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Risk Estimates for CO Exposure in Man Based on Behavioral and Physiological Responses in Rodents

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Chapter 1

INTRODUCTION

The significance of toxic combustion products as a causal factor in fire deaths is well established. In the U.S., toxicity contributes to death in approximately 34% of all fire fatalities (Radford et al., 1974). Since the widespread introduction of synthetic polymers for use in the construction, clothing, furniture, and transportation industries, both the chemical nature of the fire environment and its toxic threat have grown more complex.

Various authors (Hartzell et al., 1977; Barrow et al., 1978; Alarie and Anderson, 1979; Kourtides and Gilwee, 1978; Hilado et al., 1977) have proposed a number of behavioral or physiological indices for the evaluation of the toxicity of combustion products. Frequently, hazard rankings of various synthetic and natural materials are generated on the basis of these studies, with the most common toxic endpoints being time to incapacitation or time to death of the test species in the fire atmosphere. However, both the types and amounts of chemicals produced by thermal combustion of either synthetic or natural materials are very much dependent on fire conditions and the fire environment. Such variables as ventilation, temperature of the fire, and whether combustion is flaming or non-flaming, can significantly alter the chemistry of a fire atmosphere and thus its toxicity. This fact leads to serious inconsistencies between hazard rankings obtained under different combustion conditions. Since the parameters of a real fire cannot be predetermined, nor can all possible fire

conditions be simulated experimentally, it seems advisable to investigate the toxic risk associated with some of the compounds most common to a majority of fire environments. One such compound is carbon monoxide (CO).

Carbon monoxide is ubiquitous in fire atmospheres since it is produced by the incomplete combustion of any carbon-containing material. Without interfering with respiratory mechanics, carbon monoxide acts as chemical asphyxiant (Casarett, 1975). By combining with hemoglobin to form carboxyhemoglobin, which is inefficient in oxygen transport, CO causes anoxia by interfering with the normal oxygen-carrying capacity of the blood. Additionally, the presence of carboxyhemoglobin in the circulating blood alters the shape of the oxygen dissociation curve of normal oxyhemoglobin, so that a smaller amount of blood oxygen is released in tissue capillaries (Bartlett, 1968).

A large portion of the extensive literature on CO is devoted to chronic toxic effects that are associated with low-level exposures of long duration, such as those which may result from urban air pollution or cigarette smoking. Less information is available regarding the acute toxic effects of CO on mammals, at concentrations which mimic the high levels often present in fires. Necessarily, very little is known of quantitative human responses at these same high levels.

In conjuction with NASA-Ames Research Center, San Jose State University's Department of Biological Sciences has developed a modular system to assess select, sub-lethal, toxic effects in rodents caused by acute exposures to combustion products. The modular system was designed to interface with the NASA Radiant Panel for the generation

of polymeric combustion products, or with a gas delivery system for testing individual combustion components such as CO. The system has the advantage of incorporating multiple behavioral and physiological indices in a small-scale combustion toxicity test. For reasons previously discussed, carbon monoxide was chosen for the purpose of validating the system.

The characteristics and limitations of behavioral tests in current use by combustion toxicologists have been extensively reviewed by Winslow (1981). Briefly, the behavioral assay utilized in the modular system is a pole-jump response to detect changes in operant or motivated behavior of mice. The pole-jump apparatus quantifies discrete trial, avoidance-escape behavior throughout the course of a toxic exposure. A trial consists of a conditional stimulus (a tone or light cue), followed by unconditional stimulus (an electric shock), and an inter-trial pause. The mice are trained to avoid the shock by jumping on the pole during the conditional stimulus period; if the mouse fails to avoid the shock but is able to jump to the pole during the unconditional stimulus period, the response is termed an escape rather than an avoidance. Two toxic endpoints, the initial behavioral change and the loss of escape, are derived during combustion exposures from a series of repetitive, one-minute long trials. The initial behavioral change occurs at the first trial the mouse fails to avoid the shock, followed by failure to avoid in at least 4 of 6 subsequent trials. Loss of escape is defined as the first of 3 consecutive trials in which the mouse fails to escape the shock. For complete details of the behavioral system, the reader is referred to Winslow (1981).

The physiological assay incorporated in the modular system consists of electrocardiographic and respiratory monitoring of rats during the course of exposure, and post-exposure analysis of selected serum enzyme activity levels. The presence of intracellular enzymes in extracellular fluids is generally accepted as an indication that cell death has occurred. The enzymes chosen for analysis are associated with organ systems or tissues which are particularly sensitive to CO-induced anoxia, because of their high energy requirements or low anaerobic capacity. Creatine phosphokinase (CPK) in the adult rat is distributed pr. rily in the central nervous system (Booth and Clark, 1978), in the cardiovascular system, and throughout skeletal muscle (Ziter, 1974). The major lactate dehydrogenase (LDH) isozymes are localized in the heart, skeletal muscle, and liver of rats (Penney et al., 1974). Alpha-hydroxybutyrate dehydrogenase (HDBH) represents the cardiac fraction of the LDH isozymes. Using rat heart cell cultures, van der Laarse et al. (1979) have demonstrated a linear correlation between the amount of HBDH released, the extent of cell death, and loss of contractile function in response to anoxia. Penney and Maziarka (1976) have investigated the time course of changes in serum enzyme activity levels of CPK and LDH in rats exposed to 1500 ppm CO for 2 hours. The tissue distribution and chemical characteristics of CPK, LDH, and HBDH in the rat are similar in man (Bio-Science Laboratories, 1978).

Data from the behavioral and physiological assays cited above provide information on the quantitative relationship between CO concentrations ("dose") and the probability of occurrence of selected toxic

"responses" in rodents. A number of general, theoretical models have been proposed to describe mathematically the way in which the probability of response, or risk, varies as a function of the dose of a toxicant. The purpose of such models is to provide a means of extrapolation from animal studies to a predictive statement of relative risk for man, when quantitative toxicity information for man is necessarily limited or unavailable. The derivation and application of these models will be discussed in the literature review. The risk-estimate models which are most suitable for the interpretation of the acute toxic effects elicited by CO will be used to analyze the rodent behavioral and physiological dose-response data. With the application of appropriate interspecies conversion factors, the "dose" of CO associated with a very low risk (1 in 10^6) of occurrence of similar toxic responses in man will be estimated. Finally, the reliability of the human risk estimates derived from these models will be evaluated by comparison with available literature on equivalent human responses to acute carbon monoxide exposures. The implications of these risk estimates for combustion toxicology will be considered.

Chapter 2

LITERATURE REVIEW

Risk Assessment and Dose-Response Theory

The concept of risk assessment is inherent to virtually all toxicologic experimentation. The value of observing or measuring the effects of a toxicant in test populations lies in the ultimate application of this information to predict the response of naive populations to a given exposure of the same toxicant. Obviously, the toxicologist is primarily concerned with estimating potential toxicity in the general human population, based on laboratory data from other species, or from epidemiologic data when it is available. It has long been recognized that the dose of a compound determines the nature and extent of toxicity. Therefore, to assure reliable risk predictions, the quantitative relationship between the dose administered and the toxic response must be investigated.

In the discussion to follow, a number of aspects of basic dose-response theory and their relevance to quantitative risk estimation will be introduced. The way in which a toxic response is quantified, whether on a graded or a dichotomous scale, or as time-to-occurrence, is basic to understanding the content of dose-response curves used in risk estimation. The nature of the toxic response (e.g., reversible, heritable) is also important for risk assessment, particularly as it pertains to the question of the existence of toxic thresholds. Next, a number of theoretical models which have been developed to describe

mathematically the relationship between dose and response, and which are the basis on which risk estimates are established, will be presented. Finally the biological processes which influence the shape of the dose-response curve by helping transform an administered dose into an effective dose will be considered.

The Quantification of Toxic Response

O'Flaherty (1981) has discussed the various types of quantitative dose-response data, which include graded response data, dichotomous or quantal response data, and time-to-response data. In a graded response system, the toxic response as a function of dose is measured on a continuous scale of intensity of effect; increasing dose is associated with increasing severity of response. The toxic effect can be thought to be mediated by the interaction of the chemical with a reactive biological entity or "receptor"; the magnitude of response is therefore proportional to the number of affected receptors.

In dichotomous or quantal response systems, a toxic response criterion is established. In this case, the toxic response is defined as an all or none event and various degrees of intensity of response as such are not considered. The proportion of individuals in a population responding or not responding to the criterion at a given dose can then be determined (O'Flaherty, 1981). The response criterion chosen, termed the quantal response, may be the appearance of a particular, sublethal pathology or lethality. Since quantal dose-response curves relate the dose of the toxicant with the proportion of the

population responding (the rate of response in the population), quantal transformations of dose-response data are very important for the estimation of risk, or the probability that a toxic response will occur at any given dose of a toxicant.

For those compounds capable of producing irreversible toxicity with long delayed onset, the time to response can also be considered. Fishbein (1980) has reviewed the current status of time-to-occurrence dose-response modelling which is based on the important finding of Druckrey (1967) that increasing dose is associated with decreasing latency (Dose x time^{n} = constant). Necessarily, this type of system is applicable only to certain classes of toxicants, particularly carcinogens, mutagens, or agents causing chronic, degenerative, injury.

The Threshold Concept: Implications for Risk Estimation

The existence of individual thresholds has been generally accepted for most types of toxicological responses (Lehring and Rao, 1960). A threshold dose for a toxicant is defined to be the dose above which a given toxic response will occur and below which it will not. A threshold is dependent not only on the chemical itself, but upon the complex interactions of a number of physiologic factors specific to the individual at risk - sex, age, diet, stress, and general health to name a few. In small, experimental populations, where many of these variables have been eliminated, it may be possible to establish an effective population threshold, below which an infinite number of population members may be exposed without ill effect. However, in

large and unrestricted populations, a few individuals may be so sensitive or susceptible to the effects of a given toxicant, that any dose of the agent is associated with a small be measurable population risk (IRLG, 1979).

Much controversy currently centers on whether effective population thresholds, such as those which are generally agreed to exist for certain "nonstochastic" effects of toxicants, also exist for "stochastic" effects. Nonstochastic effects are those for which severity of the toxic effect is proportional to the dose (e.g., liver damage), and for which there may exist a no effect level; for stochastic effects (e.g., tumor development) the probability of occurrence varies with dose (Butler, 1978).

The most prevalent toxicants in the latter category are carcinogens and mutagens. It is frequently argued that because of the established relationship between dose and incidence of effect for these classes of compounds, there is some finite probability of occurrence associated with any dose, no matter how low (Butler, 1978). Maugh (1978), Gehring and Blau (1977), and Schneiderman and Brown (1978), have extensively reviewed the evidence supporting arguments both for and against the existence of thresholds for stochastic effects, particularly carcinogenesis. Briefly, those who argue for the existence of thresholds cite a variety of repair, membrane barrier, or immunosurveillance mechanisms that are available to prevent or arrest the toxic disease process. Those who argue against thresholds presume that initiation of the toxic disease process can occur by a single, effective hit of a single, susceptible target, following which,

progression of the injury is self-sustaining. At least one author feels that for stochastic toxic effects, thresholds may indeed exist but at the level of numbers of molecules of the toxicant, not numbers of grams (Rall, 1978). Ultimately, resolution of the threshold debate for stochastic and certain irreversible, nonstochastic effects of toxicants depends on the further elucidation of the mechanisms underlying the toxic disease process.

Because of the differences presumed to exist between their toxic mechanisms, particularly with regard to the threshold issue, different methods for determining acceptable exposure levels have developed to treat either stochastic or nonstochastic effects of chemicals. Traditionally, the approach which has been used for nonstochastic toxicities is the establishment of "SNARL" values, the "suspected-no-adverse-response-level" (NAS-NRC, 1977). SNARL values were established in an effort to provide guidelines for drinking water standards; a similar approach was adopted by the World Health Organization to establish acceptable daily intakes (ADI) of toxic residues in food. The SNARL or ADI value is obtained by applying safety and interspecies conversion factors to the no-observed-effect-level (NOEL) in appropriate animal studies. These safety or uncertainty factors vary widely. The approach adopted by the National Academy of Sciences - National Research Council (1980) has been to use a safety factor of 10 when good human exposure data is available and supported by data in other species, a factor of 100 when good data is available from one or more species, and a factor of 1000 when data is limited or incomplete. Not only do these safety factors differ from one author to the next, but

the subjective interpretation of what constitutes good data allows considerible room for variance in the evaluation of SNARL values.

Because of the inability to establish conclusively whether delayed onset stochastic effects exhibit thresholds, a more conservative approach to risk assessment is warranted. This method, termed the risk estimate method, involves the following:

1) selection of an appropriate experimental bioassay
2) selection of a theoretical dose-response model, and estimation of its parameters from responses at all dose levels
3) statistical extrapolation of the experimental results to low doses outside the experimentally observable range
4) extrapolation of the estimated results in animals at the low dose level to man, taking into account the inter- and intraspecies biological variables which influence toxicity (Hoel et al., 1975)

For those toxicants (e.g., potent carcinogens) for which a threshold has not been demonstrated, the dose-response relationship is extrapolated to a level of "acceptable risk", a near-zero lifetime risk first proposed by Mantel and Bryan (1961), and recently adapted by the Food and Drug Administration (Federal Register, 1977) and the Interagency Regulatory Liason Group (1979), to be a lifetime response level of 1 in 10^6. In the U.S., this elevation in lifetime risk would result in 3 cases per year; the dose corresponding to this lifetime risk is called the VSD, or virtual safe dose (Scientific Comittee of the Food Safety Council, 1980).

