were prepared in 0.1 M NaOH, and the standard electric potentials produced by the cyanide specific ion electrode were read on a Beckman pH meter.
2. A plot of electrical potential versus CN^- concentration was made and demonstrated that change in potential was linear from 10^{-5} to 10^{-3} M CN^- .
3. During a test, a known volume of gas from the response chamber was pulled through a NaOH scrubber. This was accomplished by removing the CO septum and connecting the inlet side of the scrubber to the septum arm of the exhaust tee and the outlet side of the scrubber to a small pump.

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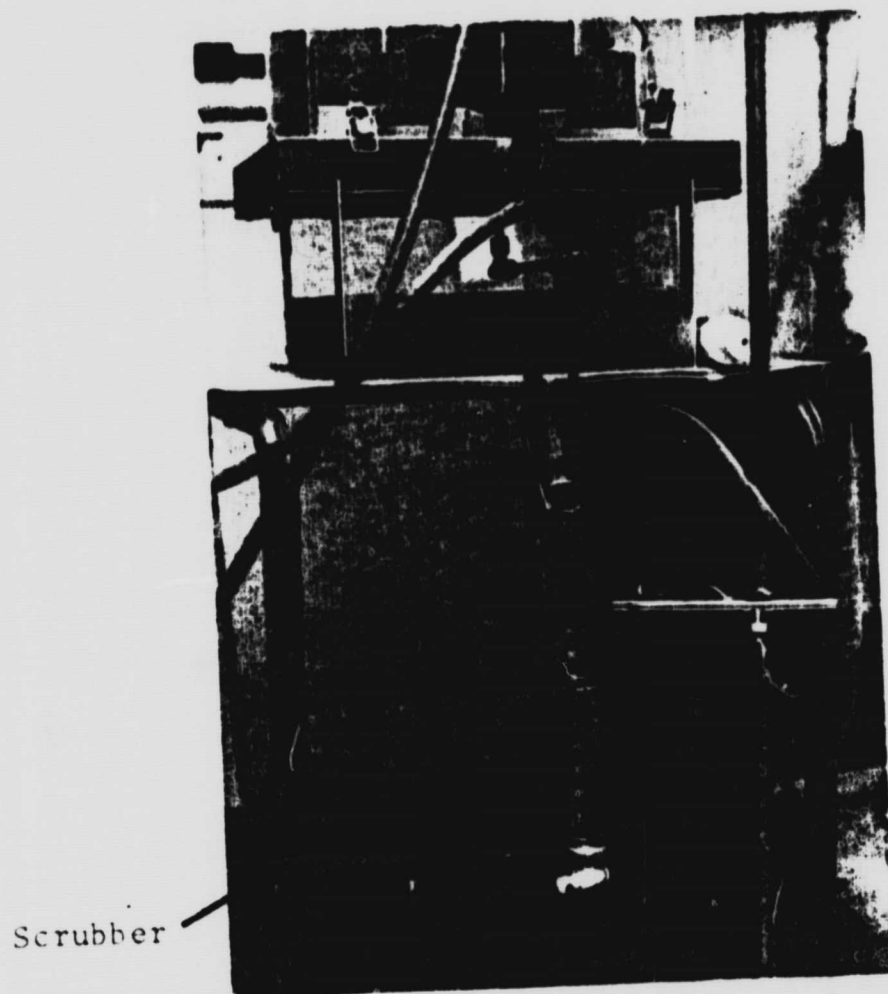


Figure 18
NaOH Scrubber for HCN Determination

4. Following the time schedule mentioned previously, a 10 ml sample of the scrubber solution was drawn to analyze for CN^- concentration.
5. After each sample was taken, gas flow through the scrubber was checked with a bubble column.

The scrubber solution, which was 0.1 M in NaOH and 10^{-5} M in CN^- , contained enough CN^- , so that the CN^- concentration of the first sample taken was in the linear region of electrode response. The acid HCN gas was neutralized by the NaOH in the scrubber, and the increase in CN^- concentration in the scrubber solution was converted into HCN concentration in the vented gases.

Toxic Gas Testing with CO and HCN

Subjects

Subjects were male Swiss Webster mice 8-10 weeks of age (35-40 gm). On receipt from the animal colony, the mice were housed in rodent cages in a sound attenuated plywood box. The box had a fan that circulated air at a maximum rate of 50 cu ft per minute. A florescent light automatically operated on a 12 hr light-dark cycle. The animals were provided food and water ad lib.

Carbon Monoxide-Dynamic Concentration Methodology

Using the following procedure, individual mice were exposed to increasing concentrations of CO that approximated the production of CO in the NASA Radiant Panel Test System. After placing the gas apparatus outlet hose in the walk-in hood suction vent, the apparatus was adjusted to deliver a predetermined CO concentration and allowed to equilibrate. Concurrently, a trained animal was placed in the response chamber, the fan was switched on and the paradigm was begun to allow the animal 5-10 warm up trials. The warm up insured the animal was at base line when the experiment began (Dilley et al. 1977). At zero time, the gas apparatus outlet hose was placed in the chamber inlet fitting, and the exposure began.

The CO concentration of the inflowing mixture was such that the response chamber contained 600-3000 ppm CO within 3-15 min. The desired experimental CO conditions were randomized by listing them on separate pieces of paper. The slips of paper were placed in a basket, and the tests were drawn each day until the basket was empty. All testing occurred between 13:00 and 16:00 hours.

Exposure was terminated by either of two contingencies: 1) the animal failed to escape the shock three consecutive times and had diminished postural tonus (Figure 25, page 82), or 2) thirty minutes elapsed. The 30 min

time limit was similar to that used in the radiant panel burns. Contingency 1 applied in 26 of the 28 experiments. In addition to the loss of escape, the initial behavioral change (i.b.c.) was also determined. This change was defined as: 1) the first trial the animal failed to avoid the shock (avoidance block) that was followed by loss of avoidance until the end of the experiment, or 2) the first trial the animal failed to avoid the shock that was followed by 4 avoidance blocks in the next 6 trials. Figure 24 (page 81) is an example of situation 1, and Figure 25 shows situation 2.

Recovery of Conditioned Behavior after CO Intoxication

In three of the dynamic concentration tests, the trained animal was removed from the pole-jump apparatus immediately post-exposure, the chamber was flushed with air for 5 min and the atmosphere was sampled for residual CO. The animal was then put back in the chamber and periodically tested for recovery of pole-jump behavior until its response returned to base line.

Carbon Monoxide Static Concentration Methodology

Static exposures, to determine the variation in time to the behavioral end points among animals in the test population, were done at two CO concentrations. The static

and dynamic methodologies differed in the following respects. As is the dynamic tests, a trained animal was placed in the pole-jump apparatus, the apparatus was sealed, the fan activated and the paradigm begun. At the same time, the gas apparatus was adjusted to deliver the appropriate CO concentration and allowed to equilibrate. After approximately 5 min, however, a calculated quantity of 99.5% CO was injected (30 sec) into the response chamber in a location that caused it to pass through the fan before reaching the animal. The amount of CO necessary to produce the desired chamber CO concentration was determined by a number of preliminary tests.

In the preliminary tests, a predetermined quantity of 99.5% CO was injected into the chamber, and the resulting CO concentration was analyzed by GC. Adjustments were made in the quantity of CO introduced, and the process was repeated until the desired chamber CO concentration was attained.

After injection of the 99.5% CO was complete (40 sec), a CO-air mixture equal to the chamber in CO concentration was diverted into the chamber from the gas delivery apparatus at a rate of 6 l/min. The exposures were terminated by the dynamic methodology criteria (page 56) except that the time limit was 60 min. The longer limit was necessary, because the low CO concentration used in one

set of experiments required, in some animals, more than 30 min to cause loss of escape. All testing was done between 9:00 and 12:00 hours.

Carboxyhemoglobin Concentration at Loss of Escape and Death

The percent COHb was determined by the method of Williams et al. (1960) at the second behavioral end point, loss of escape. Two paradigm trained animals were sacrificed, using CO₂, immediately after the first trial they failed to escape the shock. Both animals exhibited reduced postural tonus when sacrificed.

To compare the percent COHb at loss of escape with that existing at death, 3 untrained mice were exposed to 6000 ppm CO until their respiration ceased. They were immediately removed from the chamber and blood analyses for percent COHb were done.

Half Life of COHb in Mice

In 4 experiments, groups of 8 mice were exposed to increasing concentrations of CO (0-3000 ppm) for 20 min in a cubicle of the pole-jump apparatus. After exposure, the group was removed from the apparatus and one mouse was sacrificed every 5-15 min using CO₂, a blood sample (0.5 ml)

was collected by intracardiac puncture, and the percent COHb was determined

Hydrogen Cyanide

There is little information in the literature concerning levels of HCN that cause behavioral changes in mice. In order to find a reasonable range of HCN concentrations to work with, 17 trained mice previously exposed to CO were used first. After this range was determined, a list of desired HCN concentrations was prepared and placed in the basket. Naive conditioned mice were then exposed to HCN concentrations drawn from the basket using the static CO methodology. 1) The subject was placed in the box, 2) the paradigm was begun, 3) a predetermined amount of 2.13% HCN was injected and 4) the HCN-air mixture was started into the chamber. The static CO methodology end point criteria were used. All testing was done between 10:00 and 14:30 hours.

