BEHAVIORAL TECHNOLOGY AND ITS APPLICATION TO FIRE TOXICOLOGY RESEARCH

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ABSTRACT

The application of behavioral technology to the toxicity testing of pyrolysis/combustion (P/C) products is discussed and two categories of behavioral tests commonly employed in fire toxicology programs are reviewed. Data are presented from a comparison of carbon monoxide (CO) induced incapacitation in rats performing in a rotating wheel or under a Sidmon free-operant schedule of shock avoidance. Rats performing in the rotating wheel were behaviorally incapacitated at CO concentrations and carboxyhemoglobin levels significantly lower than those which incapacitated operant avoidance animals. It is concluded that different measures of behavioral incapacitation may vary since incapacitation is a function of the particular toxic mechanism at work and the behavioral requirements of the specific task employed in the test procedure.

The National Research Council's Committee on Fire Toxicology recently suggested that traditional toxicity measures of lethality and organ pathology are necessary, but not sufficient for the toxicological evaluation of the pyrolysis and combustion (P/C) products of commonly employed construction materials.[1] Certainly any material whose P/C products are highly lethal or seriously damaging to bodily organs or tissue would be less desirable than one whose products were less toxic in terms of such effects. However, it is possible that the P/C products of a candidate material may be relatively safe in terms of these traditional measures of toxicity, yet at the same time, be behaviorally disabling, and, therefore, potentially dangerous in the event of fire. The relevance of behavioral measurements in toxicity evaluation procedures is further emphasized by recent statistics which show that impairment of escape capability due to smoke inhalation is a significant factor in a majority of fire-related deaths.[2] The logical conclusion is that the effects of P/C products on escape behavior and, therefore, on survival capability, must be an integral part of any complete fire toxicology evaluation.

In accordance with such reasoning, a number of different measures of behavioral incapacitation has been employed in the toxicity evaluations of P/C products during the past decade. One category of behavioral techniques involves simple visual monitoring of animal subjects during exposure to the P/C products of materials.

One such test is the USF/NASA procedure involving visual observation of free-moving mice with incapacitation defined as loss of equilibrium, prostration, collapse, or convulsions. Another test method in this same category employs performance in a motorized wheel with incapacitation defined as an inability to keep pace with the rotating wheel. Both of these procedures are relatively simple in terms of required test equipment and pre-test training time, with the USF/NASA procedure requiring no training of test subjects prior to P/C product exposure. However, each of the tasks requires visual observations and each employs admittedly subjective reports in determining incapacitation time.

On the other hand, a second category of behavioral techniques utilizes objective measurement of shock escape and avoidance behavior as a measure of incapacitation. Tasks of this type involve the leg-flexion response, performance on a rotorod, or the use of a pull rod or lever for operant manipulation. These latter techniques require more elaborate equipment and varying amounts of animal training prior to test exposure. However, none of these latter techniques depends upon subjective visual reporting and all permit a dichotomy of behavior into escape and avoidance components.

Despite the usage of this variety of behavioral techniques, little research has been conducted which allows a comparison of different behavioral end points. Hilado, Cumming, and Packram[3] report a comparison of end point measurements using two different

species of subjects (mice and rats), two different behavioral techniques (the USF/NASA and leg-flexion methods), and the pyrolysis effluents of two different test materials (polycarbonate and wool). The results indicated a close correlation between end point measurements of the two methodologies in both species and materials tested.The investigators concluded that much of the differences seen in the literature between various combustion toxicological methods may be due to differences in pyrolysis techniques rather than differences in behavioral methodologies.

These findings are in contrast to those of Fitzgerald, Mitchell, & Packam[4] who reported significant differences between rotorod and leg-flexion incapacitation induced by carbon monoxide. While average CO concentration was 1947 ppm, animals performing the rotorod task displayed behavioral incapacitation after shorter exposure times and at lower carboxyhemoglobin levels than animals performing the leg-flexion task.

The contrasting results of these two studies point out the need for further investigation into the relative contribution of different behavioral methodologies to the variability of findings in fire toxicology. Furthermore, comparisons such as these can provide useful information about the susceptibility of different behaviors to toxic incapacitation, supply further knowledge about specific mechanisms of incapacitation, and ultimately provide a guideline by which appropriate behavioral end points may be chosen.