Despite the functional distinctions made between these two classes of toxic effects, the Scientific Committee of the Food Safety Council (1980), has recently argued that the risk estimate approach should be applied to nonstochastic as well as stochastic toxicities. The committee stated that for nonstochastic responses, a distribution of unique thresholds exists within a population, the minimum of which cannot be determined a priori. Thus, the risk estimate method, which incorporates information from the shape and slope of an experimental population's dose-response curve, is deemed more appropriate to give a best estimate of an effective population threshold for noncarcinogens. The committee generally rejects the practice of establishing NOEL and SNARL values, which disregard numbers of experimental animals and the slope of the dose-response curve. Additionally, many of the biological phenomena which greatly influence the functional shape of the dose-response curve, especially metabolic activation and deactivation processes, are common to both stochastic and nonstochastic responses. Cornfield (1977) has expressed the opinion that dose-response curves for carcinogens and noncarcinogens alike reflect the saturation of protective biological processes, in which case differences between the dose-response curves for acute or chronic, reversible or irreversible toxicities may primarily reflect kinetic differences rather than differences in kind.

Mathematical Models of Dose-Response Curves for Quantitative Risk Estimation

As mentioned previously, one of the primary steps in the derivation of a quantitative risk estimate is the specification of a theoretical dose-response curve for extrapolation to low dose exposures. Numerous models have been proposed to describe how the probability of a toxic response occurring, P, varies as a mathematical function of the dosage, D. The following discussion of these mathematical models will consider in detail only the form of each model which is most commonly used to interpret quantal dose-response data. Although most of the models can be adapted to include a time-to-response term, since this thesis is concerned with the acute nonstochastic toxicity of carbon monoxide, time-to-response will not be discussed any further. The types of models presented can be categorized as either tolerance distribution models or hit-theory models.

Tolerance Distribution Models

Theoretical dose-response models which are derived from the assumption that individual threshold values or tolerances vary among a population according to some probability distribution are classified as tolerance distribution models (NAS-NRC, 1980). Since a few individuals possessing thresholds at the extremes of the distribution will cause it to "tail" extensively, the threshold doses are frequently measured on a log dose scale. These models assume the general form

$$P(D) = F(\alpha + \beta \log D)$$

where P(D) is the probability of response at dose D, F represents the frequency function which describes the population's distribution of thresholds, α is a location constant, and β is a scale constant. The two most common examples in this class are the probit model and the logit model. The distribution of log tolerances, F, is defined as the normal (Gaussian) distribution for the probit model, and as the logistic distribution for the logit model (Hoel, 1980). The mathematical equations of the resultant dose-response curves are given by

Probit: $P(D) = \Phi(\alpha + \beta \log D)$
Φ = standard cumulative Gaussian distribution

Logit: $P(D) = 1 + \exp -(\alpha + \beta \log D)^{-1}$

Both models are characterized by a sigmoidal dose-response curve. The effect of changing the location constant, α, in the probit model can be seen graphically in Figure 1. For a constant value of β ($\beta = 4$), changes in α shift the dose-response curve along the x - axis; α thus locates the mean of the threshold distribution (i.e., the dose corresponding to 50% response) along the x - axis. Changing β however, while holding α constant ($\alpha = -1$) causes the slope of the probit curve to vary in steepness as shown in Figure 2. The logit model differs little from the probit model in the observable experimental range, but at the extreme of the distribution approaches zero response less rapidly than does the probit, and therefore produces more conservative

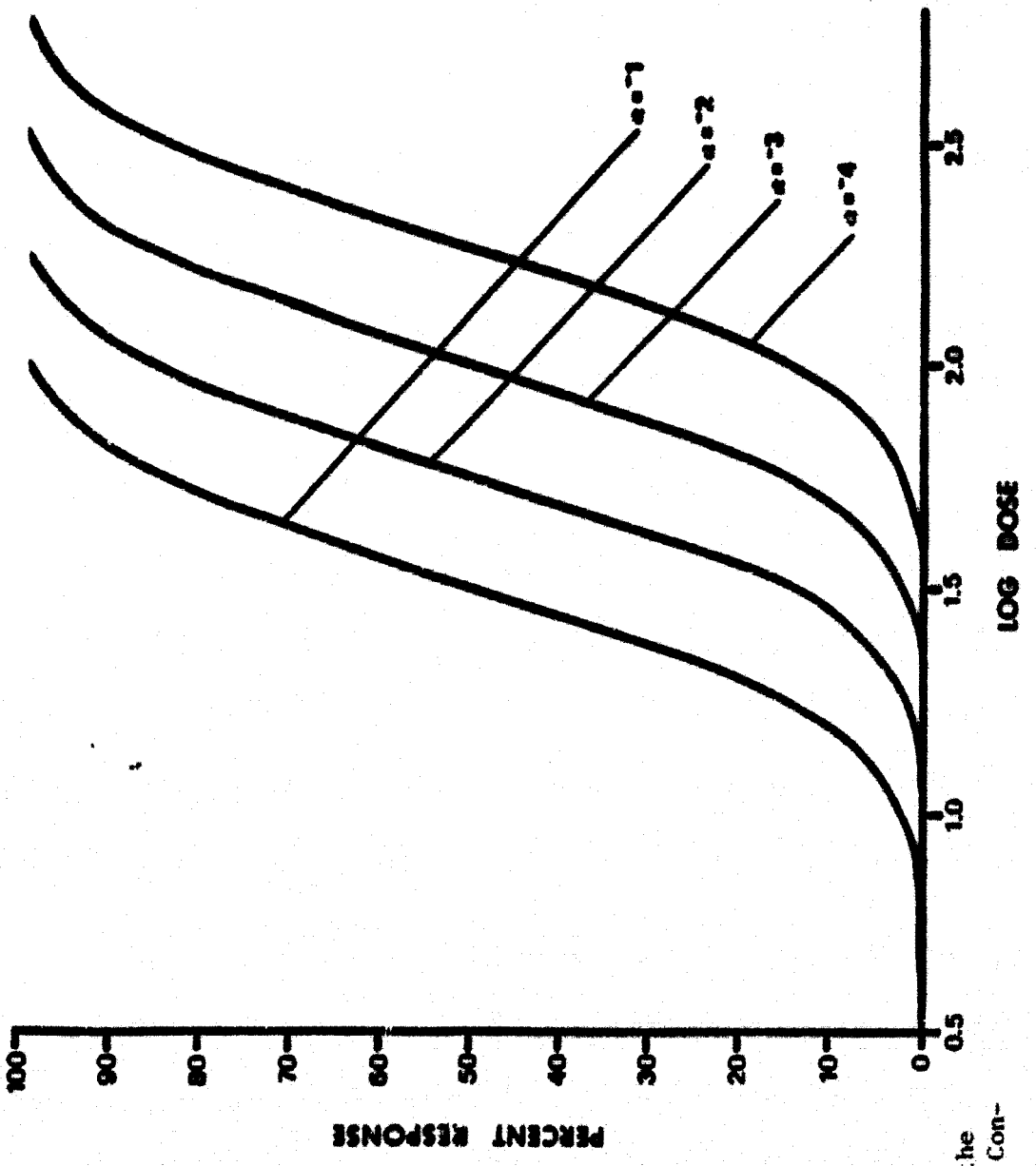


Figure 1

Significance of α in the Probit Model; β Held Constant (β = 4).

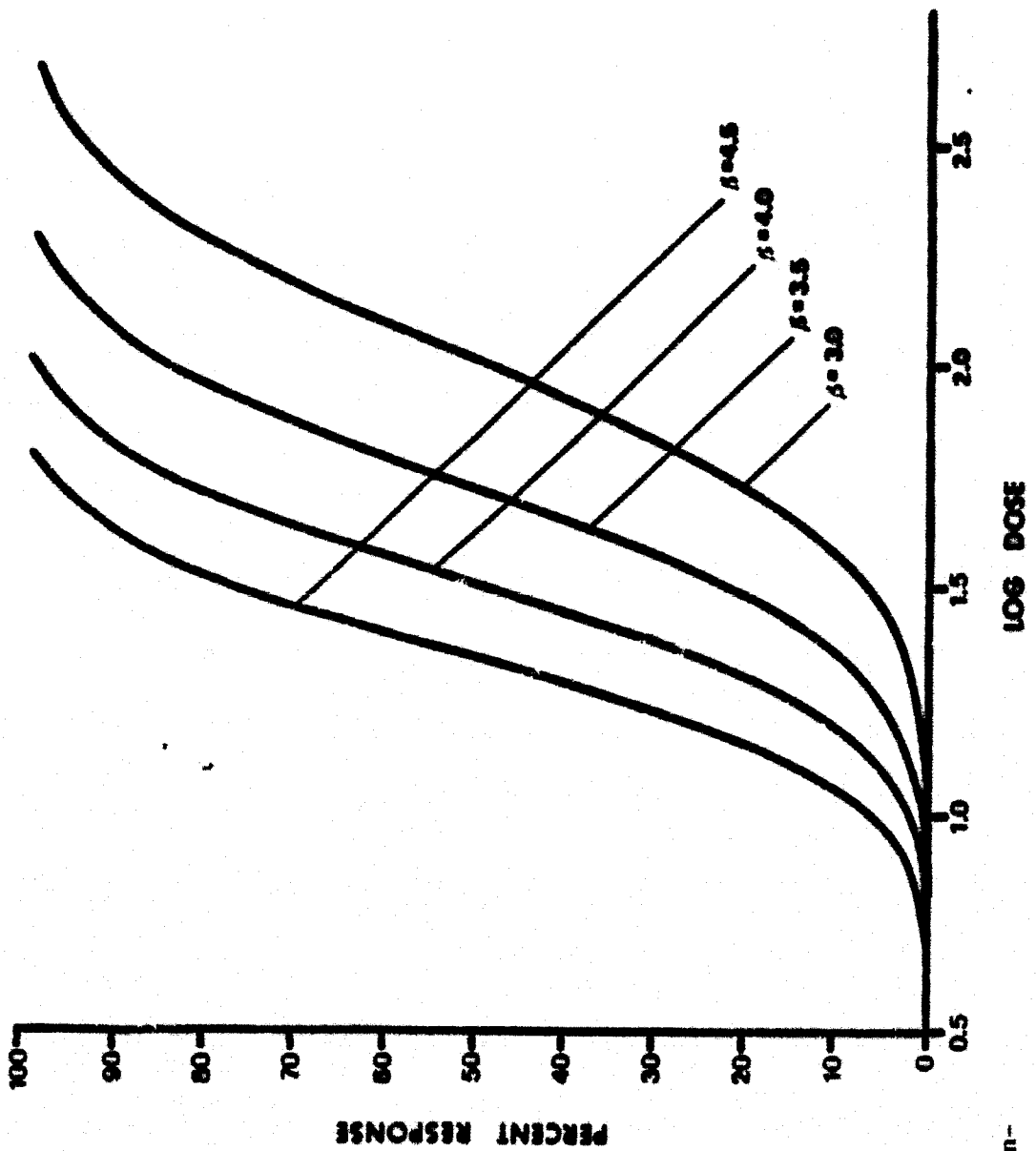


Figure 2

Significance of β in the Probit Model; α Held Constant (α = -1).

VSD's (Fishbein, 1980). Neither choice of distribution has strong biological or mechanistic justification in this context, but both have been used extensively since they "fit" a large body of observed dose-response data from a variety of toxicants.

The probit sigmoidal curve can be made linear by plotting the dose-response data on a log-probability scale. The utility of this transformation has been discussed by O'Flaherty (1981). The probit transformation is derived from the established relationship between the distance in standard deviation units from the mean of a normal distribution and the percent area under the cumulative normal curve. Using this log-probit scale, the probit lines corresponding to the sigmoidal curves of Figures 1 and 2, are presented in Figures 3 and 4 respectively. The slope of the probit line is equal to $1/\sigma$, and is therefore indicative of the degree of variability in population sensitivity to the toxicant; the steeper the slope, the less variable the population response at a given dose.