Chapter 4

RESULTS

Base Line Response

Figure 11 (page 43) is a typical learning curve for 9 mice trained in the pole-jump apparatus. As shown in the figure, all 9 mice were trained on days 1 and 2. After day 2, 4 of the 9 mice were near base line response; as a result, on day 3 only the 5 slower learners were trained. On day 4, 4 of the 9 mice were randomly selected and trained, and the balance were trained on day 5. Hence, the 9 animals were at base line response by days 4 and 5. The average base line response for 89 animals trained was 9.9 ± 1.0 SEM seconds. Three animals were eliminated, because they learned to jump up and cling to the pole lever apparatus when shocked.

Dynamic Concentration Tests with CO

Table II is a summary of the 28 dynamic CO pole-jump experiments. In the table CT, listed in columns 2 and 5, is the product of the average CO concentration in ppm times the exposure duration in minutes. CT was determined by plotting the dependent variable CO concentration against time and integrating the area under the curve from zero time to the end points (Figure 19). The average CO

TABLE II

Summary of Dynamic CO Exposure
Pole-Jump Experiments

Experiment #	Initial Behavioral Change (CT)	Time to Initial Change (min)	Average CO at Initial Change (ppm)	Loss of Escape (CT)	Time to Loss of Escape (min)	Average CO at Loss of Escape (ppm)
M-24	203	2.2	3000	7203	2.2	3000
OB-3	10125	4.5	2250	11913	5.5	2166
M-19	11866	5.5	2157	11866	5.5	2157
OB-9	6972	4.2	1160	12924	6.5	1988
M-12	5652	3.4	1662	9079	4.6	1974
M-20	11187	5.8	1945	11187	5.8	1945
OB-6	9503	5.3	1810	9503	5.3	1810
OB-5	11544	7.0	1649	14387	8.2	1760
M-18	10428	6.4	1604	10428	6.4	1604
M-7	3925	2.5	1570	3925	2.5	1570
OB-2	8183	6.0	1363	13361	7.9	1564
OB-7	10882	8.0	1360	15267	10.3	1489
OB-8	11544	8.4	1374	13820	9.7	1429
M-17	11347	8.2	1390	11347	8.2	1390
M-8	11792	8.4	1403	13241	9.6	1379
M-6	9500	7.2	1319	9500	7.2	1319
M-5	9000	6.9	1304	9000	6.9	1304
M-21	14043	11.6	1210	29286	24.0	1220
M-22	5433	4.5	1207	5433	4.5	1207
M-16	13510	12.4	1089	20138	17.1	1191
M-11	4181	6.0	697	20497	18.6	1101
M-23	6832	6.8	1012	6832	6.8	1012
M-15	15367	17.0	904	22074	23.2	953
OB-1	9830	12.0	819	14915	17.0	887
M-13	10360	12.3	842	15891	19.7	812
M-14	9349	11.7	801	9349	11.7	801
M-25	-----	-----	-----	-----	none	797
M-26	19300	25.0	772	-----	none	772

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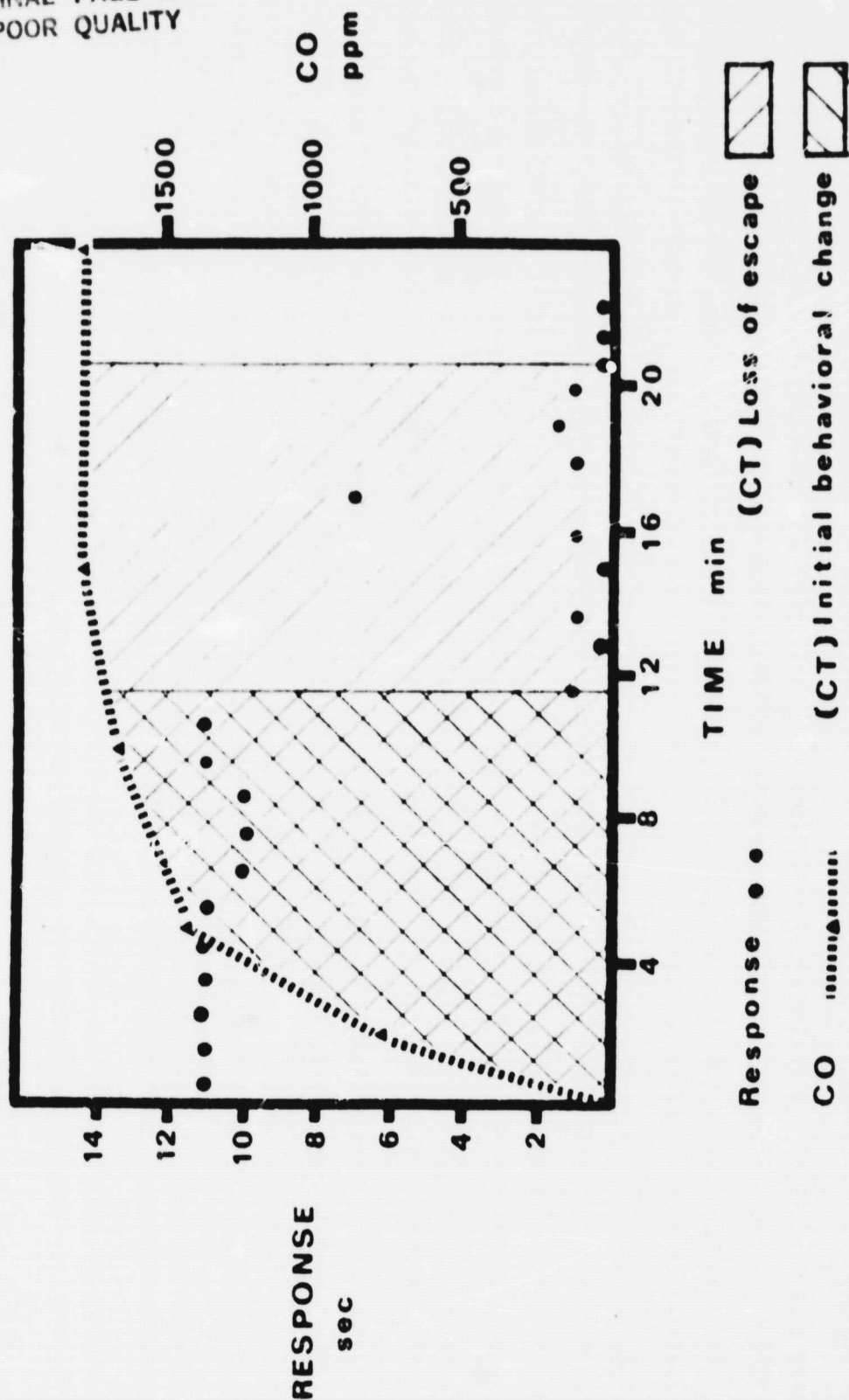


Figure 19

Typical Integration of CO Plots to Determine CT at Behavioral End Points.

concentrations, columns 4 and 7, were calculated by dividing the CTs in columns 2 and 5 by the end point times in columns 3 and 6. In experiments M-25 and M-26 avoidance was affected, however the escape response was never lost within the experimental time limit.

Figure 20 depicts the dose response relationship of the average CO concentration versus time to behavioral end point data of Table II. The lines, dashed for the initial behavioral change and solid for loss of escape, are the best least squares fits of the data points. The analytical program solved the equation:

$$T = (q/C)^{1/b}$$

where T is time at the end point and C is average concentration for values of q, a and b, so that the sum of squares (SS) of the following relationship was minimized.

$$SS = [T_{real} - T_{predicted}]^2$$

The program required a beginning experimental value for time and concentration and a guess of the value of q. Various combinations were tried. A concentration of 953 ppm, a time of 23 min and q equals 1000 provided the minimum sum of squares. The equation was solved for loss of escape using 3 criteria: I) exponents a and b were restricted to a value of 1, II) a was restricted to 1 and b