One aspect of the fire toxicology program at the Johnson Space Center has been an assessment of the applicability of two different behavioral methodologies to the toxicological evaluation of P/C products. The following experiment, as part of this assessment process, was designed to compare the course of changes in two behaviors in animals exposed to increasing concentrations of CO. Specifically, the experiment was designed to compare CO-induced incapacitation of simple motor behavior in a rotating wheel with CO-induced changes in a more complex operant avoidance behavior. Behavior in the rotating wheel was selected for this study because of its history of frequent usage in fire toxicology evaluations while Sidman avoidance behavior was chosen because the many measurable parameters of this behavior allow multiple points of comparison and because its suitability for toxicity evaluations has yet to be tested. CO was selected as the incapacitating agent because it is a universal pyrolysis product and its quantity in the blood, in the form of carboxyhemoglobin (COHb), can be measured and correlated with overt behavior.

METHOD

ANIMALS. Sixteen naive, Sprague-Dawley rats ranging in age from 60-120 days old and in weight from 340-460 gms served as subjects. Throughout the course of the experiment, the subjects were housed individually or in groups of 2-3 in 30.5 cm x 35.6 cm lucite cages and given free access to food and water.

APPARATUS. Ten subjects were exposed to CO in an enclosed wire mesh wheel, measuring 27.9 cm in diameter and 8.9 cm in width, which rotated at a rate of 8 rev/min. The remaining 6 subjects were trained and exposed to CO in a 20.33 x 20.33 x 18.36 cm operant chamber equipped with a grid floor through which 70-80 volt AC shock could be delivered.

TRAINING. Prior to CO exposure all subjects were trained until a stable baseline performance was achieved. Operant subjects were trained on a Sidman avoidance schedule with a response-shock interval of 20 sec, a shock-shock interval of 5 sec, and shock duration of 1 sec.

CO EXPOSURE. CO was supplied to either the wheel or operant chamber through a flow regulator from a pressurized cylinder containing 3430 ppm CO mixed with air. On days of exposure, samples were drawn from the chambers at the end of each 5 min of exposure. Exposure duration was 20, 25, 30, 35, or 45 min for operant subjects and lasted until

incapacitation was evident for wheel subjects. At end end of each exposure session, the subject was removed from the apparatus and a venous tail sample of blood was obtained for COHb determination. CO concentrations were derermined by standard gas chromatographic techniques and COHb determinations were performed on an Instrumentation Laboratories Model 182 CO-oximeter precalibrated for rat blood.

RESULTS AND DISCUSSION

Figure 1 illustrates the concentration of CO in the rotating wheel as a function of increasing exposure time. Each point on the curve represents the mean and standard error of 10 samples taken from the wheel at each of the indicated exposure times and at the incapacitation end point. The mean concentration of CO in the wheel was 1407 ± 54 ppm at incapacitation.

Figure 2 shows the level of CO in the blood as % COHb under control conditions, at the point of incapacitation, and as a function of time since incapacitation after exposure on the rotating wheel. The mean level of COHb under home cage control conditions was 2.6 ± .6% compared to 48.6 ± 1.4% at incapacitation. As the slide illustrates, the exponential decay of COHb blood levels depicts a first order rate of CO elimination.

Figure 3 presents the mean CO concentration at the end of each 5 min. of exposure and mean blood COHb level after 20, 25, 30, 35, and 45 min. of exposure in the operant chamber. The decreasing increments which occurred in these two measures as a function of time can best be described by exponential functions. For instance, though mean COHb level rose to 58% during the first 25 minutes of exposure, the mean level increased only from 58% to 66% during the last 20 minutes of exposure.

It is important to note that after 20 minutes of exposure in the operant chamber, both mean CO concentration and blood COHb

levels were higher than the corresponding concentrations and levels present at the point of incapacitation in the wheel. After 20 minutes of exposure, mean COHb level in operant Ss was 50% at a CO concentration of 1761 ppm. At incapacitation in the wheel, mean CO concentration and COHb levels were 1407 ppm and 48.6%, respectively. These comparisons assume significance when the course of CO-induced changes in avoidance and escape behavior is evaluated.

AVOIDANCE BEHAVIOR - Figure 4 illustrates the inverse relationship between mean avoidance response rate and average inter-response time as a function of CO concentration. Average inter-response time was significantly increased as CO concentration reached 2208 ppm and blood COHb levels rose above 60%. This increase in average inter-response time is due almost exclusively to a significant decrease in avoidance response rates since escape response rates were not significantly affected at this concentration. Both the decrements in avoidance behavior and the increments in inter-response times remained statistically significant at all concentrations of CO greater than 2200 ppm.