Hit-theory Models

The theoretical basis for "hitness" models is the assumption that the site of toxicity has some number of critical targets which must be "hit" or altered by a toxic agent before a toxic response is elicited (NAS-NRC, 1980). The concept of hit here implies any of a number of dose-dependent, biochemical events or transitional stages between initiation and expression of the toxic effect. For these models, the probability of response varies directly with dose, due to an increased chance of a critical target being hit at higher dosages, rather than to

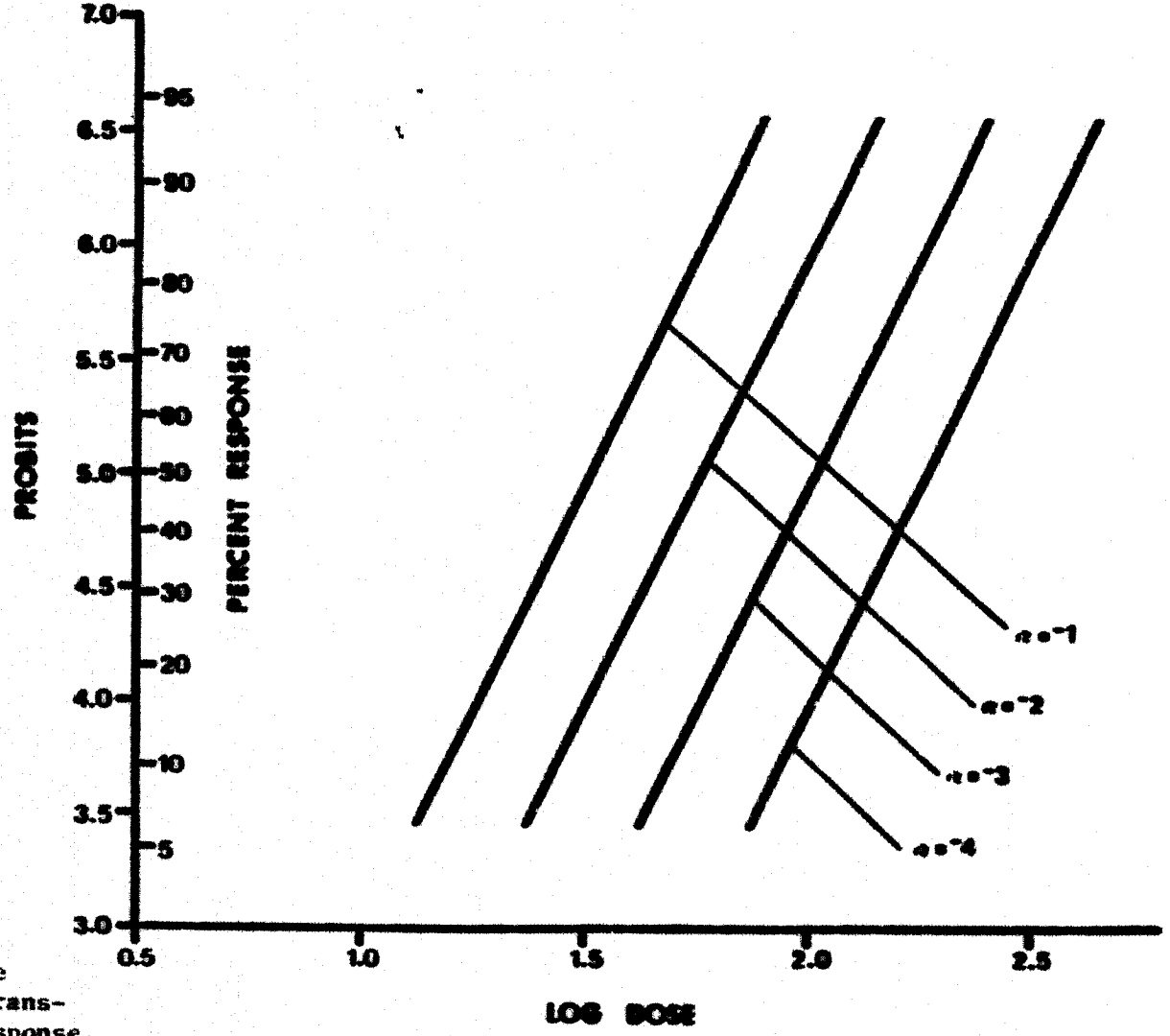


Figure 3

Significance of α in the Probit Model; Probit Transformation of Percent Response.

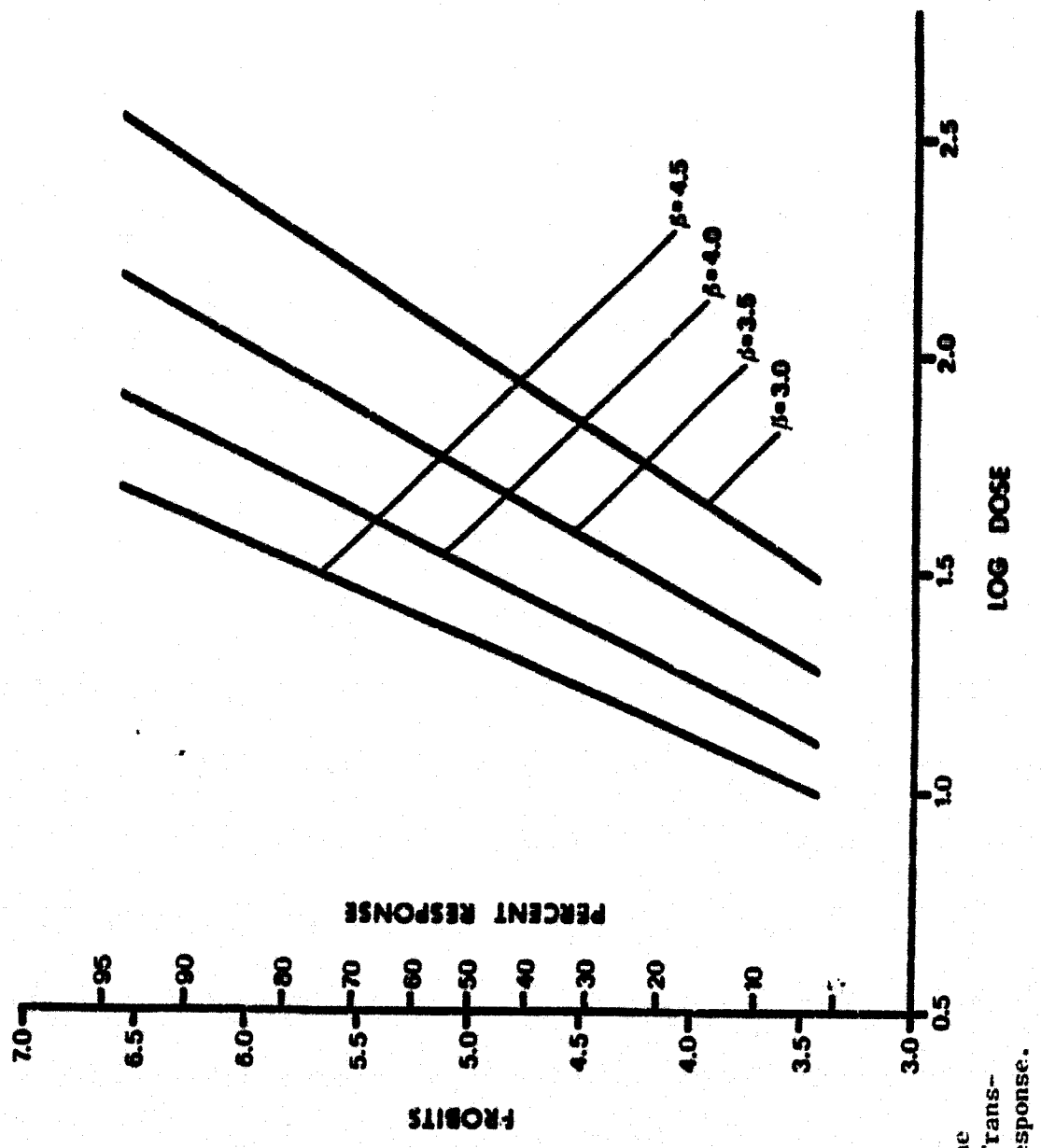


Figure 4

Significance of β in the Probit Model; Probit Transformation of Percent Response.

a larger proportion of the population being above threshold. Although this group of models assumes the nonexistence of a population threshold, several will fit data with an apparent threshold, and have been used to treat data from non-carcinogens as well as carcinogens (Scientific Committee - Food Safety Council, 1980).

The simplest of the hit-theory models is the one-hit or linear model first proposed by Arley and Iversen (1952). The one-hit model assumes that the toxic mechanism is a one-step transition occurring at a single target or receptor which has been exposed to a single, effective, dose unit of a toxic agent. The equation of the dose-response curve can be written as

$$P(D) = 1 - \exp(-\lambda D)$$

where λ is a constant equivalent to the slope of the function at low dose levels; increasing λ increases the steepness of the exponential curve between 0 and 100% response. In the low dose region, the curve is essentially linear and the probability of response is directly proportional to dose (i.e., $P(D) = \lambda D$) (IRLG, 1979; Fishbein, 1980; Hoel et al., 1975). Since the one-hit has only one disposable parameter, λ, it often fails to provide a suitable fit to data in the observable or experimental range of the dose-response curve. It is generally the most conservative hit-theory model; if the true shape of the dose-response curve is sigmoidal, the linear model will overestimate the response rate at low doses by 200 to 400 percent (Butler, 1978).

A generalization of the one-hit model is the multi-hit or k-hit model, for which at least k hits of a receptor are required to produce a response. The dose-response curve for the model is approximated by

$$P(D) = \frac{(\lambda D)^k}{k!}$$

For small values of λD^k, this approaches $P(D) = \lambda D^k$, which is equivalent to $\log P(D) = \log\lambda + k\log D$; k then is equal to the slope of log P(D) versus log D (Fishbein, 1980). Generally, the k-hit model gives the highest VSD among the hit-theory models.

A generalization of the multi-hit model is the multi-stage model; due to its greater utility, the multi-stage model is more commonly used than is the multi-hit. The model, originally proposed by Armitage and Doll (1954, 1957), was developed to account for the observation that adult human cancer incidence is approximately proportional to a high power of age. Like the multi-hit, the multi-stage model is based on the assumption that a single cell must undergo several, successive, stable changes prior to tumor development; since the probability of occurrence for each stage is time dependent, the overall incidence rate thus increases as a power of age. Unlike the multi-hit, the model assumes that only some of these transitional events depend on the carcinogenic agent, while the rest occur spontaneously at a given rate. This feature makes the model consistent with human and animal data which suggests cancer incidence is proportional to dose or the square

of dose, but no higher powers (NAS/NRC, 1977). The equation for the curve may be expressed as

$$P(D) = 1 - \exp-(\alpha_1 + \beta_1 D)(\alpha_2 + \beta_2 D)\ldots(\alpha_k + \beta_k D)$$

where the important parameter, k, is interpreted as the number of events or stages in the carcinogenic process, α is the spontaneous occurrence for each stage, and β represents a dose-dependent proportionality constant for each stage (Fishbein, 1980). Though the assumptions of the multi-stage model are drawn from stochastic responses with long latent periods, the model has been applied to acute lethality data for botulinum toxin (Scientific Committee - Food Safety Council, 1980).

Carlborg (1981b) has discussed several drawbacks to the multi-stage model, the most significant being that it is mathematically undefined with regard to the choice of terms in the polynomial exponent. Carlborg has demonstrated that there are innumerable ways to fit the multi-stage model to a given data set, leading to uncertainty about both the number of stages, k, and the risk outside the observable response range. This lack of definition, he argues, does not occur with the Weibull model, another generalization of the one-hit model.

For the Weibull model, the probability of response as a function of dose can be written as

$$P(D) = 1 - \exp^{-}(\alpha + \beta D^m)$$

The parameter α represents the spontaneous background response rate (i.e., the response rate observed in a control population). Figure 5 illustrates how changes in α alter the Weibull dose-response curve, when β (β = .005) and m (m = 5) are held constant; the value of α primarily effects the extent of tailing of the function as zero response rate is approached. The parameter β is a scale parameter which primarily alters the slope or steepness of the Weibull curve without affecting the basic shape of the curve (e.g., sigmoidal). This relationship is shown in Figure 6 for constant values of α (=.01) and m (5). The critical parameter, m, determines the shape of the Weibull curve. The importance of m is illustrated in Figure 7 (adapted from Carlborg, 1981a); in Figure 7, α is constant (= .0009), but the slope parameter, β was varied to keep the curves on the same scale. For values of m $<$ 1, the dose-response function is concave (high response rates even at low doses), linear for m = 1 (the one-hit model), and sigmoidal to convex for m $>$ 1 (low response rates even at high doses). The latter characteristic accounts for the model's ability to fit data with an apparent threshold, despite the hit-theory assumption of no threshold (Carlborg, 1981a). The VSD predicted by this model generally falls between the values for the multi-hit and the multi-stage models (Scientific Committee - Food Safety Council, 1980). At present there is no physiological interpretation of the important shape parameter, m, although Carlborg (1981b) has suggested that the m term in the Weibull equation may be related to the number of stages, k, in the multi-stage model by the relationship k/2 = m.