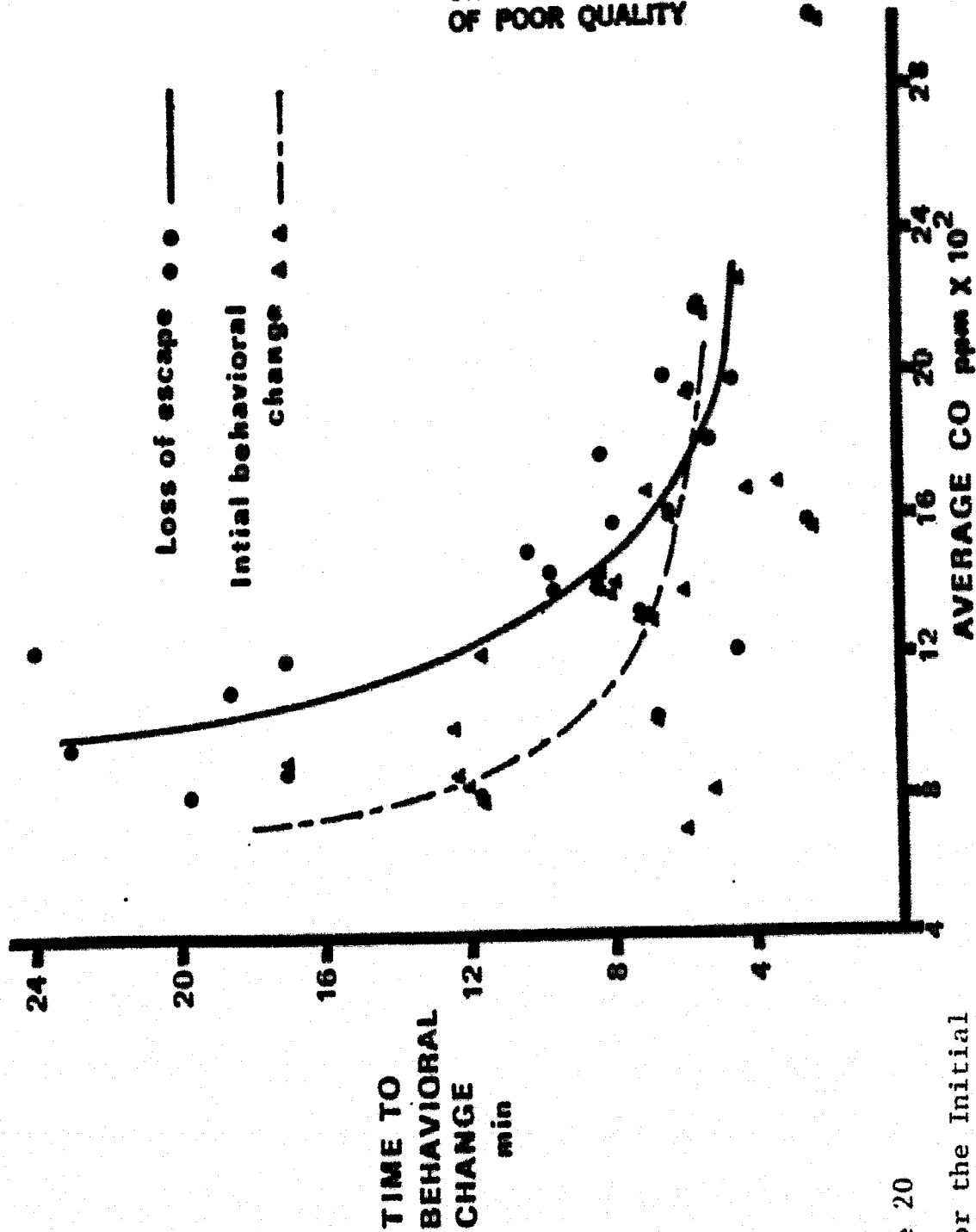


Figure 20

Dose-Response for the Initial Behavioral Change and Loss of Escape in the Dynamic CO Exposures.

to 0.5, and III) a and b were between the values of -5 and 5. The equation was also solved for the initial behavioral change using criterion III. The results of this analysis are listed in Table III.

Recovery of Conditioned Behavior After CO Exposure

The times to return to pre-exposure pole-jump response levels were determined in 3 of the dynamic experiments (Table IV). In the 3 animals tested, recovery, defined as 3 consecutive base line responses, was complete within 80 minutes. The animal exposed in OB-2 was tested for 2 days post-exposure and showed no decline in its base line response.

Static Concentration Tests with CO

The tests with two static carbon monoxide concentrations, designed to show the variation in time to the behavioral end points, are summarized in Table V. One group of animals (n=11) was tested at 1091 ± 67 ppm, however 3 animals did not reach the experimental end point of interest, loss of escape, in 60 min and were eliminated. At the higher concentration, 1626 ± 81 ppm, all animals stopped responding and were included in the table.

TABLE III

Analysis for Best Fit Curves to Describe the CO
Concentration and Behavioral End Point Data of
Table II and Figure 20

Behavioral Change	$T = (q/C^a)^{1/b}$ Conditions Placed on a and b	Predicted Value		Sum of Squares
		a	b	
Loss of Escape	a and b = 1	1.0	1.0	570
Loss of Escape	a = 1, b = 0.5	1.0	0.5	476
Loss of Escape	-5 < a < 5 -5 < b < 5	1.0	0.3	444
Initial Behavioral Change	-5 < a < 5 -5 < b < 5	1.0	0.3	149

TABLE IV
Recovery of Pole-Jump Behavior
After Loss of Escape

Experiment #	Time to First Pole-Jump Post-Exposure (min)	Time to Apparent Recovery (min)
OB-2	37	65
OB-6	47	80
OB-9	14	27

TABLE V

Static Exposure Pole-Jump Experiments
at Two Carbon Monoxide Concentrations

Sample Size	CO Concentration (Mean $\pm$ SD ppm)	Time to Initial Behavioral Change (Mean $\pm$ SD min)	Time to Loss of Escape (Mean $\pm$ SD min)
8 ^a	1091 $\pm$ 67	13.4 $\pm$ 4.0	20.2 $\pm$ 6.3
8	1626 $\pm$ 81	8.0 $\pm$ 1.8	10.3 $\pm$ 1.6

* The actual sample size was 11, however 3 animals failed to stop responding in 60 min and were not included in the calculations.

Carboxyhemoglobin Concentration at Loss of Escape
and Death

In order to compare carboxyhemoglobin at loss of escape and at death, blood analyses were done in 5 animals. The 2 trained animals of experiments OB-7 and OB-8 sacrificed with CO₂ after the first trial they failed to escape had 51 and 58% COHb. The 3 randomly chosen untrained mice killed with an exposure to 6000 ppm CO had 89, 76 and 81% COHb.

The Half Life of COHb in Mice

The elimination of carbon monoxide from human and animal blood is a first order process (Peterson and Stewart 1970; Korobov and Filippov 1979). For this reason, the half life of COHb in each of the 4 groups of mice exposed to CO was calculated by plotting the natural log of the group members' percent COHbs versus their sacrifice times post-exposure. The slope of the best least squares fit straight line (Figure 21) through the group data points was divided into the natural log of 0.5 to yield the half life. The half lives of COHb in the 4 groups of mice were 21, 40, 14 and 18 min. In total, 32 mice were analyzed for percent COHb in this study. Evidence for the rapid elimination of CO was substantiated by the fact that, even though the first animals sacrificed in the 4 groups had approximately

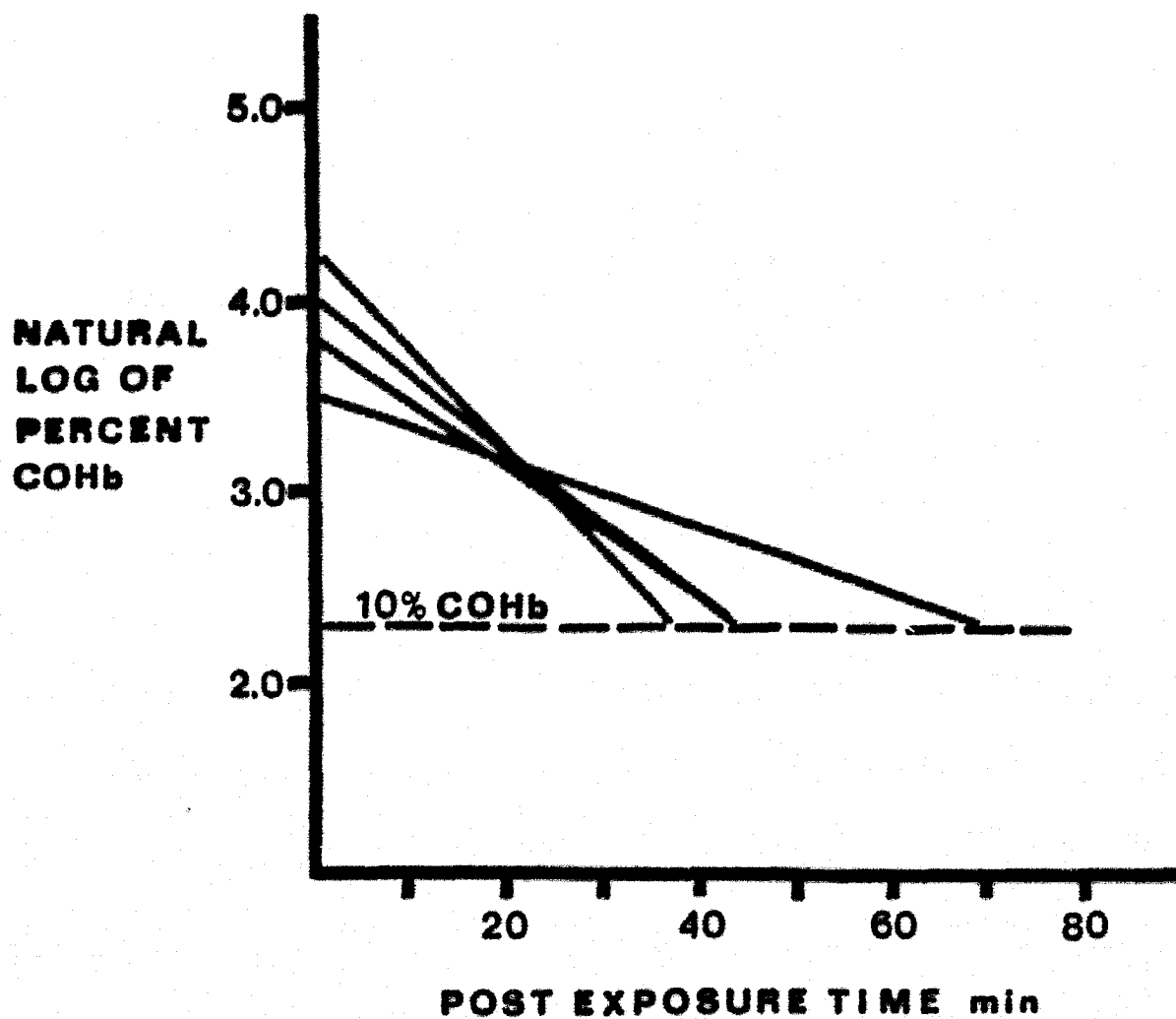


Figure 21

Decay of COHb in 4 Groups of Mice
After a 20 Minute Exposure to CO.

50% COHb, in 19 of 20 animals sacrificed 44 or more minutes post-exposure, the COHb was less than 10%.