It is interesting to note the temporary but significant decline in avoidance response rates which occurred during the first 5 minutes of exposure when CO concentration remained below 600 ppm. This initial decrement in avoidance behavior was not due to any incapacitating effect of CO since avoidance responding quickly returned to control levels and remained stable until the CO concentration rose above 2200 ppm.

ESCAPE BEHAVIOR. Figure 5 depicts the changes occurring in escape response and shock rates as a function of CO concentration. The significant increase in shock rate during the first 5 minutes of exposure (resulting from the previously discussed decline in avoidance response rates) was paralleled by a significant increase in escape response rate. Since escape impairment would be reflected by the failure of escape response rates to increase directly with any increase in shock rates, no impairment in escape functioning is evident at CO concentrations below 2000 ppm. However, at CO concentrations between 2100 and 2900 ppm and at COHb levels above 60%, significant increases in shock rate were not paralleled by any significant change in escape responding. At CO concentrations above 2900 ppm, as shock rate continued to increase, a significant decrease occurred in escape response rates. Thus, the impairment of escape functioning which was first evident as CO concentration rose above 2000 ppm was clearly established at 2900 ppm.

The consistence in the results of Experiment 2 is apparent in Table 1 which summarizes the CO-induced changes in operant performance. With the exception of the temporary decrement in avoidance responding during the first 5 minutes of exposure, other measures of performance show that significant behavioral impairment began as CO concentration rose above 2200 ppm and as COHb levels rose to 63%. These results are in contrast to the findings of Experiment 1 which demonstrated that behavioral impairment in the rotating wheel occurred at concentrations of CO below 1500 ppm and at COHb levels below 50%.

The present experiment demonstrates that considerable variability in measurements of time to behavioral incapacitation may occur if different behavioral tasks are employed in toxicological evaluations of pyrolysis and combustion products. Thus, caution is warranted in interpretating the incapacitation measures of any single behavioral task. For instance, to conclude from the wheel performance data that all escape functioning is impaired at CO concentrations of 1500 ppm and COHb levels of 50% would be inconsistent with the operant results which demonstrate that animals are capable of maintaining baseline rates of escape/avoidance behavior in the presence of CO concentrations up to 2000 ppm and at COHb levels up to 60%.

It is clear from the contrasting results of this experiment that behavioral incapacitation in any pyrolysis product evaluation procedure will be a function of two interacting factors: (1) the particular mechanism of incapacitation of the pyrolysis products, and (2) the behavioral requirements of the specific task employed in the test procedure. Marked differences in end point measurements due to these two factors are possible whenever different behavioral screening tasks are employed. For example, impairment in the rotating wheel appears to be due primarily to a loss of motor function. Performance of this task is particularly susceptible to the incapacitating effects of CO because of the continuous muscular activity required by the task. Data from preliminary studies indicate less susceptibility to CO-induced impairment in the rotating wheel when

motor requirements are reduced. In contrast, the pressing of a lever in an operant avoidance task requires considerably less muscular activity and possibly more involvement of higher CNS functions. This contrast in task requirements probably contributes significantly to the differences in the end point measurements of this experiment.

In conclusion, these results indicate that the factors which determine time of useful function are specific to the incapacitating agent and to the behavioral task employed and that these factors may cause considerable variability whenever different end point measurements are used. The selection of a particular behavioral task for the toxicological screening of pyrolysis and combustion products requires a careful consideration of these factors as well as a concern for the degree of relevance which any particular behavioral task may have for human fire escape and survival capabilities.