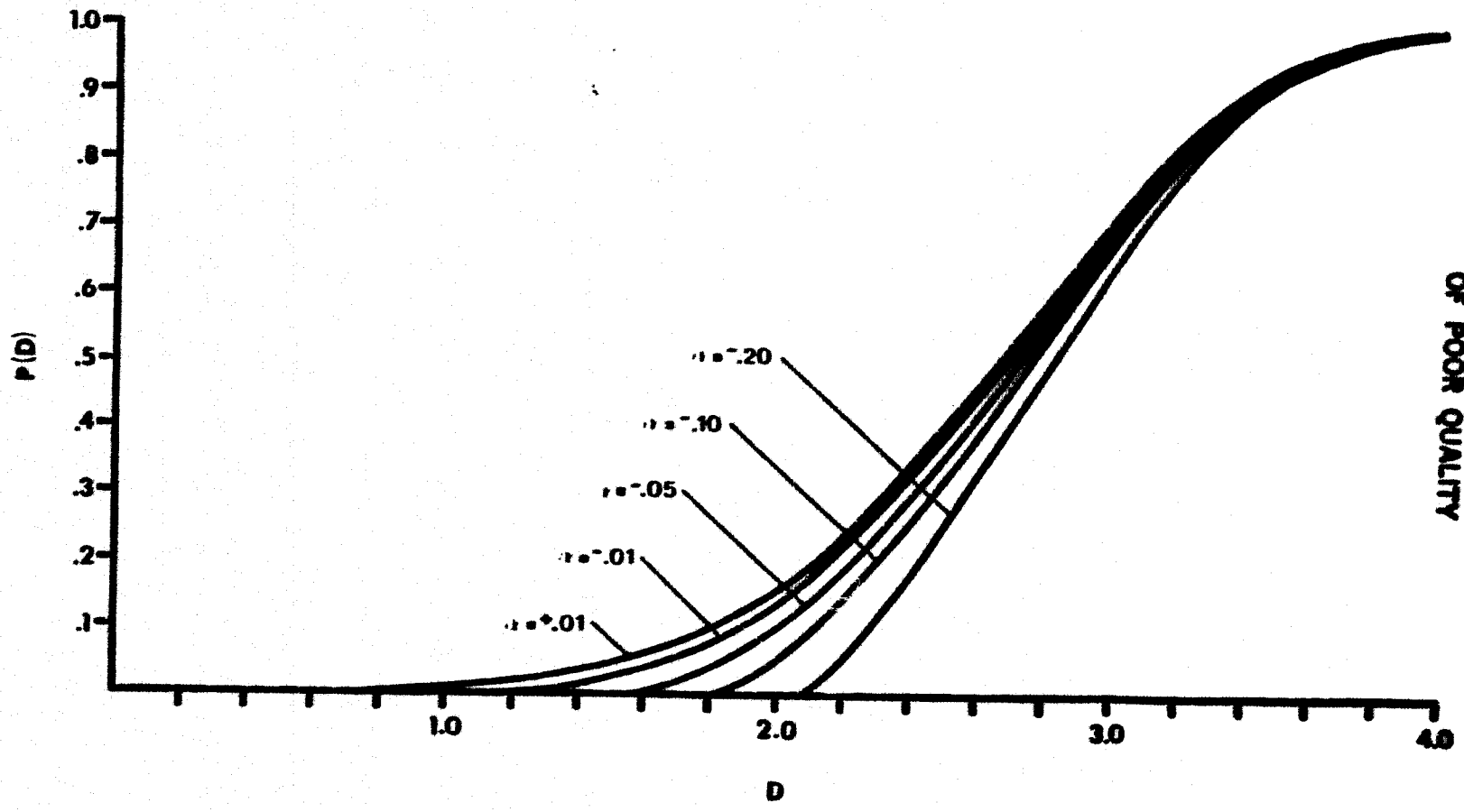


Figure 5

Significance of the α Parameter in the Weibull Model; β and m Held Constant.

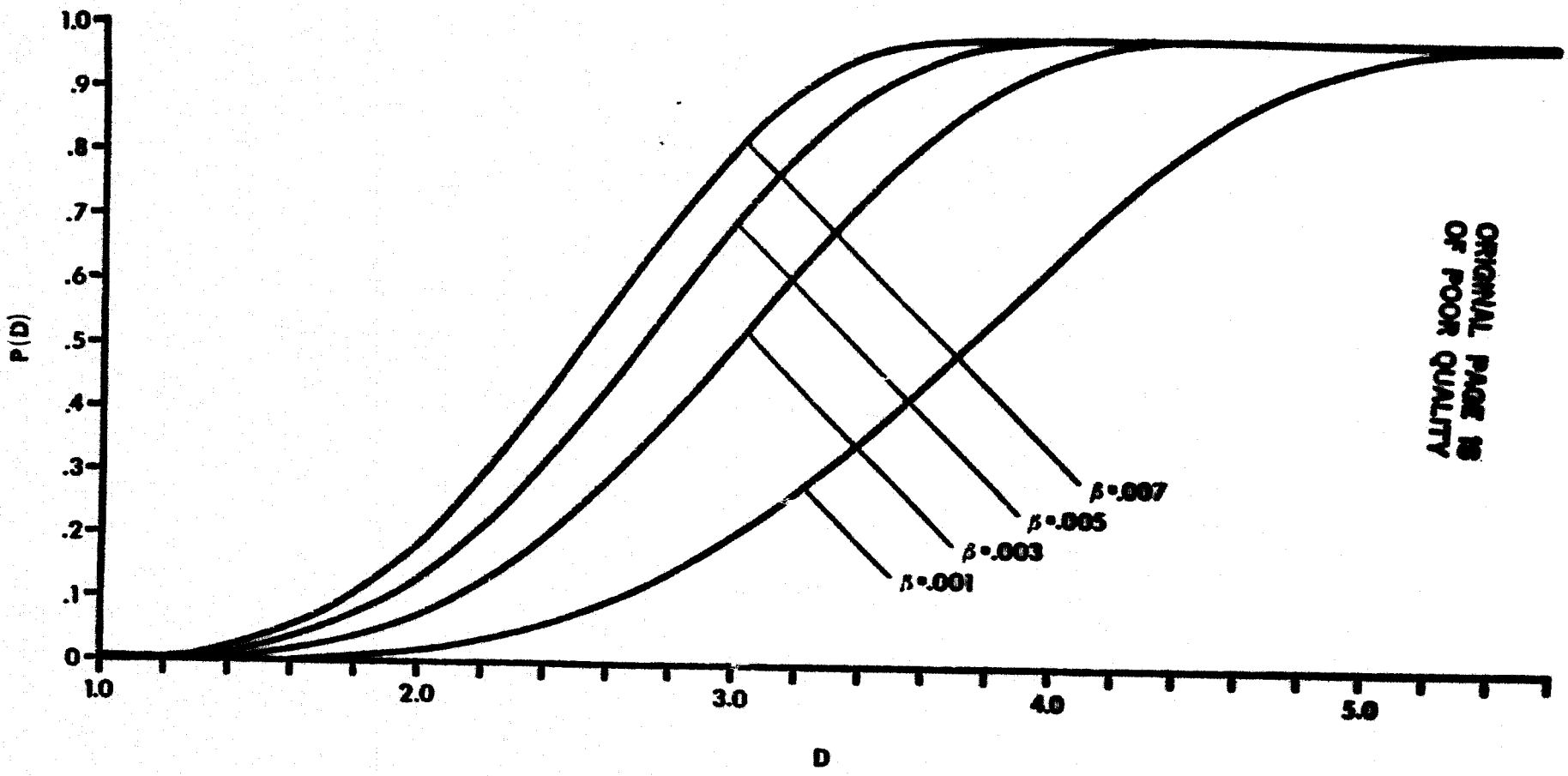


Figure 6

Significance of the β Parameter in the Weibull Model; α and m Held Constant.

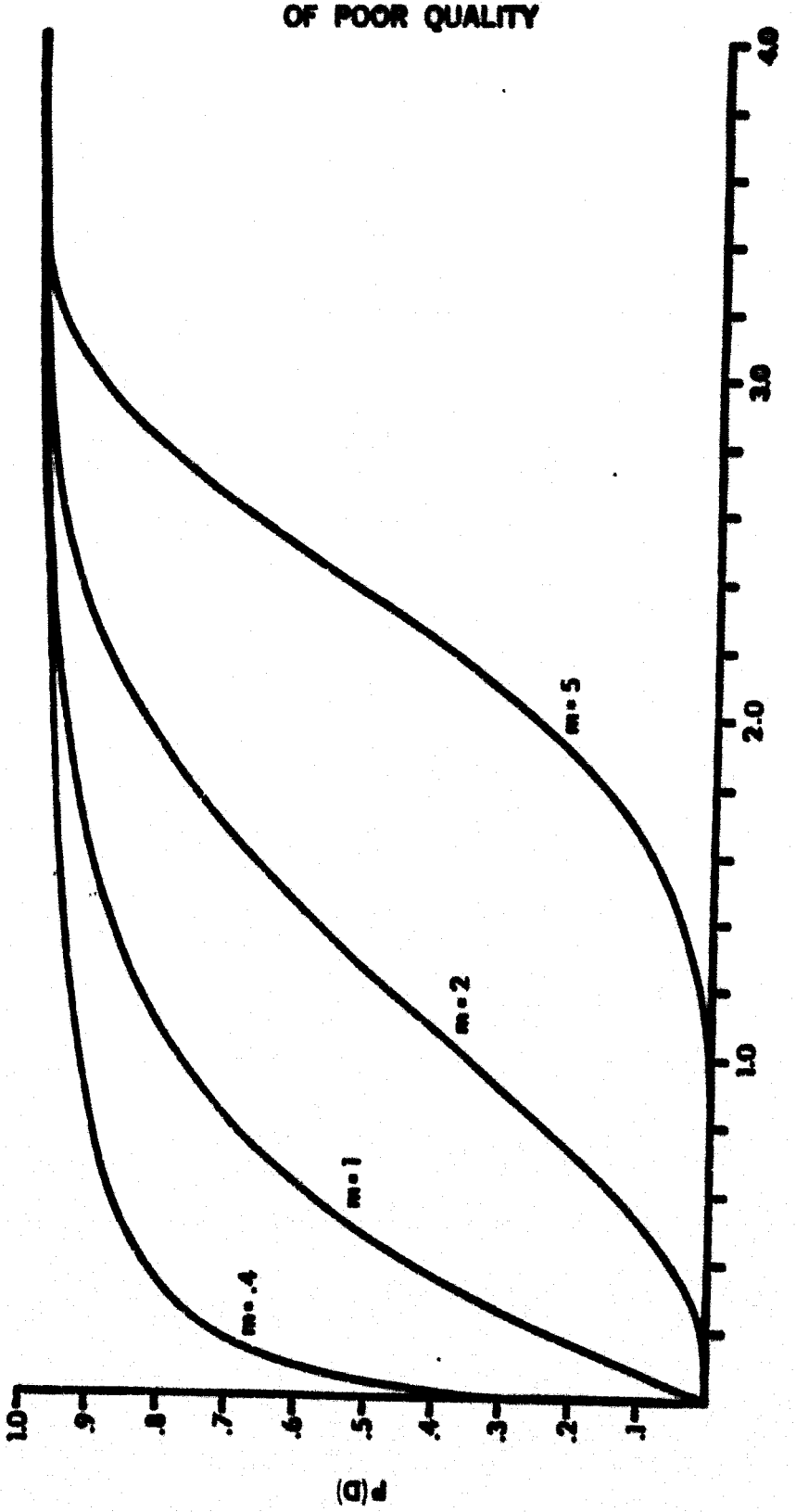


Figure 7

Significance of the m Parameter in the Weibull Model; α Held Constant.

Biological Considerations for Species-to-Species Extrapolation

It is apparent from the preceeding discussion that numerous theoretical models have been proposed to describe the true shape of dose-response curves at low dose levels from assumptions about the nature of the toxic event. However, the various dose-response curves may not relate solely to different modes of action at receptors; differences in how an administered dose becomes an effective dose must also be considered. It is an oversimplification to equate the term dose, the amount of a chemical administered, with the critical concentration of the toxicant at the receptor, which gives rise to a toxic effect. The latter is a complex function of both the actual exposure, and the biochemical and physiological characteristics of the host (Schneiderman and Brown, 1978). The compound dose-response curve then depends on the relationship between dose and the effective concentration at the receptor, and between the effective concentration and response (Nordberg and Strangert, 1978). The biological processes which are involved in the conversion of an administered dose to an effective dose include absorption, distribution, metabolism, and excretion. Of these, metabolism is often the most significant with regard to risk assessment.

Metabolism can transform some or all of the administered dose of a xenobiotic into innocuous or readily excretable compounds, or may produce highly toxic metabolites via the same degradative pathways. To assure reliable risk estimates, not only must allowances be made for interindividual variability in metabolism, but interspecies

differences in metabolic pathways or efficiency must be considered (Ramsey and Gehring, 1980). Confidence in quantitative inferences drawn between species is highest for direct-acting agents; chemicals requiring bioactivation are subject to species' variation in saturability and end-products of metabolic processes (Nelson, 1978).

Substantial interspecies differences in the absorption, distribution, or excretion of a chemical also can and do occur, which may significantly affect the shape of the dose-response curve, particularly at low doses. Generally these phenomena are considered on a case by case basis and may be incorporated into the risk estimate process in the form of additional safety or coversion factors.

Occasionally, certain genetic, nutritional, sexual or developmental characteristics can predispose a few individuals or an entire species to display a hypersensitive response to a given toxicant. The prevailing regulatory assumption for the purpose of estimating risk to the general human population, is that man must be considered the most sensitive species until experimental evidence proves otherwise (IRLG, 1979). If such evidence is not available, the Environmental Protection Agency has proposed as a rule of thumb, that man may be tenfold more sensitive than the experimental animal used, and that there may be a tenfold variation in sensitivity between individuals (Cornfield, 1977).

Additionally, safety factors such as those recommended by the National Academy of Science for establishing SNARL values, may be applied to VSD's obtained from the risk estimate approach. The magnitude of these factors will depend on a subjective interpretation

of a number of biological and experimental variables, including:

1) quality of the experimental design and consistency of the biological data
2) availability of supporting evidence from other in vitro, epidemiologic, or structure-activity studies
3) observation of similar qualitative response in other species
4) conservatism warranted by the nature of the toxic response and size of the population at risk.