Hydrogen Cyanide

The behavioral changes in mice exposed to HCN are listed in Table VI and plotted as a dose-response relationship in Figure 22. The table, which includes an in-depth description of the adverse behavioral changes occurring during HCN exposure, reveals the complex and significantly different behavioral pattern compared to that of CO. The test end points were the same as described for the static CO study.

TABLE VI

Summary of Behavior During
Hydrogen Cyanide Exposures

HCN (ppm)	Test #	Time to Initial Behav- ioral Change (min)	Time to Loss of Escape (min)	Description of Behavior During Exposure Including Occurrence of Temporary Loss of Escape
130	M-45	2.1	2.1	prostrate on grid, very deep breaths
101	M-52	2.2	2.2	prostrate on grid, very deep breaths
80	M-54	5.2	7.2	loss of escape from 5-7 min one escape at 7.2 min
78	M-58	5.0	13.8	loss of escape from 5-12 min one escape at 13.8 min
77	M-60	4.0	9.0	loss of escape from 4-6 min avoidance block 6-9 min
69	M-49	4.0	35.0	loss of escape from 4-11 min avoidance block from 11-35 min
68	M-47	22.0	26.3	erratic response with inter- mittent blocked avoidance during entire exposure
66	M-59	9.0	28.5	avoidance block 9-19 min then recovery, avoidance block 25-28.5 min
65	M-55	5.0	46.0	loss of escape from 5-12 min avoidance block from 12-46 min
64	M-56	37.0	58.0	avoidance block 37-43 min then recovery, avoidance block 52-58 min

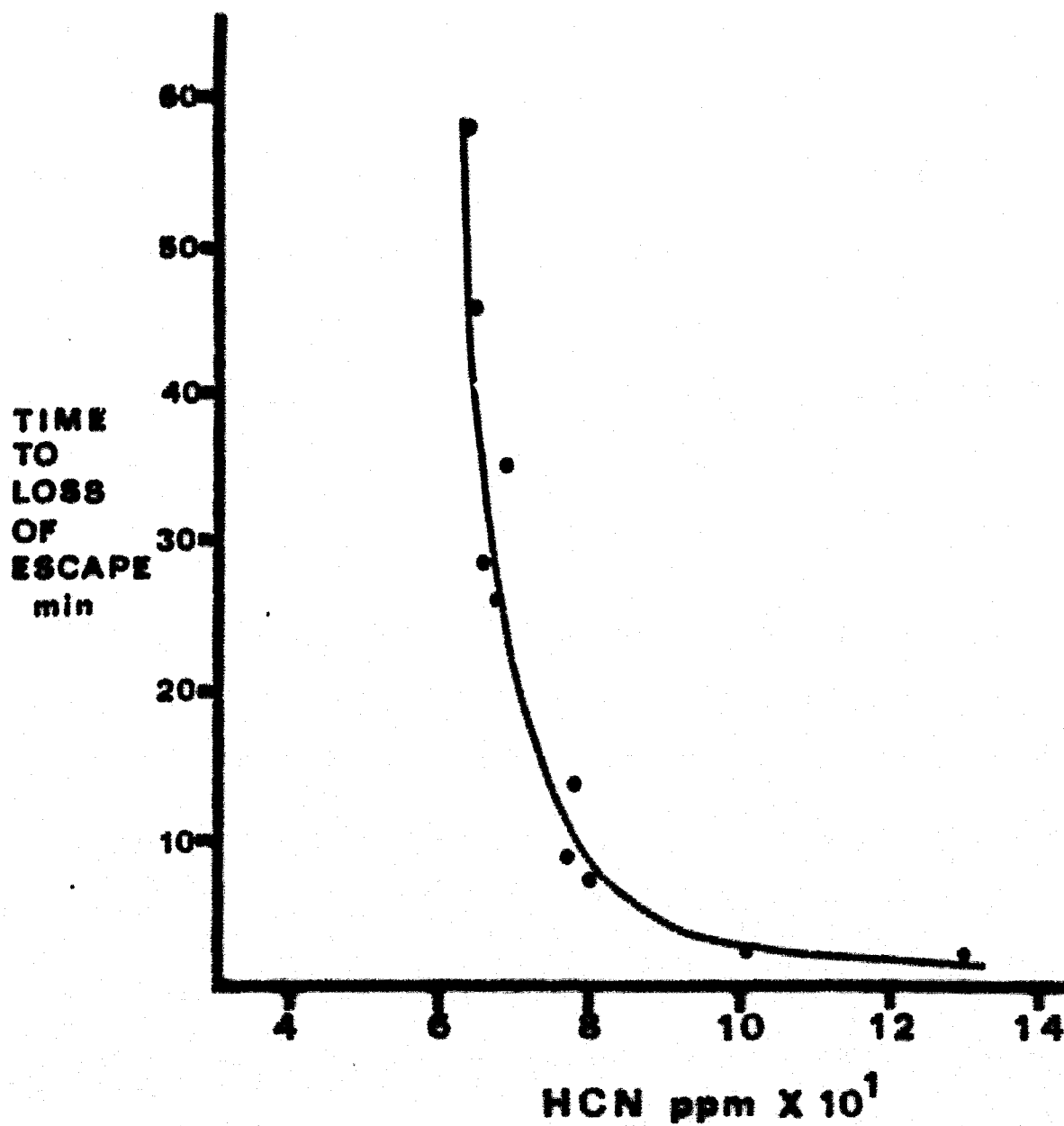


Figure 22

Dose-Response for Loss of Escape
in Hydrogen Cyanide Exposures.

Chapter 5

DISCUSSION

Rationale for Development of the Pole-Jump Apparatus

The recent catastrophes in Las Vegas are striking examples of the potential for disaster in fires. The results were as familiar as they were tragic. Many victims were overcome by toxic combustion products before they could escape or be rescued.

A hypothetical progression of toxic symptomology in a fire would include many aspects. Initially, the victim might experience burning and irritation in the eyes and nasal mucosa caused by gases, such as SO_2 and NH_3 . At the same time, asphyxiants, like CO , H_2S and HCN could affect the central nervous system changing breathing rates and higher order thinking processes. Exposure to increasing concentrations of irritants could completely close the victims eyes and make breathing very difficult. In time COHb concentration could build causing loss of motor function and incapacitation. In the end, if outside help did not come, death would occur.

At some point in this scenario, the victim lost the ability to save himself. When this occurred has not been clearly defined, however a victim who has lost motor function is obviously in trouble. For this reason, many

authors have used motor function incapacitation as the critical end point in combustion toxicology studies: including Packham et al. (1976), Crane et al. (1977), Saito (1977) and Kanakia et al. (1980).

Assessing incapacitation in a rotating cage or similar device is a simple task that produces time to incapacitation data. Unfortunately, no insight is gained into adverse changes occurring before loss of motor function. It is also necessary to relate loss of wheel running ability to what is really of interest, namely loss of avoidance and escape behavior.

For these reasons a group at NASA, using the pole-jump response described by Cook and Weidley (1957) and modified by Dilley et al. (1977), developed an instrumented pole-jump avoidance-escape response apparatus to assess combustion product toxicity. This apparatus allows observation and quantification of behavioral changes as they occur and does not rely on a single end point. In addition, a species specific survival behavior is tested that more closely approximates the situation of the human fire victim than approximated by the rotating cage.

The pole-jump apparatus was designed to train and test 4 animals simultaneously with lights as the CS or 1 animal with the tone as the CS. After a number of experiments, it was apparent that mice learned to avoid the

shock more rapidly using the tone rather than the lights. Because understanding combustion product effects on avoidance-escape behavior was of immediate interest, the apparatus was used with the tone. Modifications to make the lights an efficient CS are discussed in Chapter 6.

As mentioned previously, the tone was a very effective CS. Many animals learned to avoid the shock the first training day, and all learned by day 4. Figure 11 is a typical example. Once an animal reached base line, its response was extremely stable. Periodically, however, each trained animal tested the system to see if not jumping would be followed by a shock. This phenomenon is normal in conditioned animals and is the first step in unlearning a behavior that is no longer reinforced (Fantino and Logan 1979). Because animals do periodically fail to avoid a shock, the initial behavioral change was defined by multiple failures to avoid that occurred only during toxic gas exposures.

It was obvious from the frequency of defecation and urination that the pole-jump chamber was a stressful environment the first training day. Once avoidance was learned, however, the amount of feces and urine declined significantly. Preliminary experiments showed that a 10-15 min recess during the first day of training helped facilitate learning which minimized stress.

A final characteristic of pole-jump to be considered is secondary responses, which occur during the ITI. Initially, when avoidance was learned, secondary responses were common; but by training day 3, their number approached zero.

Dynamic Concentration Tests with CO

In the radiant panel system, burning materials normally produce up to 3500 ppm CO. During a burn, the CO increase profile can approximate a smooth hyperbolic function; or after a short delay, the increase may be almost instantaneous before leveling off. The dynamic concentration tests with CO, listed in Table II, simulated a range of CO increase profiles that resulted in mean CO concentrations between 700-3000 ppm.

The pole-jump test with mice worked well in the desired concentration range of 800-3000 ppm as shown by the dose-response relationship of Figure 20. Below a mean CO concentration of 800 ppm, the mice escaped successfully for more than 30 min, and the experiments were discontinued. Above a mean CO concentration of 3000 ppm, loss of escape occurred in 2-3 minutes.

Carbon monoxide is an unusual toxic gas in the respect that there is abundant information on its behavioral effects on both humans and animals (Latis and

Merigan 1979). From human and animal data, it is obvious that there is a progression of increasingly distressful symptomology associated with the duration and concentration of CO exposure.