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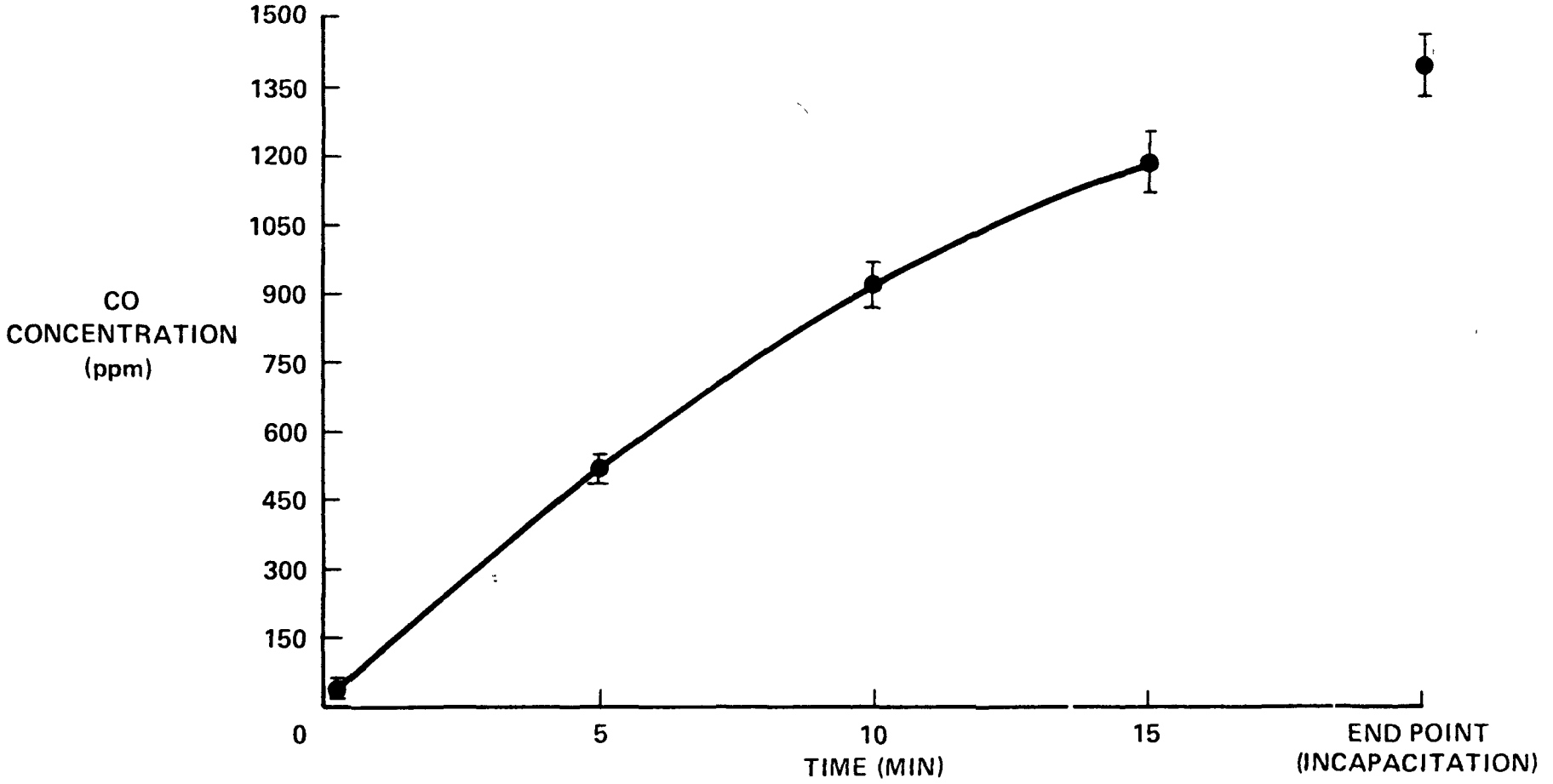

FIGURE 1.- CO CONCENTRATIONS IN THE ROTATING WHEEL AS A FUNCTION OF EXPOSURE TIME. EACH POINT REPRESENTS THE MEAN OF 10 EXPOSURE SESSIONS ± 1 SE.