Chapter 3

MATERIALS AND METHODS

Mouse Behavioral Assay - Adapted from Winslow (1981)

Experimental Procedure

Free-running, male, Swiss-Webster mice weighing approximately 35-40 grams were exposed to various concentrations of CO in air for up to 30 minutes. During exposure, the mice were continuously monitored for their ability to carry out a learned avoidance task. Mice were trained to jump to a pole upon presentation of a conditional stimulus (a tone cue) in order to avoid an unconditional stimulus (an electric shock). If the mouse fails to avoid the shock but can still jump to the pole during the shock period, the behavioral response is termed an escape rather than an avoidance. During exposures, one-minute long trials consisting of the conditional stimulus followed by the unconditional stimulus and an inter-trial pause were repeated continuously. The initial behavioral change is defined as the first trial the mouse fails to avoid the shock, followed by failure to avoid in at least 4 of 6 subsequent trials. Loss of escape occurs at the first of 3 consecutive trials in which the mouse fails to escape the shock. The exposure was terminated once loss of escape was established. For further details of the experimental design of the behavioral assay, the reader is referred to Winslow (1981).

Behavioral Response Chamber

Complete details of the pole-jump chamber specifications and operating procedures are provided by Winslow (1981). A picture of the mouse pole-jump in use is presented in Figure 8.

Rat Physiological Assay

Experimental Procedures

Restrained, female, Sprague-Dawley derived rats (Simonsen Labs, Gilroy) weighing approximately 225-275 grams were subjected to 20 minute long, nose-only, inhalation exposures of various concentrations of CO in air. Exposures were conducted at the same time $\pm$ 1/2 hour each day, since Cinkotai and Thompson (1966) have reported a diurnal variation in pulmonary diffusing capacity for CO in humans.

During exposures, electrocardiographic and respiratory records were obtained. At two hours after the exposure was terminated, survivors were sacrificed with CO_2 and cardiac blood samples (3 ml) for enzyme analysis were collected by cardiac puncture. The two hour post-exposure sampling time was chosen on the basis of a preliminary study of the time dependence of enzyme release in rats (n = 27) exposed to approximately 5100 ppm of CO for 20 minutes. Serum enzyme activities immediately after exposure were significantly increased but had not peaked, while enzyme activities in samples collected after the 2 hour post-exposure sample, exhibited greater interindividual variation.

Asphyxiation by CO_2 was chosen as the method of choice for termination rather than cervical fracture, since in a preliminary study, the mean serum enzyme levels from previously untreated rats killed by

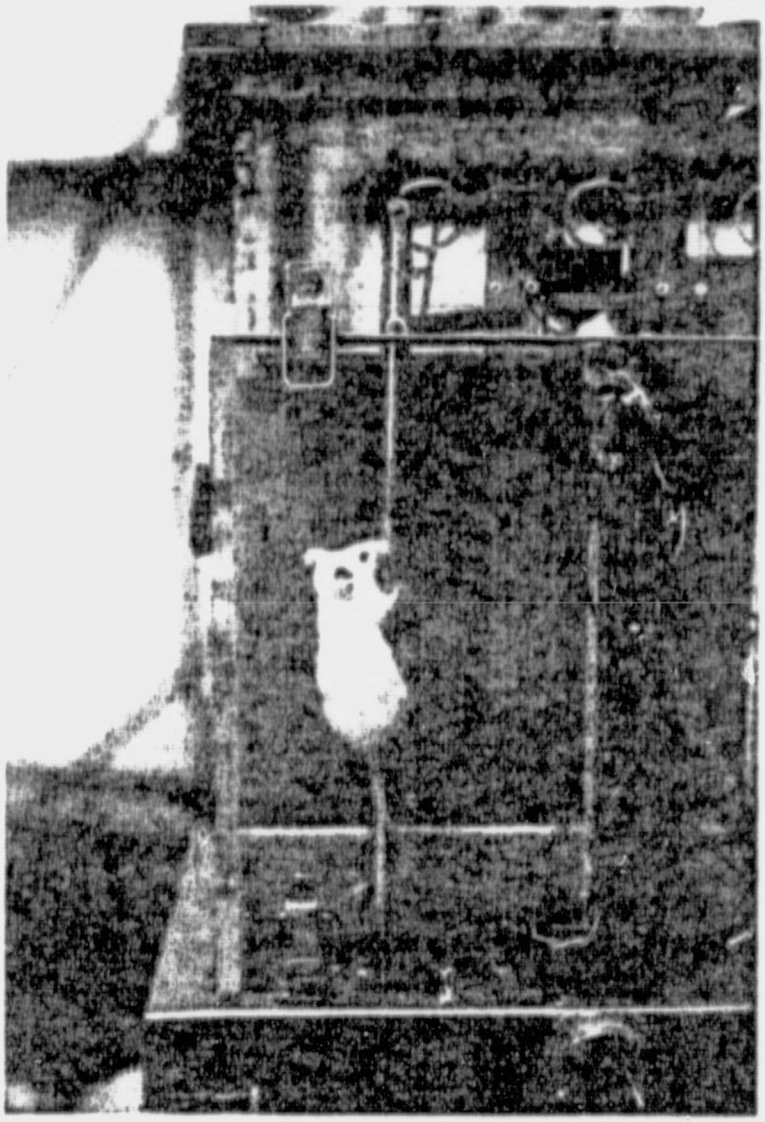


Figure 3

Mouse Pole-Jump

cervical fracture (n = 11) were from 2 to 10 times greater than those observed for untreated rats killed by CO_2 asphyxiation (n = 11).

Electrocardiographic and Respiratory Monitoring

Continuous respiratory and electrocardiographic records of restrained rats during exposures were obtained from a pair of transthoracic, surface, disk electrodes. The electrode contact area on the chest of the rat was shaved at least 24 hours prior to CO exposures. The electrodes were held in place on the rat by a latex and velcro jacket (Figure 9 and 10). The jacket was designed to allow secure and correct placement of surface electrodes on unrestrained and unanesthetized rats. Electrocardiographic and respiratory responses are monitored on a NARCO Physiograph Model 6-B, equipped with impedence pneumograph couplers (respiration) and hi-gain couplers (ECG), and on a GRASS Model 79D Polygraph. Samples of control ECG and respiration records are reproduced in Figure 11.

Serum Enzyme Analysis

Cardiac blood samples were collected and allowed to clot in labelled, serum separator tubes (Microtainer™, Becton-Dickinson) for 30-60 minutes at room temperature. They were subsequently centrifuged at 5°C for 5 minutes at top speed in a desk top centrifuge. Individual serum samples were divided into aliquots and then transferred to labelled glass ampules for storage at 4-8°C (maximum stability for creatine phosphokinase (CPK) and alpha-hydroxybutyrate dehydrogenase (HBDH)) and at approximately 23°C (maximum stability for lactate

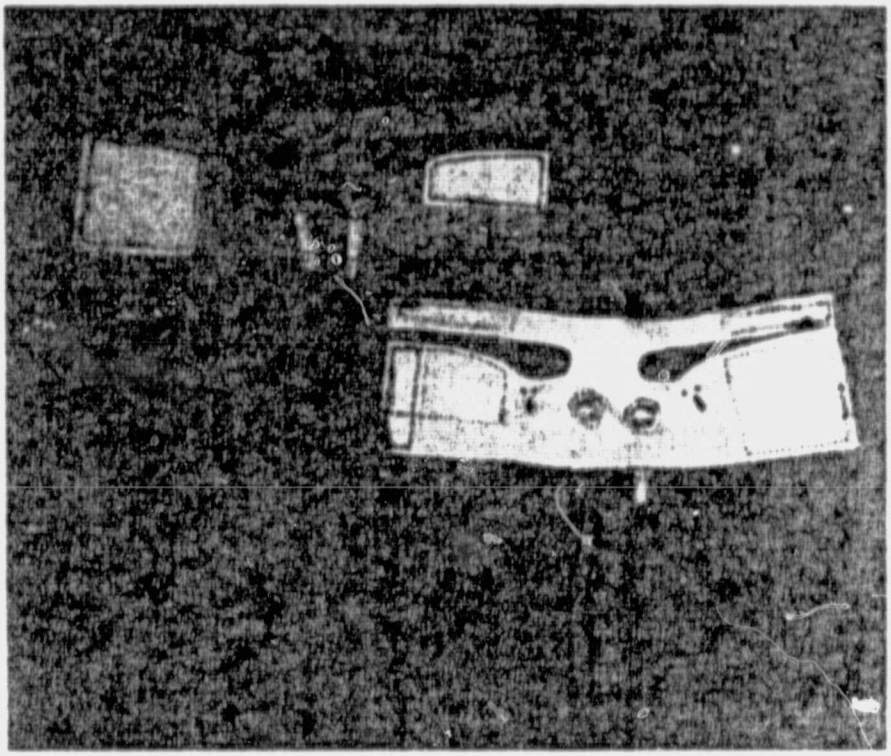


Figure 9

Electrode Jacket for Electrocardiographic
and Respiratory Monitoring

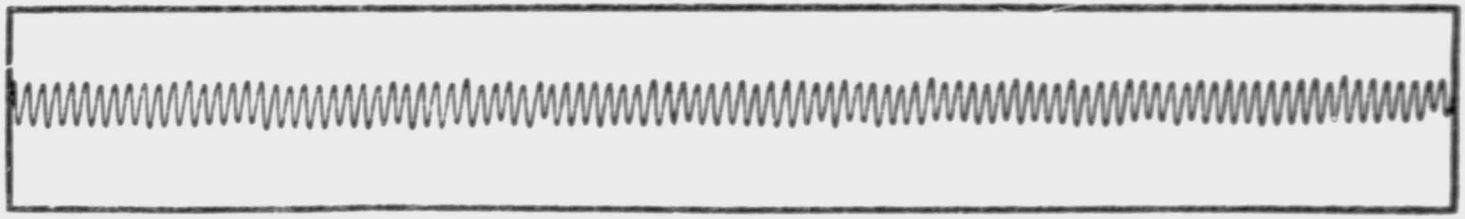
Figure 10

Electrode Jacket on Rat

NORMAL ELECTROCARDIOGRAM

5 mm/sec 25 mm/sec

NORMAL RESPIRATION



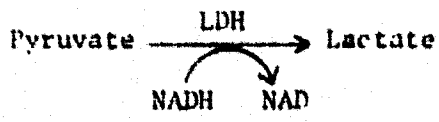
5 mm/sec

Figure 11

Sample Electrocardiographic and Respiratory Records.

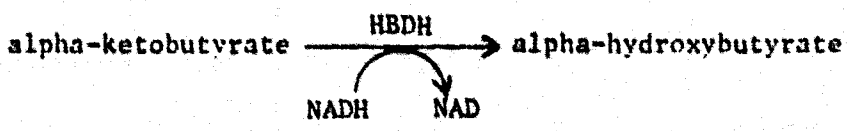
dehydrogenase (LDH)). Enzyme analyses were conducted within 48 hours of serum collection. Only non-hemolyzed serum samples were used for serum HBDH and LDH determinations, since both enzymes are released from lysed red blood cells; hemolysis has no effect on serum CPK activity (Frank et al., 1978).

Calbiochem Behring S.V.R.^{TM} (single vial reagent) diagnostics were used for the determination of all enzyme activities. All reagents and cofactors necessary for each assay were pre-packaged in single vials, and were prepared fresh every 48 hours as needed by reconstitution with an appropriate volume of distilled water. The LDH assay is modified from the procedure of Wacker et al. (1956). The LDH reaction measured is the following:



(high A_{340})

The progress of the reaction is followed spectrophotometrically at 340 nm. The HBDH assay is based on the methods of Rosalki and Wilkinson (1964). The HBDH reaction



(high A_{340})

is followed spectrophotometrically at 340 nm. The CPK assay is based on the procedures of Oliver (1955) and Rosalki (1967). The CPK reaction

$$\text{creatine phosphate} + \text{ADP} \xrightarrow{\text{CPK}} \text{creatine} + \text{ATP}$$

is linked in the assay to two other enzyme reactions which result in the generation of NADPH from NADP; the formation of NADPH is followed spectrophotometrically at 340 nm.

The U.V. spectrophotometer used in all cases was a double-beam Varian Technitron Model 635, equipped with a chart recorder and circulator to maintain temperatures in the cuvette compartment at an optimum 30°C throughout the reaction period. Three ml of prepared reagent and 50 µl of serum were incubated separately for 5 minutes in a water bath at 30°C, then combined, transferred to a quartz micro-cuvette, and placed in the spectrophotometer to be read against a water blank for 7 to 10 minutes. The enzyme activities of the samples were determined from the rate of change of absorbance at 340 nm per minute, measured in the linear portion of the enzyme reaction curve. Enzyme activity is expressed in mIU of enzyme activity per ml of serum. An IU, or International Unit, represents the amount of enzyme which converts one micromole of substrate in one minute; under standard conditions.

A quality control check was conducted daily using control sera (Calbiochem-Behring CALTROL™ I, Sigma Enzyme Control 2-E) with established enzyme activity levels.