It is apparent from Figure 20 that the pole-jump test reveals two concentration dependent behavioral changes with carbon monoxide. It is also apparent that the relationship between the two end points is concentration dependent. For example, in the high CO concentration exposures where the animal responded less than 6 min, response was base line and then abruptly dropped to zero. A typical example is shown in Figure 23. In exposures lasting 6-12 min, response was base line until the final 1-3 trials when the animal jumped to the pole only when shocked (Figure 24). In low concentration experiments, where the animal responded for 13-25 min, the initial behavioral change occurred typically at about one half the time required to cause complete loss of escape (Figures 25 and 26). Following the initial behavioral change, response was characterized first by escapes mixed randomly with avoidances, followed by complete avoidance block and finally by loss of escape. The pattern, then, seems to be that at high CO concentrations the animal is overcome rapidly, as evidenced by an abrupt loss of escape and postural tonus. Below mean CO concentrations of 1600 ppm, however, the initial

CO ppm

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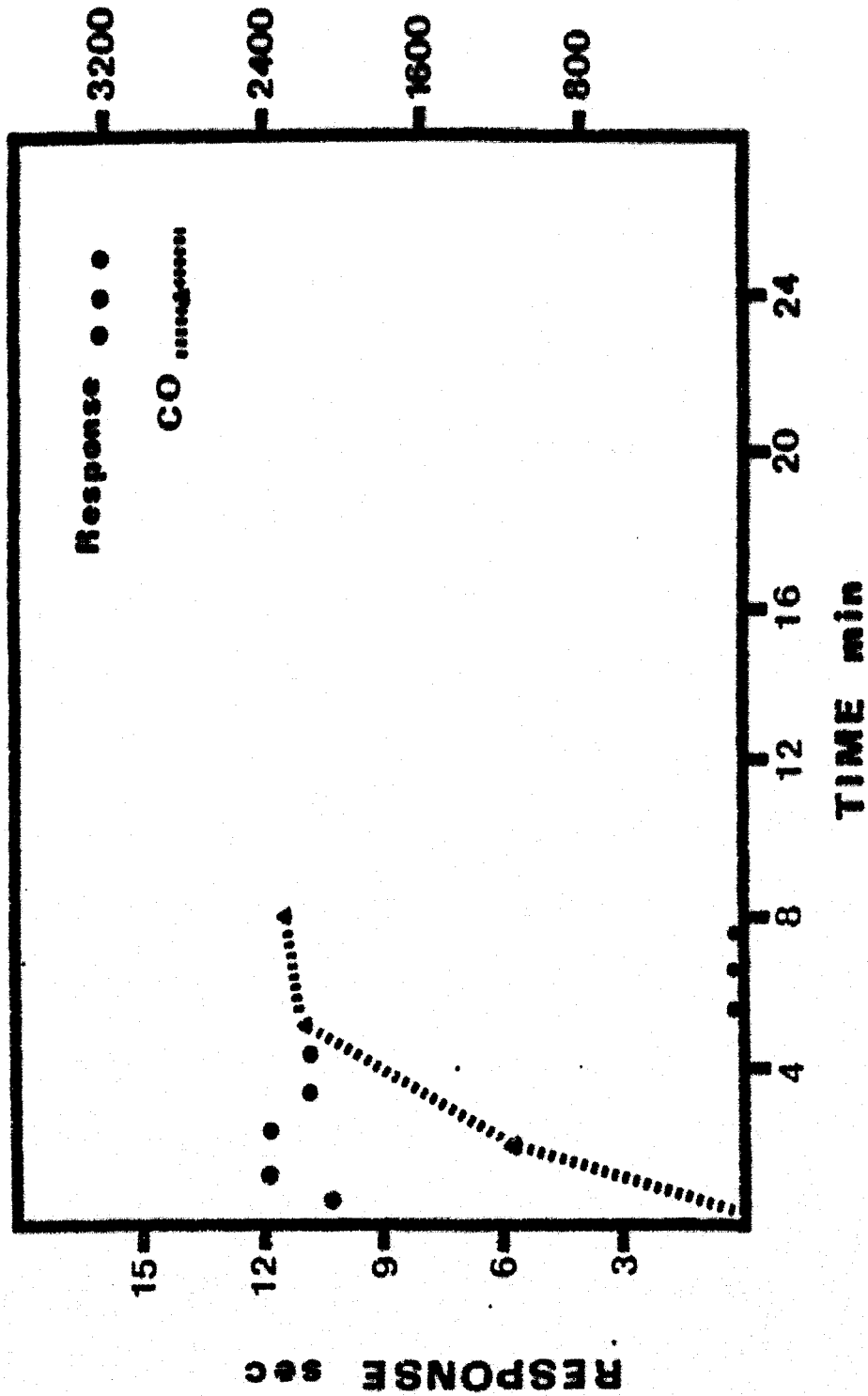


Figure 23

Typical Example of Loss of Escape in
Less Than 6 Minutes.

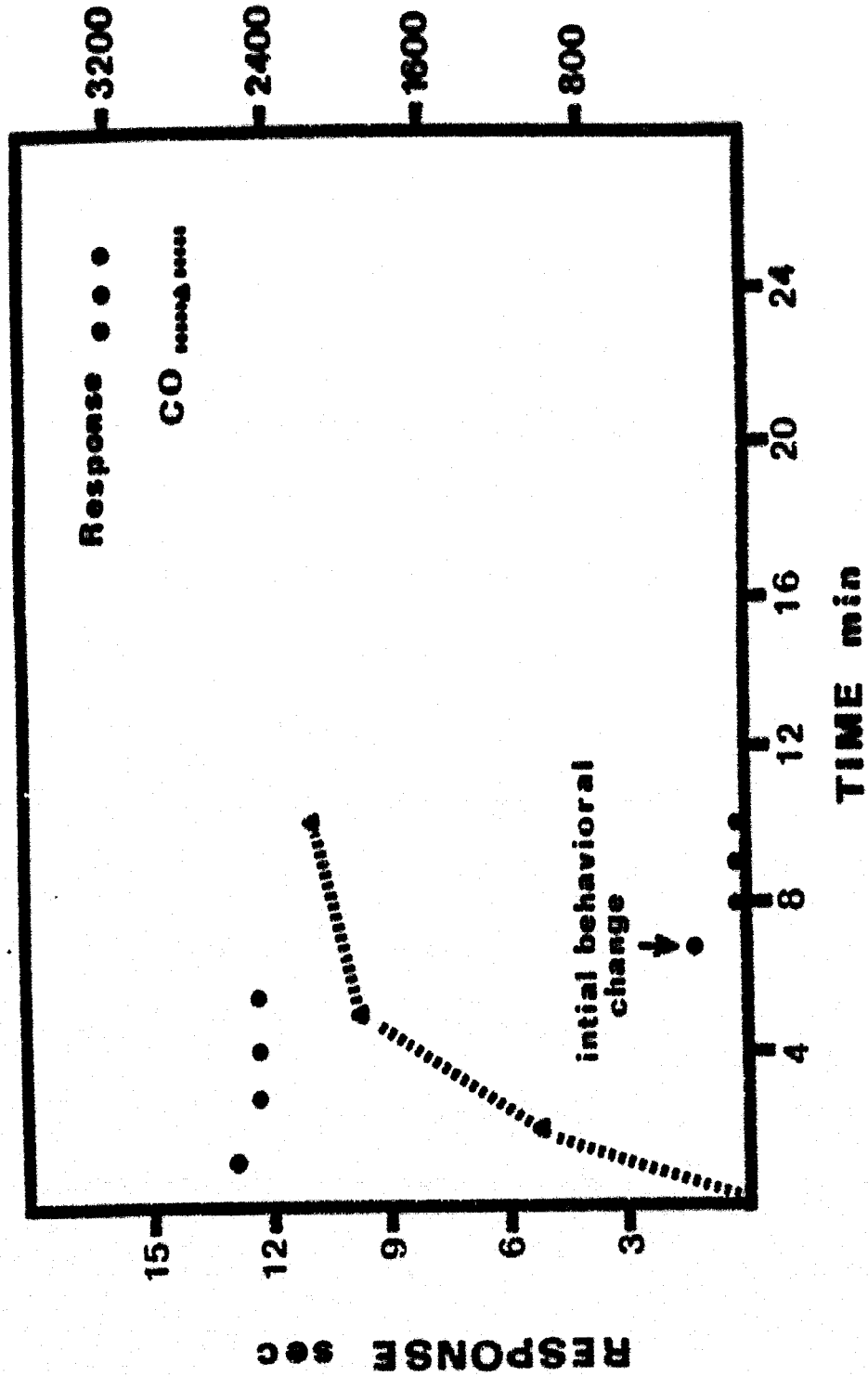


Figure 24

Typical Example of Loss of Escape
in 6-12 Minutes.