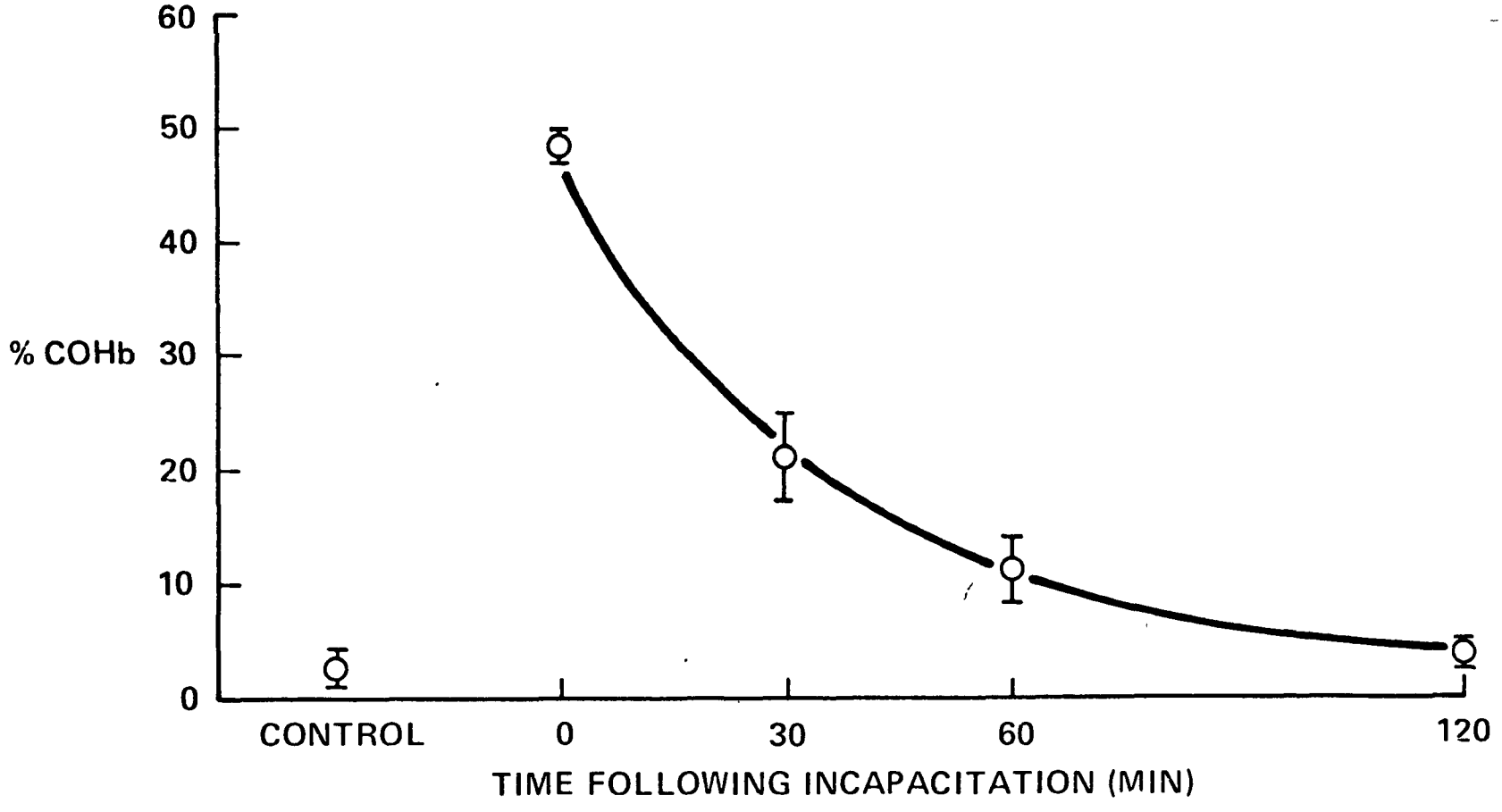

FIGURE 2.- BLOOD COHb CONCENTRATIONS OF CONTROL RATS AND RATS EXPOSED TO CO IN THE ROTATING WHEEL. COHb CONCENTRATIONS OF EXPOSED ANIMALS WERE MEASURED AT INCAPACITATION AND 30, 60, AND 120 MIN FOLLOWING INCAPACITATION. EACH POINT REPRESENTS THE SAMPLE MEAN (N = 7–9) ± SE.