Physiological Response Chamber

The physiological response exposure chamber is a modular unit designed to interface with the behavioral response chamber, and either the NASA Radiant Panel Furnace (for thermal combustion of polymers) or a pure-gas delivery system (for CO exposures). The chamber consists of a rectangular, plexiglass sleeve, 12 in. long, 8.5 in. wide, and 4.5 in. deep. The sleeve is punctuated by twelve, level, 1 in. diameter holes which interface with individual rat restraint tubes (Figure 12). The restraint tubes are constructed of 9 in. lengths of 2 in. (inner diameter) plexiglass tubing (Figure 13). The head end of the tube is fitted with a 1.25 in. deep cone, which has a 2 in. inner diameter proximally, and a 0.5 in. diameter distally. This nosepiece effectively allows one to channel the head of the rat forward in the tube, so that an inhalation-only exposure can be obtained (Figure 14); only the nose and mouth of the rat project from the cone through the openning in the sleeve, and into the exposure chamber (Figure 15). An adjustable teflon stopper at the tail end of the restraint tube accomodates the length of the tube to different weight animals.

Carbon Monoxide Delivery and Analysis

For inhalation exposures of CO, the behavioral response chamber and physiological response chamber were sealed off and connected to a gas delivery apparatus. Tanks of 99.5% CO (Matheson certified standard) and breathing air were connected via a latex plumbing system to two, separate, gas-flow controllers. Gases from the controllers were

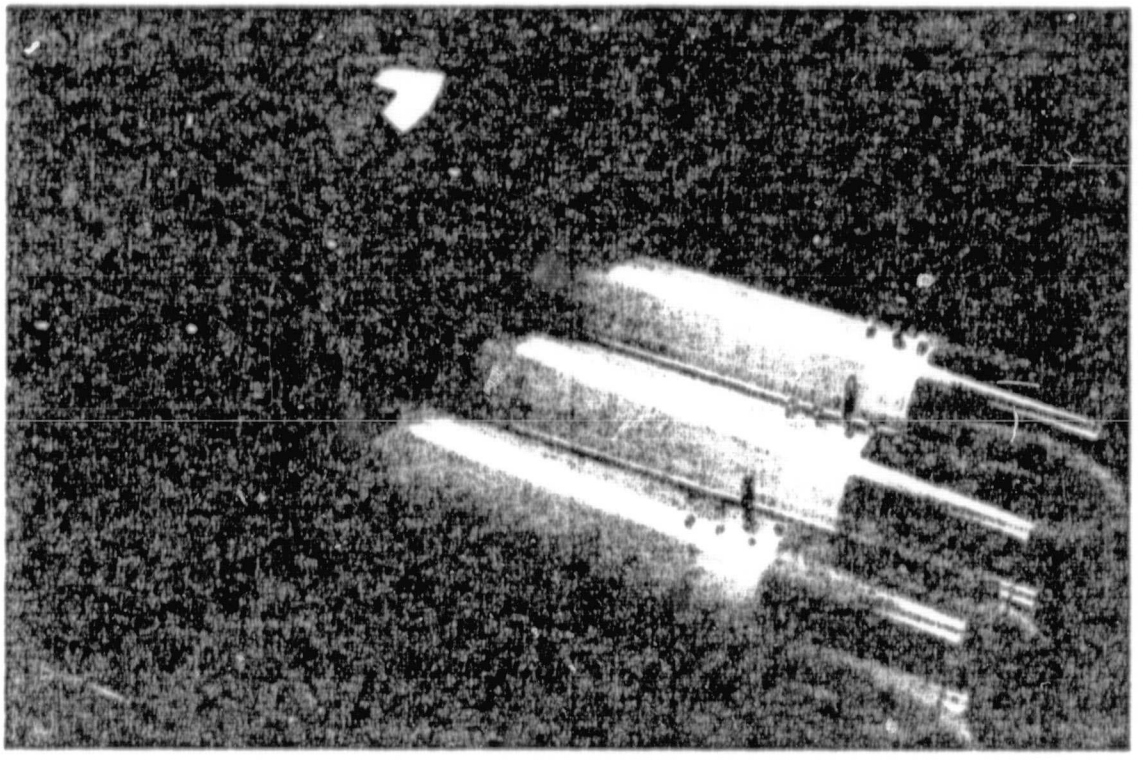


Figure 12

Physiological Response Exposure Chamber

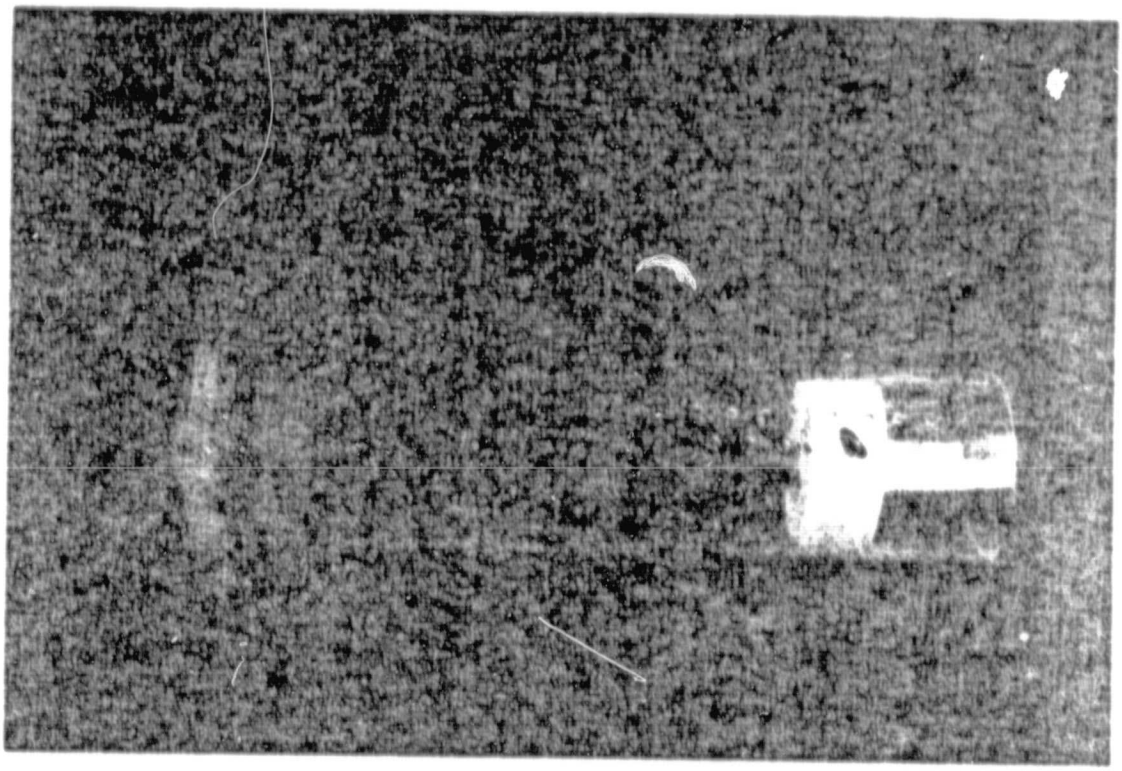


Figure 13

Individual Rat Restraint Tube

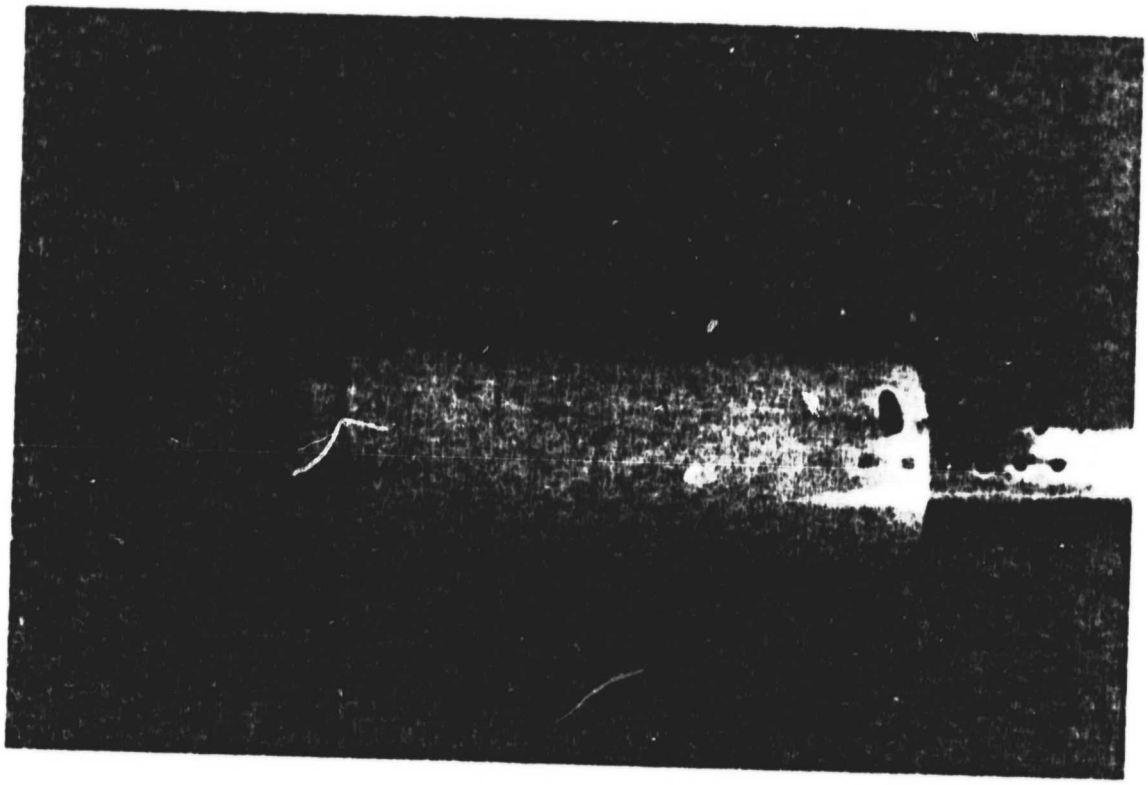


Figure 14

Rat in Restraint Tube

Figure 15

Nose-Only Inhalation Exposure

homogenized by being passed through a 3 cm diameter by 40 cm long glass tube filled with 3 mm glass beads. Any number of homogenized gas mixtures could be generated in line by virture of altering flow rates. The homogenized gas mixture was delivered to an inlet in the top of the exposure module; a 4 in. fan located at the gas inlet ensures rapid and complete mixing of the incoming gases with the chamber atmosphere (Figure 16).

The gas mixture was delivered under slightly positive pressure and was continually supplied throughout the course of exposure to maintain relatively constant CO concentrations. Spent gas was vented through an exhaust tube at the chamber bottom. At time zero, a small volume of 99.5% CO was injected into the gas inlet near the fan, just before initiating delivery of the homogenized gas mixture, in order to bring the chamber atmosphere up to the desired CO concentration quickly (within 2 minutes). Samples of the exposure atmosphere were withdrawn by syringe through an air-tight septum in the chamber wall, just below the level of the rats' noses. The CO concentration of samples taken at 2, 5, 10, 15, and 20 minutes into exposure, were analyzed by molecular sieve chromatography against a standard curve prepared daily from known concentrations of CO.

The response chambers and gas delivery system were operated at all times under a walk-in fume hood, to prevent accidental exposures.

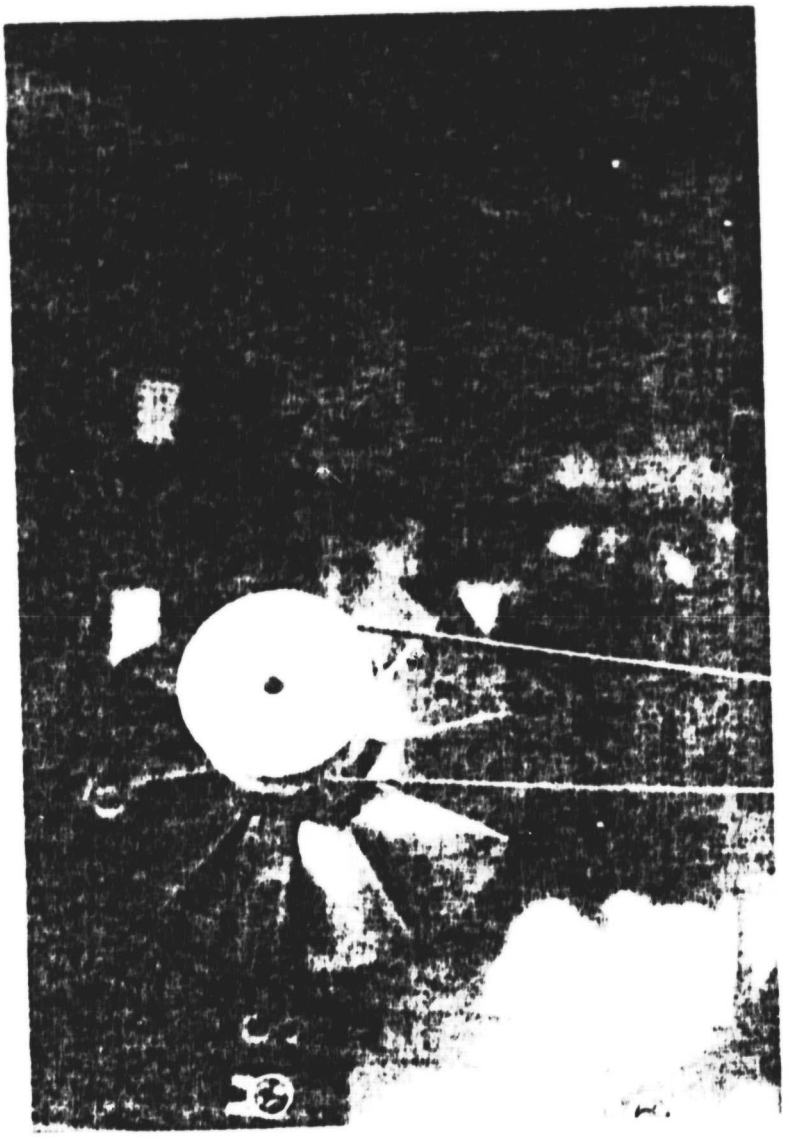


Figure 16

Mixing Fan at Gas Inlet

Data Analysis

The theoretical models chosen to be fitted to the behavioral and physiological dose-response data for CO were the one-hit, probit, and Weibull. The probit model is a tolerance distribution model in wide use currently, and is not as conservative as the logit model. The one-hit model is the most basic and conservative of the hit-theory models, while Weibull derived risk estimates generally fall between those for the multi-hit and multi-stage models. The Weibull is also better defined mathematically than the latter as discussed in Chapter 2.