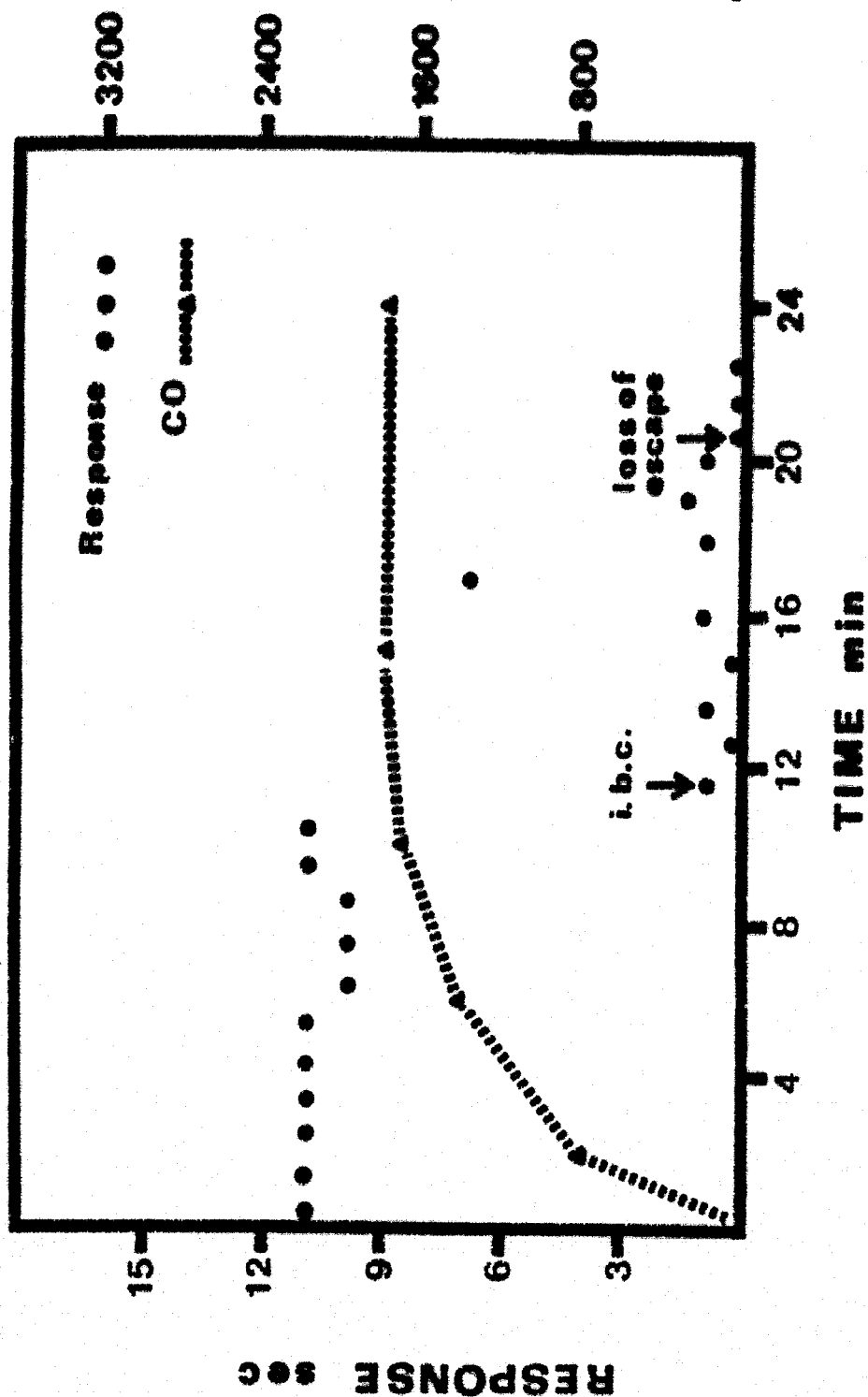


Figure 25

Typical Example of Loss of Escape
in 13-25 Minutes (1).

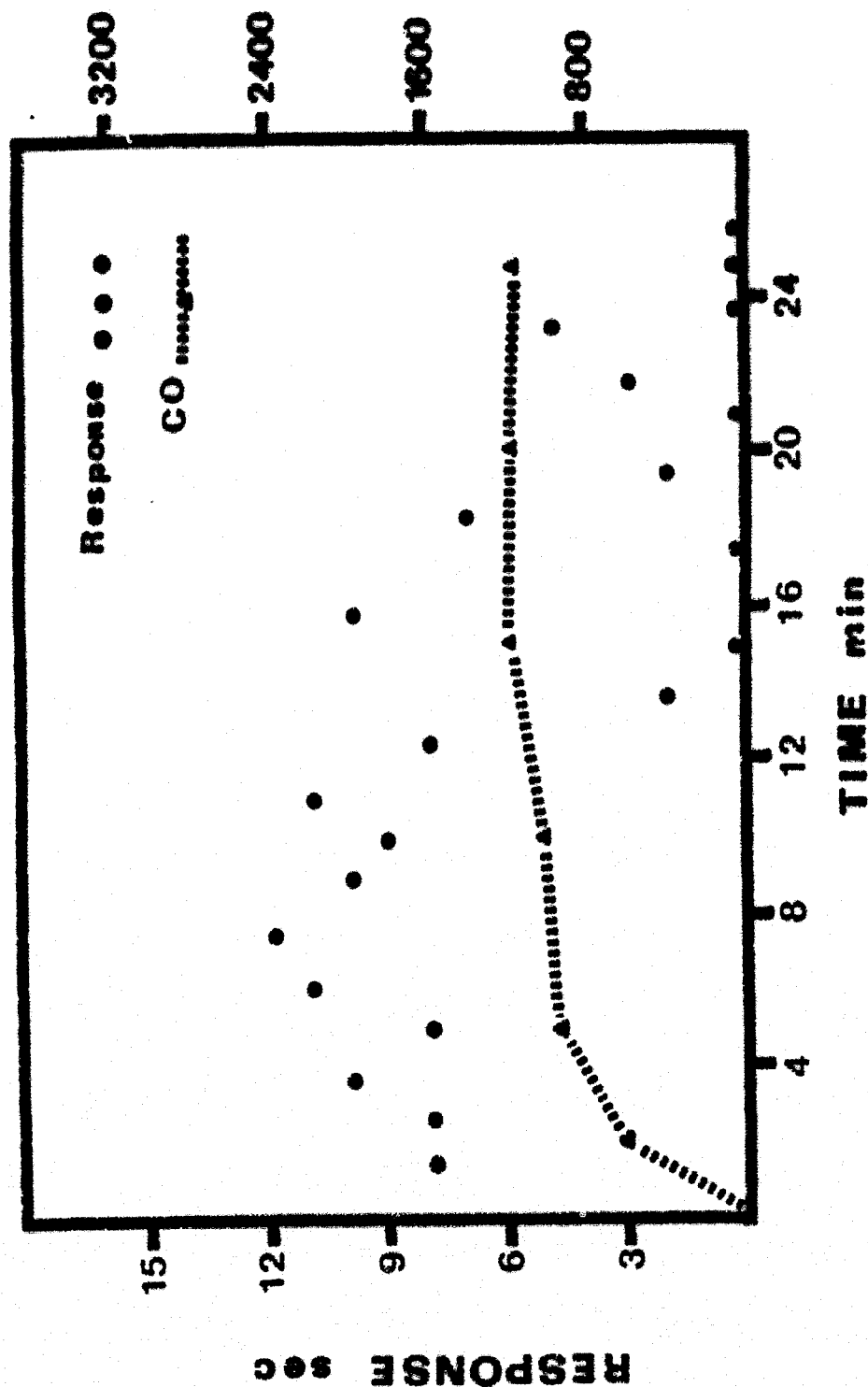


Figure 26

Typical Example of Loss of Escape
in 13-25 Minutes (2).

behavioral change becomes increasingly separated in time from the loss of escape (Figure 20).

Cook and Weidley (1957) have discussed the relationship between loss of avoidance, termed avoidance block, which occurs at the initial behavioral change, and loss of escape. These authors used the pole-jump response to differentiate between certain psychopharmacological drugs that block avoidance but not escape and those that eliminate all responding. As in the Cook and Weidley (1957) study with drugs, animals that were avoidance blocked, in this case by CO, retained good motor function evidenced by their rapid, coordinated jumps to the pole when shocked. Because the animals retained good motor function after they were avoidance blocked, they probably still could have run in the rotating cage. The fact is, however, that even though the animals could run and jump to the pole, they did not respond to the tone.

By the initial behavioral change these animals had obviously passed an important milestone in their loss of survival behavior. If these events are extrapolated to the human fire victim, the victim might remain in a dangerous location even though the muscular ability to flee was still intact. These results emphasize the importance of attempting to determine the earliest significant adverse

change and the problems with using only incapacitation to evaluate toxicity.

The Relationship of CO Concentration and Duration
of Exposure to the Behavioral Changes

Inhalation studies present a unique toxicological problem in determining the dose of the toxicant. One method of describing the dose is CT, or the concentration of the toxicant breathed multiplied by the duration of exposure. Authors including Dilley et al. (1978), Hilado and Cumming (1977) and Crane et al. (1977) have used CT to describe toxicity, and Dilley et al. (1978) and Crane et al. (1977) have implied that the CT relationship can be used to equate results of different duration CO exposures.

It was apparent from an initial examination of the dynamic test data that CT for loss of escape was not a constant. For this reason, the C and T data were fit using the empirical model $T = (q/C^a)^{1/b}$ to find values for the exponents a and b that would produce the best least squares fit. The model rearranged is $C^a T^b = q$.

Hilado and Cumming (1977) used $C^1 T^{0.5}$ to describe the lethal dose of CO for rats and mice. When the C and T data were manipulated with C restricted to 1 and T to 0.5, the sum of squares was reduced compared to results obtained by using $C^1 T^1$. The best description of the dose causing

the initial behavioral change and loss of escape, however, was $C^{1.0.3}$. This relationship is similar to that reported by Hilado and Cumming (1977), but it places even greater emphasis on CO concentration as a factor in behavioral toxicity. Possible explanations for the importance of concentration are: 1) the change in rate of COHb formation near equilibrium (Forbes et al. 1945), and 2) the K_i of enzyme catalyzed reactions inhibited by CO (Chance et al. 1970).