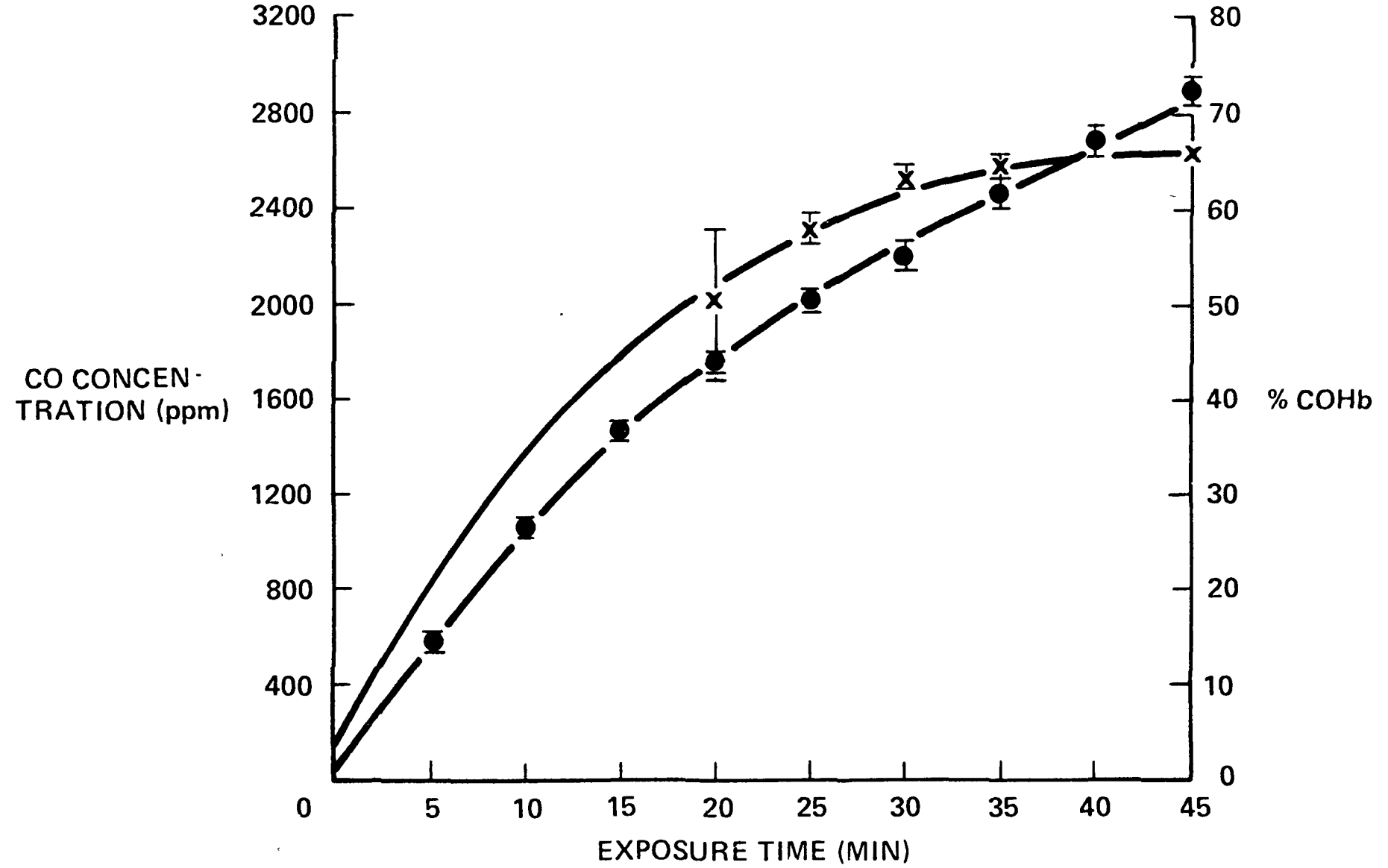

FIGURE 3.- CHAMBER CO CONCENTRATIONS (●) AND ANIMAL BLOOD COHb LEVELS (x) AS A FUNCTION OF EXPOSURE TIME IN THE OPERANT CHAMBER. EACH POINT REPRESENTS THE SAMPLE MEAN (N = 1-17) ±SE.