Parameters of the best-fit, least squares curves for the one-hit and Weibull models were determined with the aid of DYNACOMP, Incorporated's software package, Regression II (PARAFIT), on an Apple II Computer. PARAFIT employs an iterative procedure in its regression analysis to arrive at the parameters of the best, least-squares curve of the functional form entered by the user. Required inputs from the user include an upper limit on the number of iterations, initial estimates for the parameters and an approximate order of uncertainty in the estimates. The number of iterations was increased and new parameter estimates were entered until a stable value for the standard deviation of the regression was obtained.

Parameters of the maximum likelihood fit of the probit model were estimated by the method of Bliss (1938). The method is an iterative weighted regression procedure. Briefly, the data are plotted on a log-probability scale, using log units for dose, and probit units for percent response. Tables from Finney (1952) were used to convert the

percent response directly into probits. A provisional line was then fitted to the points by eye, and provisional weights for the data points on the basis of this line were determined. A series of calculations (iteration) are performed with the provisional weights and probits, which result in a corrected line closer than the provisional to the actual maximum likelihood line. If the corrected line is close to the provisional line, as determined by a χ^2 test, no further computations are necessary. If not, the corrected line becomes the provisional line and the iteration is repeated as often as necessary (Hewlett and Plackett, 1979).

Chapter 4

RESULTS

Mouse Behavioral Data

Quantal Dose-Response Curve for the Initial Behavioral Change

Data obtained by Winslow (1981) on the effects of CO on motivated behavior using the pole-jump apparatus, are reprinted in Table I. The dose of a toxicant administered during an acute inhalation exposure is commonly expressed as a concentration-time (CT) value; CT is the product of the toxicant concentration in ppm, and the duration of exposure in minutes. If the CT value adequately describes the dose of an inhalant, then the CT at which a specific toxic response is observed should be approximately constant. However, Winslow observed that CT for the initial behavioral change was not a constant. He suggests that a better description of the dose at which the initial behavioral response occurs is $CT^{0.3}$; the concentration of CO is therefore of greater import than the duration of exposure. The $CT^{0.3}$ value (listed in column 5) at which the behavioral response occurs will hereafter be considered as the dose of carbon monoxide administered.

By definition, the initial behavioral change is an all-or-none event, i.e., a quantal response. Figure 17 is a frequency histogram which relates the dose of CO, expressed as a $CT^{0.3}$ value, with response, the number of individual mice exhibiting the initial behavioral change at that dose. Arbitrary dose-increments were chosen such that all animals responding within a $CT^{0.3}$ range of 200 ppm-minutes

TABLE I

Summary of Motivated Behavior Changes
Observed During Acute CO Exposures [1]

Experiment #	Initial Behavioral Change CT (ppm-min)	Time to Initial Behavioral Change (min)	Average [CO] at Initial Behavioral Change (ppm)	Initial Behavioral Change $CT^{0.3}$ (ppm-min)
M-24	7203	2.2	3274	4148
M-19	11866	5.5	2157	3597
OB-3	10125	4.5	2250	3533
M-20	11187	5.8	1929	3269
OB-6	9503	5.3	1793	2957
OB-5	11544	7.0	1649	2956
M-18	10428	6.4	1629	2843
M-8	11792	8.4	1404	2659
OB-8	11544	8.4	1374	2602
M-17	11347	8.2	1384	2602
OB-9	6972	4.2	1660	2553
OB-7	10882	8.0	1360	2538
M-21	14043	11.6	1211	2526
M-12	5652	3.4	1662	2399
M-6	9500	7.2	1319	2385
OB-2	8183	6.0	1364	2335
M-5	9000	6.9	1304	2328
M-16	13510	12.4	1090	2320
M-15	15367	17.0	904	2115
M-7	3925	2.5	1570	2067
M-22	5433	4.5	1207	1895
M-13	10360	12.3	842	1788
M-23	6832	6.8	1005	1786
OB-1	9830	12.0	819	1726
M-14	9349	11.7	799	1671
M-11	4181	6.0	697	1193
*				

1 Data reprinted from Winslow, 1981.

* Experiment #M-25 and M-26 were omitted since times to initial behavioral change and to loss of escape were not consistently reported, precluding the determination of $CT^{0.3}$ values.

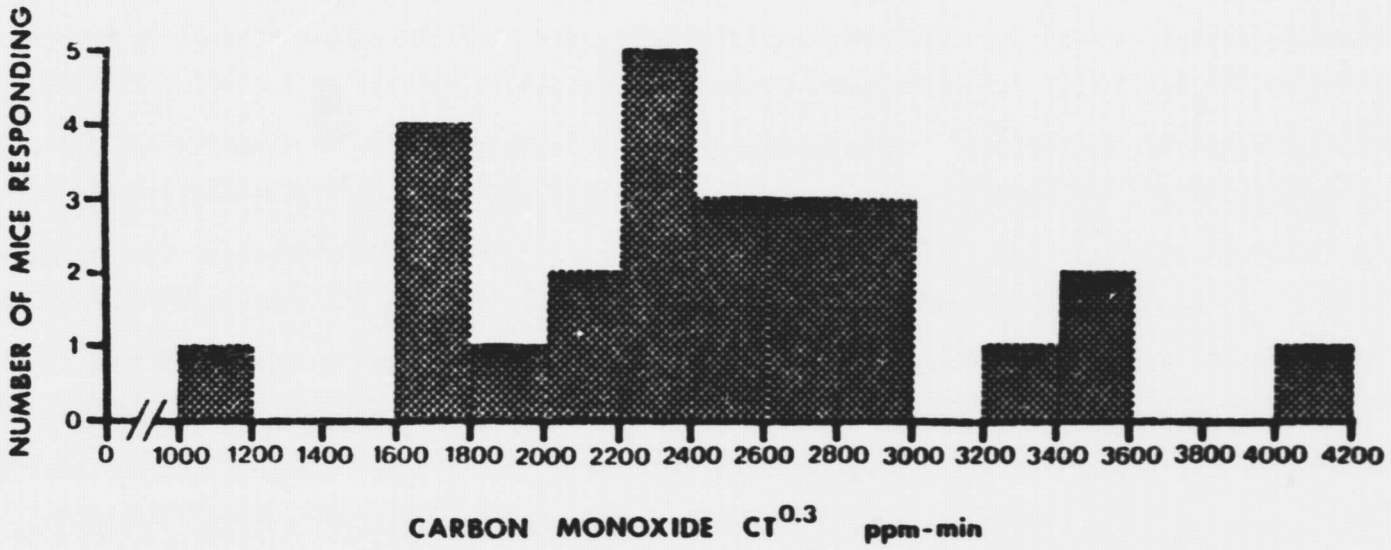


Figure 17

Relationship between Carbon Monoxide $CT^{0.3}$ and Initial Behavioral Change Response

The frequency histogram showing the relationship between the dose of carbon monoxide ($CT^{0.3}$) and the initial behavioral change response is shown. Mice were continuously exposed to various concentrations of CO until the initial behavioral change occurred. The $CT^{0.3}$ value at which each animal responded is recorded in Table 1. All animals responding between $CT^{0.3}$ increments of 200 ppm-minutes were grouped together. Original data compiled by Winslow (1981).

were grouped together. Thus, it can be seen that one mouse displayed the initial behavioral change between carbon monoxide $CT^{0.3}$ values of 1000 to 1200 ppm-minutes, no mice displayed the initial behavioral change between $CT^{0.3}$ values of 1200 to 1400 ppm-minutes, etcetera.

Figure 18 is the quantal dose-response curve obtained by a cumulative summation of the frequency histogram in Figure 17. This transformation is described by Hewlett and Plackett (1979). The curve is the result of adding together the number of animals exhibiting the initial behavioral change at or below a given carbon monoxide $CT^{0.3}$ value, and converting this number to a percentage of the total number of animals tested (n = 26). The data are presented in tabular form in Appendix 1. The percent of animals responding at a given dose hereafter will be equated with the probability of response at that same dose. If 80% of a test population responds at dose x, the risk or probability that any member of the population will response at dose x is also 80% or 0.8, provided the test population is an accurate sample of the entire population.

One-hit, Probit, and Weibull Models Fitted to Initial Behavioral Change Data

Figures 19, 20, and 21 represent respectively the best-fit curves of the one-hit, probit, and Weibull models to the quantal dose-response curve generated in Figure 18 from the initial behavioral change data. The best, least-squares fit of the one-hit and Weibull models was determined by the PARAFIT program. To prevent an overflow in the iterative program, it was necessary to divide the carbon