The relative importance of CO concentration and time to behavioral changes must be considered in terms of the Radiant Panel Test System and fire safety. Materials that produce large pulses of CO and, as a result, localized peaks in CO concentration may be more dangerous than materials evolving CO more evenly, even though by the end of a burn, they both produce the same maximum CO concentration. Thus, the fire safety of a synthetic material is related not only to the types of toxic gases produced but also, in the case of CO, to how the gases are evolved.

Static Carbon Monoxide Concentration Tests

The static testing with CO was done to determine the time variation in the initial behavioral change and loss of escape end points. The variations at two CO concentrations, reported in Table V, are reasonable and show the pole-jump test results to be reproducible. As would be

expected, animals exposed to the lower concentration had greater variability in time to both end points.

At 1091 ± 67 ppm, CO exposure caused a predictable loss of avoidance and escape in 8 of 11 animals tested. In 3 tests, however, the animals continued to pole-jump for more than 60 min, and the experiments were terminated. The curious ability of some subjects to escape at least 3 times as long as the majority of animals exposed may be explained by the percent COHb at equilibrium. A CO concentration of 1091 ppm would produce approximately 52% COHb at equilibrium, which is in the range shown to be associated with loss of escape (see page 70). As explained by dose response theory, however, animals in the test population would be intoxicated and stop escaping at different percent COHb's; so for a few animals, 52% COHb may be less than the effective concentration. A second factor may involve variability in the rate of CO transfer across membranes causing some animals to reach critical COHb concentrations before others (Cagliostro 1981).

A Comparison of COHb at Loss of Escape and at Other
Reported Behavioral End Points

The direct and indirect evidence is that loss of escape is associated with about 50-55% COHb. The direct evidence comes from the 2 trained mice sacrificed

immediately after loss of escape that had approximately 54% COHb. Support also comes from the static CO exposures at 1091 ppm. As stated previously, using a CO-hemoglobin affinity constant of 210 (Comroe 1974), 1091 ppm CO will produce approximately 52% COHb at equilibrium. In this study, 8 of 11 mice stopped escaping, so 52% COHb was the effective concentration for 73% of the population tested. Recall also that in the static study, the higher CO concentration (1026 ± 81 ppm), which would produce 60% COHb at equilibrium, caused a loss of escape in all tested animals.

The relationship between loss of escape and other reported behavioral end points can be analyzed on the basis of percent COHb. Russo and Kaplan (1978) found that rats incapacitated by CO in a wheel running device had 48% COHb. They also reported that rats trained in Sidman avoidance had greater than 60% COHb when significant response rate reductions were noted. Rats that were incapacitated by CO in rotorod and leg flexion devices had 49 and 52% COHb respectively (Mitchell et al. 1978). According to Carter et al. (1973) exposure to CO for 1.5 hr (51.6% COHb) eliminated responding of rats on a fixed ratio 15 schedule. In man, 45-50% COHb is also believed to be associated with muscular incapacitation (Schulte, 1963; Crane et al. (1977)). Based on percent COHb, it appears that loss of

escape and incapacitation are equivalent and that man as well as rodents may be similarly affected.

If incapacitation and loss of escape are equivalent, this allows, with some reservation, for a comparison of results obtained from the different models used in combustion toxicology. It is also verification that, through an analysis of the initial behavioral change and loss of escape, pole-jump provides earlier and more complete toxicological information than provided by systems such as the rotating wheel.

The reservation is that all authors do not consider percent COHb, which is the link between models, a good index of CO toxicity. For instance Plevova and Frantik (1974) found that rats exposed to 700 ppm CO for 50 min (19.6% COHb) had twice as great a decline in wheel running ability as rats exposed to 200 ppm CO for 24 hours (22.6% COHb). They concluded that concentration and time must also be considered in carbon monoxide toxicity. In another study, dogs were bled and then transfused with red blood cells containing 80% COHb yielding an average COHb of 57-64% (Goldbaum et al. 1975). Dogs exposed to CO so that their percent COHb was 54-90% died in 15 min to 1 hr, while the transfused dogs showed no apparent toxic effects. The authors concluded that the mechanism of CO toxicity is

probably the combination of dissolved CO with cytochromes rather than the reduced oxygen transport capacity of the blood due to COHb.

Half Life of COHb in Mice and Its Relationship
to Recovery of Pole-Jump Behavior

The four groups of mice exposed to CO, so that they reached approximately 50% COHb, required from 38-70 min to reduce their COHb to 10% (Figure 21). In contrast, mice which had lost the escape response, previously shown to occur at approximately 54% COHb, began recovery 14-37 min post-exposure with response returning to base line in 27-80 minutes (Table IV). These results, which should be considered preliminary, indicate recovery is not immediate upon removal from the CO atmosphere but seems to occur in the same time frame as dissociation and elimination of CO from the blood.

After CO exposure, mice removed from the chamber appeared to recover rapidly, because they regained postural tonus, were alert and began exploring in a few minutes. The fact is, however, as assessed by the pole-jump test, mice had not recovered for 27-80 minutes. If this phenomenon can be extrapolated to the human CO victim, those giving aid should be aware that complete recovery may not have occurred even though the victim seems normal.

Hydrogen Cyanide

Hydrogen cyanide is often found with CO as a component of combustion products evolved from nitrogen containing materials. HCN is classified as an asphyxiant and respiratory irritant (Casarett 1975) and is toxic at low concentrations. For this reason, it was chosen as a second pure gas to be tested with the pole-jump apparatus.

As stated previously, there were three goals to the HCN experimentation. The first goal was to bracket the working range of HCN concentrations. This range was defined at the upper end by an abrupt, permanent loss of escape and at the lower end by no behavioral change in 60 minutes. The second goal was to compare the changes in pole-jump behavior caused by HCN exposure with those caused by CO. The third goal was to subjectively observe symptomology characteristic of HCN intoxication.

The working range of HCN concentrations for mice in this study was 64-110 ppm (Figure 23). In contrast, exposure to concentrations of 83-160 ppm for 10-60 min can be dangerous or even fatal to man (Ballard et al. 1980).

The pattern of behavioral changes resulting from HCN and CO exposure differed. Gas concentrations greater than 100 ppm HCN and 3000 ppm CO caused a similar abrupt loss of escape. Below 100 ppm HCN, however, 5 of 8 mice

lost the escape response in approximately 5 min and subsequently recovered enough during the exposure to begin escaping again (Table VI). Of the 3 remaining animals, 2 had early avoidance blocks followed by temporary recovery, final avoidance block and loss of escape. The pattern of avoidance block and recovery, or loss of escape and recovery, typical of HCN, was never seen during CO exposures.

The mice seemed to be stunned by HCN, because they retained good body posture but would not jump to the pole. Often when they recovered and began to escape, the mice would wait in a position oriented toward the pole, sometimes with their paws on the pole, until being shocked.

If humans react to HCN in a similar qualitative manner, pulses of HCN produced in fires could cause dramatic changes in ability to escape. It is conceivable that one could be stunned by HCN and then fatally overcome by CO. In this hypothetical situation, the concentration of HCN in the victim's blood might not warrant its consideration as a cause of death, even though it had the initial adverse impact.

Levine and Stypulkowski (1959) observed rats that were exposed to HCN gas. They described 4 stages of intoxication. In the first stage, the rats responded by

restlessness and increasing activity leading to violent attempts to escape the chamber. In the second stage, voluntary muscular activity and postural tonus were greatly decreased. At this point, respiration varied from very deep, slow and regular to irregular with short periods of apnea. Levine and Stypulkowski's (1959) report is the only subjective description of rodent behavior during HCN exposure to compare with observations of mice made during this study.

Mice exposed to HCN also seemed restless initially. One curious and common behavior, which involved the mice placing their snouts between the grid bars and walking back and forth, was never seen in the CO exposures. After a few minutes, respiratory rate and depth changes were obvious in the mice. When the loss of escape was permanent, the animals had decreased postural tonus and were probably in Levine and Stypulkowski's (1959) Stage 2. One symptom not mentioned by Levine and Stypulkowski (1959) occurred during recovery in the more intoxicated animals. These animals experienced violent tremors, never seen following CO exposure, that lasted up to 10 minutes. After the tremors subsided, the animals were able to move about and seemed normal within 45 minutes.

How the CO and HCN Data are Used to Interpret
a Radiant Panel Burn

The final section describes a method to interpret changes in pole-jump behavior that occur during burns of synthetic materials. 1) Animals, described in this thesis, were exposed to known concentrations of pure CO and HCN to determine adverse changes in avoidance and escape behavior. 2) Using essentially the same methodology, trained animals were exposed to combustion products in the Radiant Panel Test System. 3) During the radiant panel burns, gas and vapor samples were taken to measure concentrations of atmospheric constituents, including the toxicants, CO₂ and O₂. 4) The mean CO concentration (C) and the time to the initial behavioral change and loss of escape (T) of the burn were compared to the dynamic CO data presented in Figure 20. 5) If the CO concentration in the burn adequately explained the behavioral changes, then CO was considered the primary toxicant produced by the polymer. In those cases where loss of avoidance and escape couldn't be explained by CO, the other toxicants were investigated.