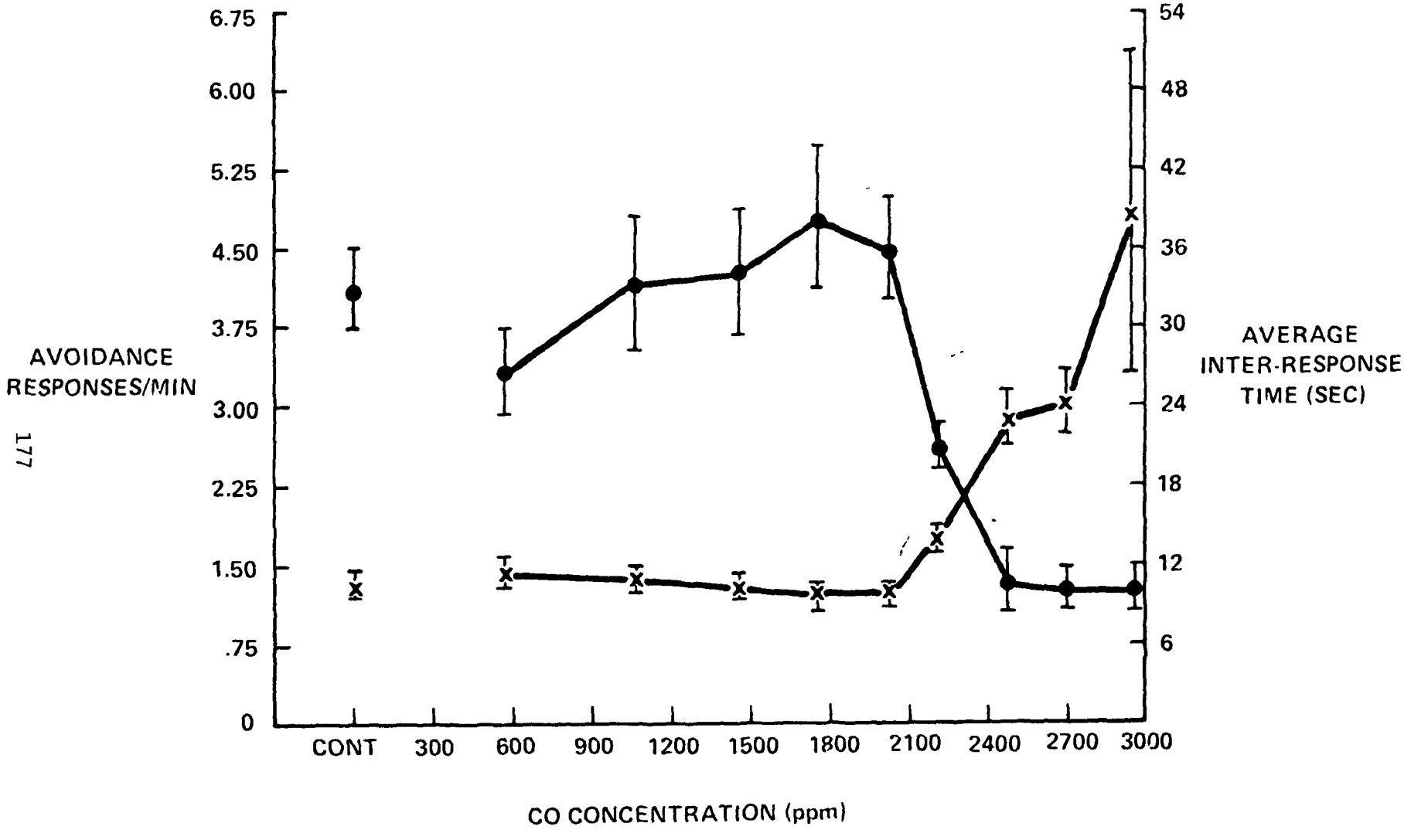

FIGURE 4.- EFFECT OF CO CONCENTRATION ON AVOIDANCE RESPONSE RATE (●) AND AVERAGE INTER-RESPONSE TIME (x). EACH POINT REPRESENTS THE SAMPLE MEAN (N = 5 – 18) ± SE.