Figure 27 shows the CO, HCN, CO₂ and O₂ concentration changes and changes in response that occurred in the course of a typical burn. Along with these gases a variety of organic vapors were produced, including aniline and quinoline. It can be seen that loss of avoidance

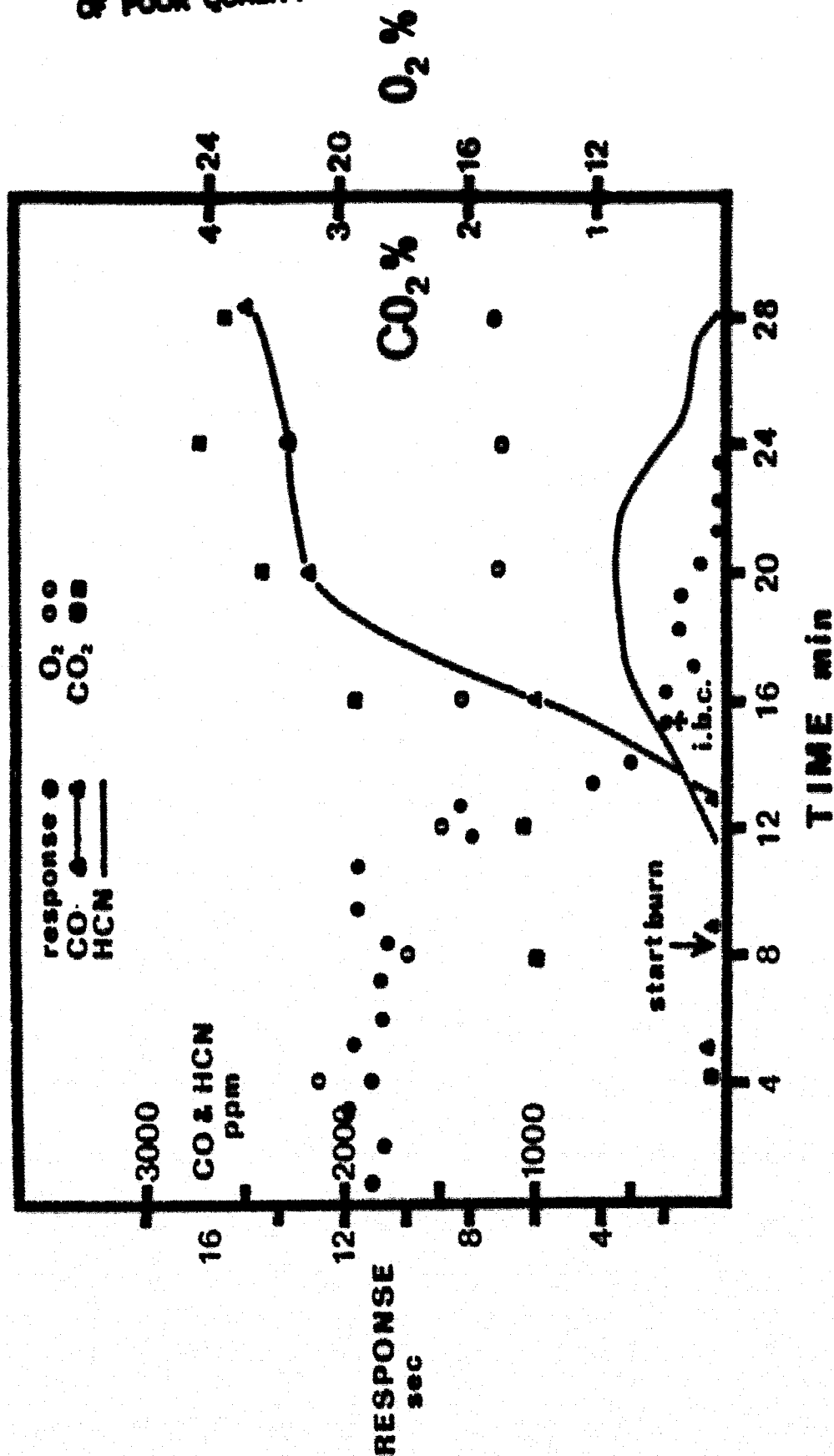


Figure 27
Animal Response and Toxicant Concentra-
tions During a Typical Polymer Burn.

(i.b.c.) occurred before a significant amount of CO was evolved. In these particular burn conditions, HCN was probably the initial primary toxicant produced by the polymer.

These conclusions, of course, are very preliminary; because the effects of low O_2 and increased CO_2 plus the individual and synergistic actions of other combustion products have not been defined. This type of analysis, however, does begin to explain the burn toxicology of a polymer on the basis of its evolved constituents. The polymer chemist can use this information to modify the chemical structure of a polymer in order to reduce its toxic threat in a fire.

CONCLUSIONS

A pole-jump apparatus was designed and constructed to test for changes in avoidance and escape behavior in mice exposed to toxic gases, vapors and particulates. The data reported in this thesis suggest that pole-jump is a valid technique to characterize and contrast the adverse impacts of toxic gases on avoidance and escape, which can be used to interface with the NASA Radiant Panel Test System. Support for these conclusions is as follows:

1. Pole-jump avoidance-escape behavior is probably a more accurate model of survival required in a fire than rotating wheels or other models currently being used in combustion toxicology.
2. A dose response in the CO concentration range produced by burning plastics in the radiant panel system was shown for loss of avoidance and loss of escape.
3. For the first time, a CO dose-response was shown for loss of avoidance and escape in the pole-jump test.
4. The studies with CO confirm what was already reported that exposure causes a progressive decline or slowing in tested behaviors. The predictable dose dependent pattern of behavioral changes varied

from rapid incapacitation at high CO concentrations, to no effect followed by the initial behavioral change, loss of all avoidance and loss of escape at lower CO concentrations.

5. An empirical model, used to show that the relationship between CO concentration (C) and time to loss of escape (T) in the pole-jump test was $C^1T^{0.3}$, revealed the toxicological importance of CO concentration. From this came the idea that materials producing CO in large pulses might be particularly dangerous.
6. That static test methodology showed that among animals in the test population variation in time to the two end points was reasonable and that results were reproducible.
7. Loss of escape was associated with 50-55% COHb. This conclusion was supported directly by blood analyses at loss of escape and indirectly by loss of escape occurring in 73% of animals exposed to CO that would produce 52% COHb. Based on percent COHb, loss of escape was shown to be equivalent to incapacitation reported by other authors, which allows comparison of different behavioral systems currently being used in combustion toxicology.

8. It follows from point 7 that pole-jump provides a significant quantifiable adverse behavioral change, loss of avoidance, occurring before incapacitation.
9. This thesis also showed that recovery of avoidance and escape behavior was probably related to decline in percent COHb and that animals that appeared to have recovered following CO exposure were still unable to avoid or escape for some time.
10. The first description of the effects of HCN on avoidance and escape was presented in this thesis. HCN produced a dose-response for loss of escape within a narrow concentration range.
11. A phenomenon described as stunning was shown for HCN that could be contrasted to the behavioral changes caused by CO. If HCN is present in a fire, it should be a suspect in causing the initial adverse behavioral change.
12. Finally, a method was described of interpreting the toxicology of combustion products produced in the radiant panel by using CO as a standard. This constituents approach may aid in the development of safer materials by revealing the primary toxicant.

Authors including Alarie (1979) and Potts and Lederer (1977) have discussed the need for research into more sophisticated behavioral systems for combustion

toxicology. The pole-jump device tested with a variety of pure gases and combustion products may provide the next step forward in understanding and improving the toxicological aspect of fire safety.

As in all research projects numerous opportunities for further study and potential for improvements in equipment were recognized. Opportunities for further research include the following:

1. Tests should be done to determine the percent COHb in mice at the initial behavioral change.
2. An attempt was made to determine how HCN and CO combinations effect avoidance escape behavior. It was not possible, however, to reproduce HCN concentrations with precision. Before CO-HCN testing is resumed, either a sophisticated mixing instrument or commercially pre-mixed gases must be purchased.
3. HCN has been shown to cause swelling and fenestrations in central nervous system tissue (Hirano et al. 1967). It would be interesting to see if a learning deficit develops in mice exposed to an acute dose of HCN. This would be very significant in understanding the long term post-exposure toxicology of people breathing HCN in a fire.
4. Because elevated CO_2 and diminished O_2 normally occur in fires, once the HCN-CO relationship is

determined, a matrix of mixtures should be tested: including CO and HCN plus increased CO₂ (4%) and low O₂ (10%) concentrations.

These basic relationships have not been investigated by combustion toxicologists; as a result, discussions of adverse changes in animal behavior remain very superficial.

5. Experimentation should continue with other common toxic gases, including the irritants, so their effects on avoidance and escape behavior can be compared to CO and HCN.

Modifications in the equipment include the following:

1. To increase the efficiency of training and exposing animals and interpreting data, the best type of light for the CS should be determined. This change could involve: i) increased light intensity, ii) darkened chamber walls to increase contrast, iii) strobe type bulbs or iv) colored light bulbs.
2. The control module was engineered, so that a jump to the pole after the CS begins only eliminates the shock while the CS is on. Because pole-jumps during the pause or UCS will not eliminate or terminate the shock, a paradigm with the UCS following the CS or with a pause between the CS and the UCS can not be used. Jumps to the pole must be associated with

elimination of shocks if rodents are to learn avoidance quickly (Fantino and Logan 1977). The system should be changed, so that any correct response from the beginning of the CS until the end of the UCS eliminates or terminates the shock.

3. The Grass SD9 stimulator, which provides constant voltage to each grid, should be modified to provide constant current. In addition, amp meters should be installed on the control module to monitor the current each animal receives.
4. Manual shock switches, a part of the control module, malfunctioned. The manual shock capability is extremely helpful in training. Animal learning is a process of shaping one desired behavior from a group of equally likely behaviors (Honig and Staddon 1977). As mentioned before, Bolles (1969) described the normal survival behaviors in rodents, including jumping, running and freezing. Mice display all of these responses until they realize that the pole will protect them from the shocks. During training a naive animal will often investigate the pole during the inter-trial interval. A shock given manually, out of sequence, to an animal that has its paws already on the pole will usually result in a successful pole-jump. It only takes a few such

escapes before the animal learns that the pole is a
safe place.

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