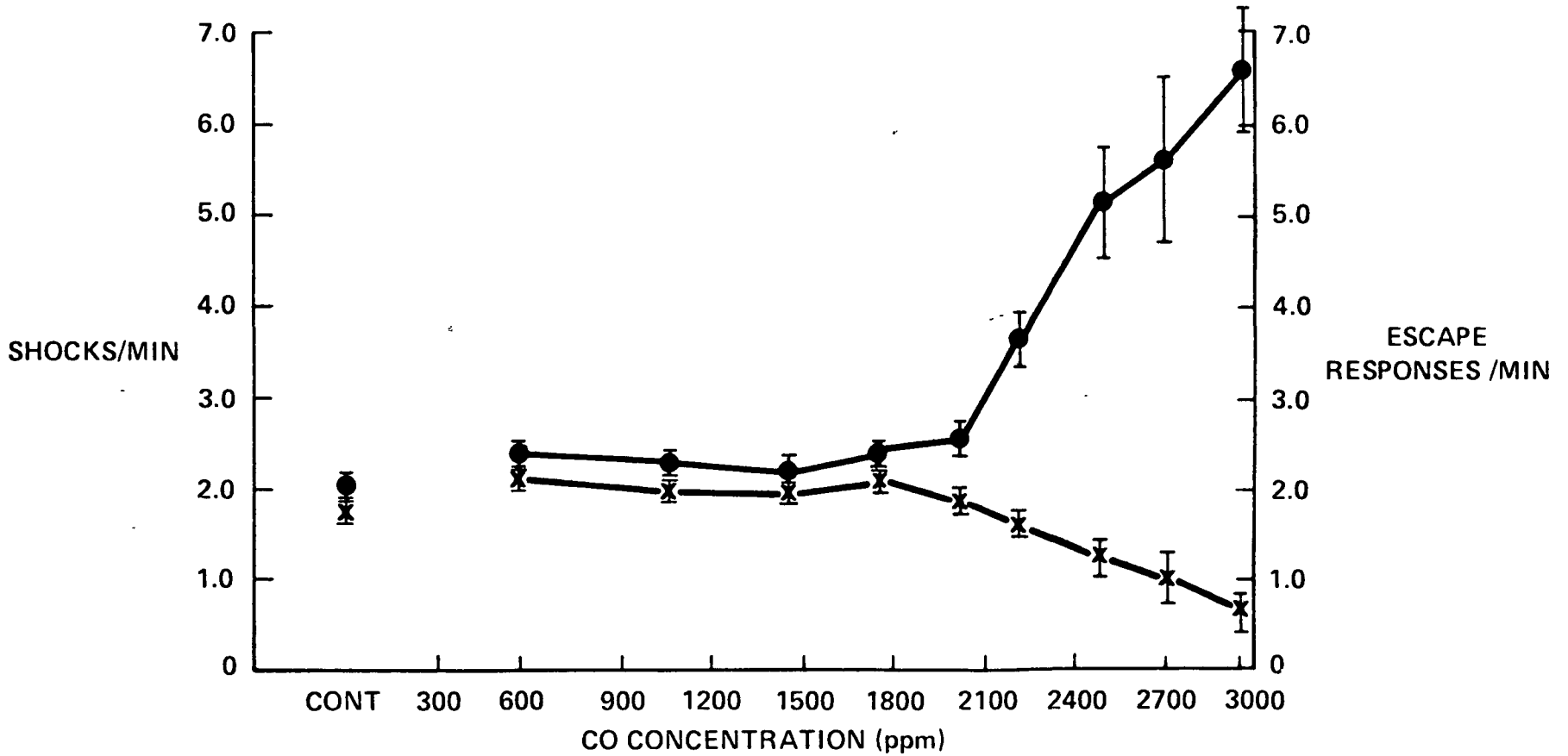

FIGURE 5.- EFFECT OF CO CONCENTRATION ON SHOCK RATE (●) AND ESCAPE RESPONSE RATE (x). EACH POINT REPRESENTS THE SAMPLE MEAN (N = 5 –18) ± SE.

CO CONCENTRATION (ppm): COHb :	571	1063	1460	1761 50%	2014 58%	2208 63%	2478 64%	2706	2938 66%
INTER-RESPONSE TIME	NS	NS	NS	NS	NS	↑	↑	↑	↑
AVOIDANCE RESPONSE RATE	↓	NS	NS	NS	NS	↓	↓	↓	↓
SHOCK RATE	↑	NS	NS	↑	↑	↑	↑	↑	↑
ESCAPE RESPONSE RATE	↑	NS	NS	↑	NS	NS	NS	NS	↓
UNESCAPED SHOCK RATE	NS	NS	NS	NS	NS	↑	↑	↑	↑
SHOCK TIME/SHOCK	NS	NS	NS	NS	NS	↑	↑	↑	↑
PERCENT ESCAPE	NS	NS	NS	NS	NS	↓	↓	↓	↓

TABLE I. CO INDUCED CHANGES IN OPERANT PERFORMANCE. EACH CELL INDICATES THE RESULTS OF A PAIRED T-TEST AS FOLLOWS:

NS: NO SIGNIFICANT CHANGE ($P>.05$)

↑: A SIGNIFICANT INCREASE ($P<.05$)

↓: A SIGNIFICANT DECREASE ($P<.05$)