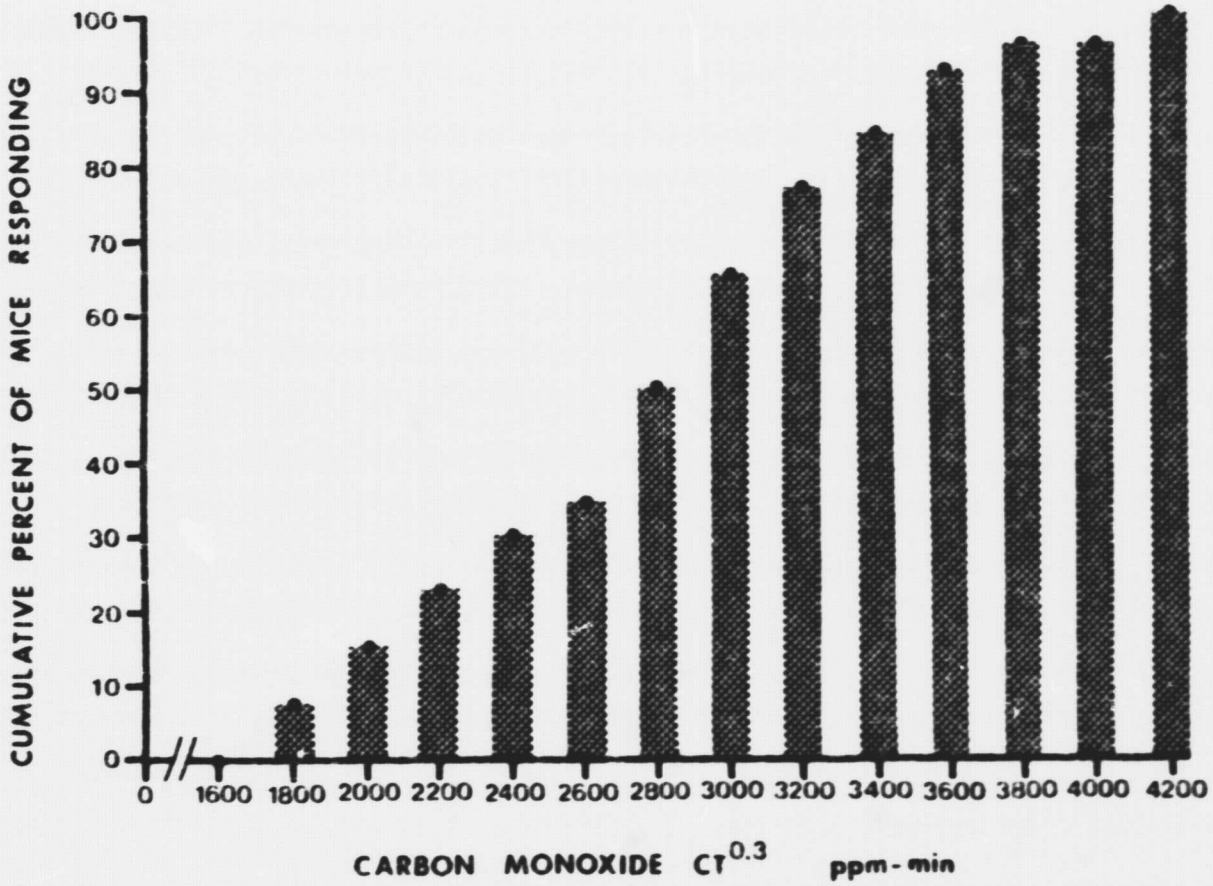
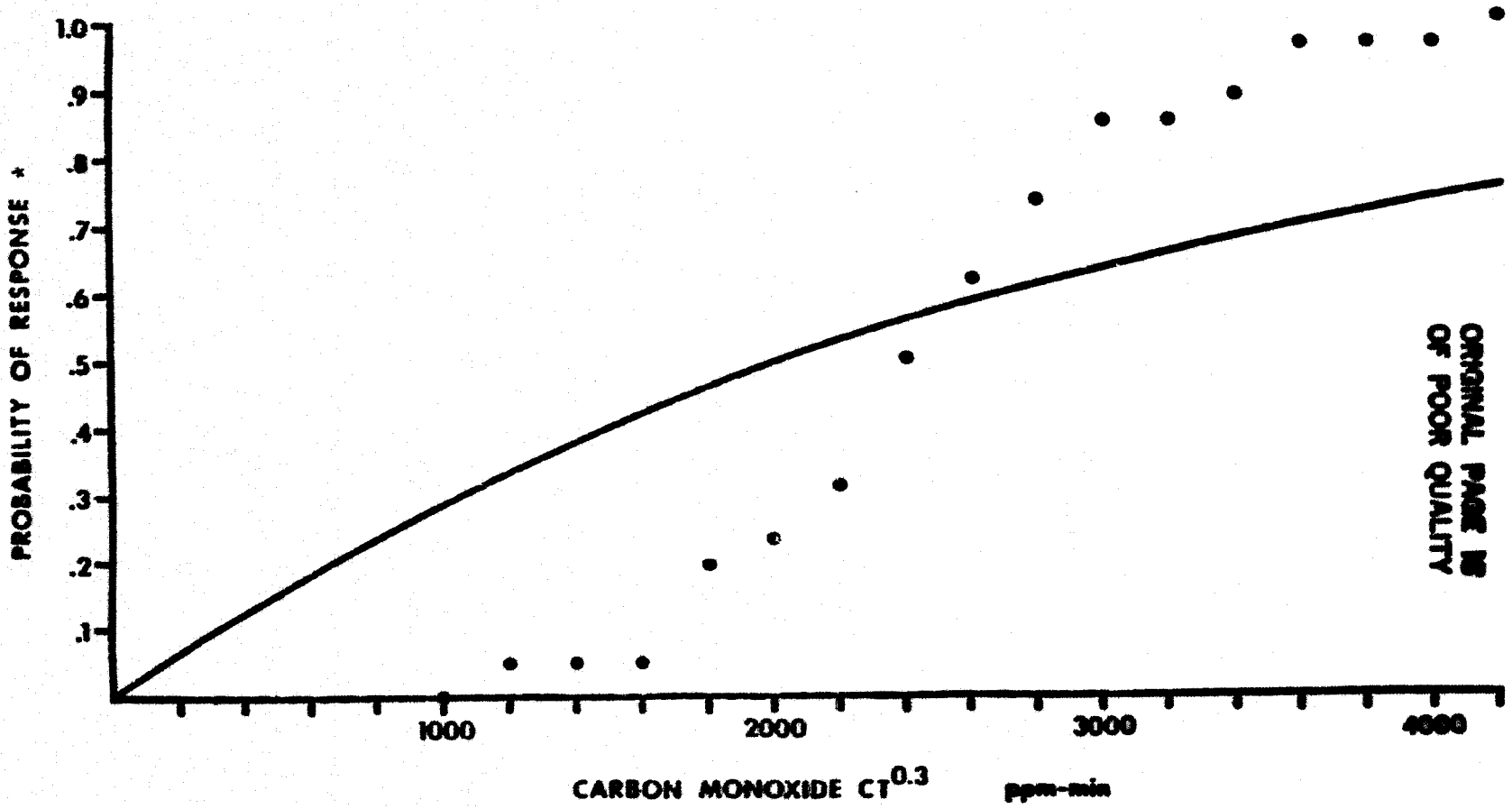


Figure 18

Quantal Dose-Response Curve for Initial Behavioral Change Data.

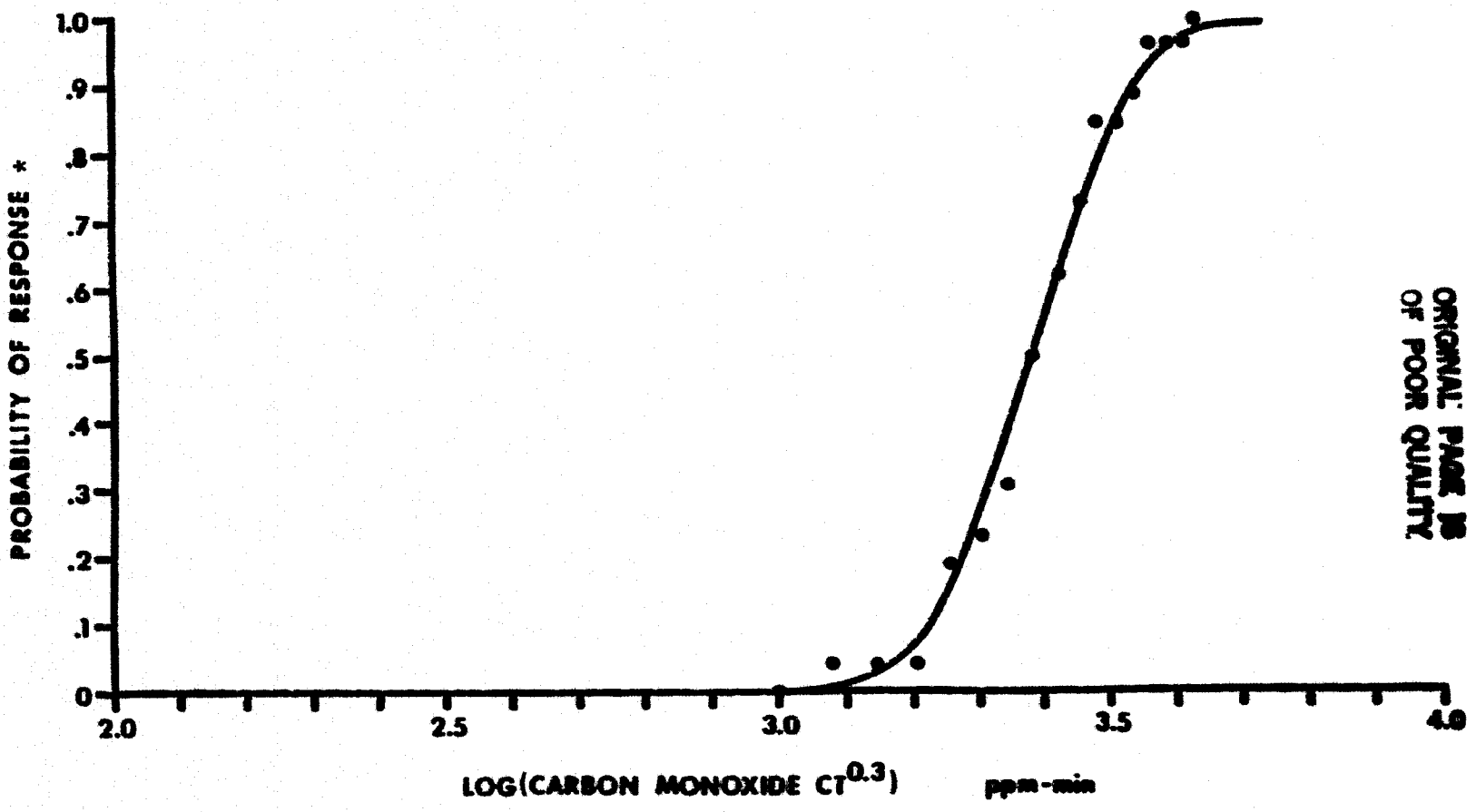
The quantal dose-response curve representing the summation of the frequency histogram in Figure 22 is shown. Every bar of Figure 22 is added to all preceding bars as the $CT^{0.3}$ increases in increments of 200 ppm-minutes. The total number of animals responding at or below a given $CT^{0.3}$ value, is plotted as a percent of total animals tested (n = 26). Original data compiled by Winslow (1981).



*The response is the initial behavioral change. Original data compiled by Winslow (1981).

Figure 19

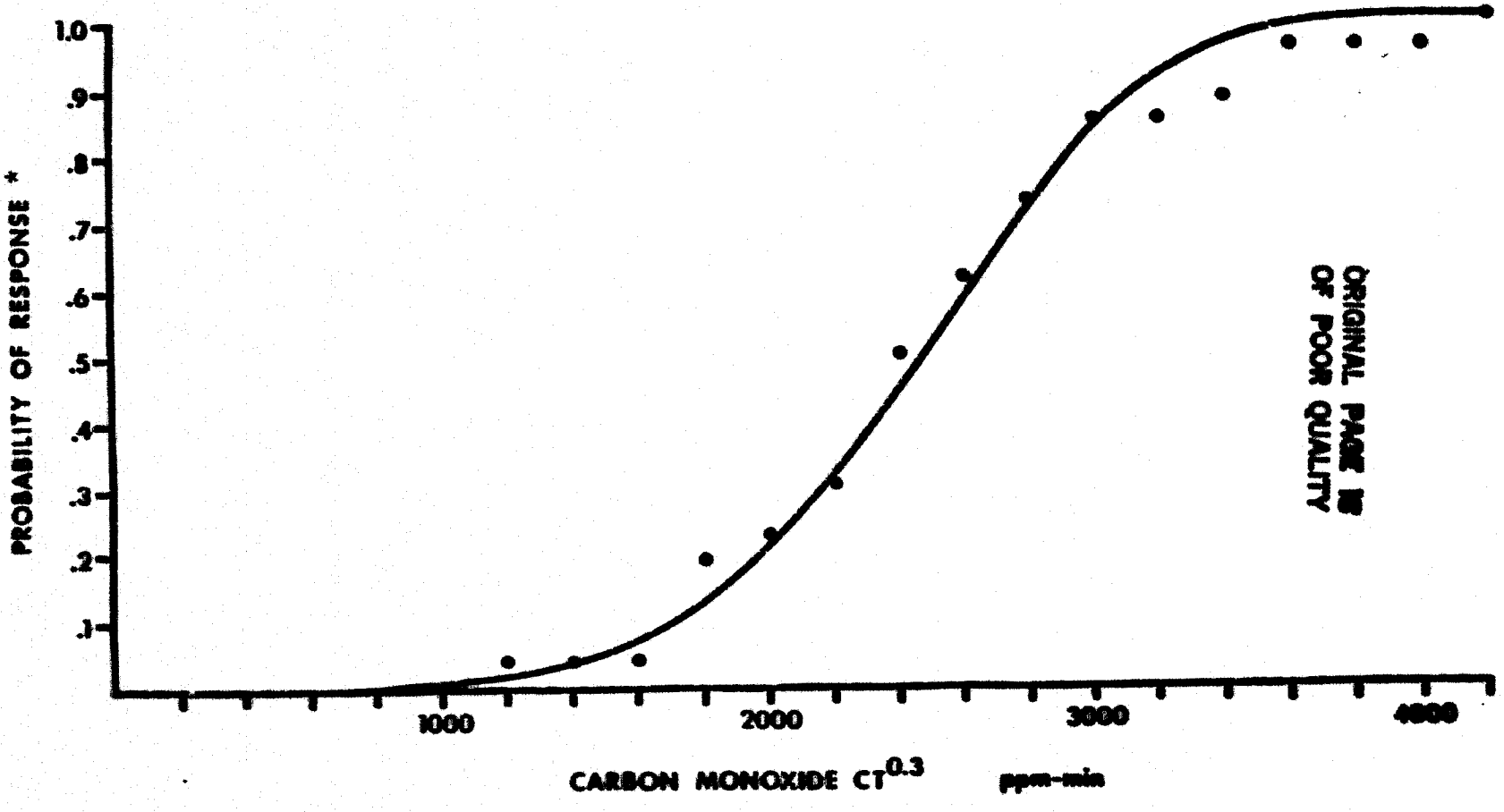
One-Hit Model Fitted to Initial Behavioral Change Data.



*The response is the initial behavioral change. Original data compiled by Winslow (1981).

Figure 20

Probit Model Fitted to Initial Behavioral Change Data.



*The response is the initial behavioral change. Original data compiled by Winslow (1981).

Figure 21

Weibull Model Fitted to Initial Behavioral Change Data.

monoxide $CT^{0.3}$ values by 1000 for these two models. The maximum likelihood fit of the probit model was determined by the method described by Bliss (1938). The calculations involved in the fitting of this probit line are included in Appendix 2.

The equations of the best-fit curves for the models are summarized in Table II. For each carbon monoxide $CT^{0.3}$ value associated with an observed probability of response from the initial behavioral change data (Appendix 1), an expected probability of response is calculated using these model equations. The expected probabilities of response as a function of carbon monoxide $CT^{0.3}$, for the one-hit, probit, and Weibull models are given in Appendix 3, 4, and 5. Differences between observed and expected probabilities of response were then analyzed for each model. The chi-square value, a measure of the goodness of fit of the model to the initial behavioral change data, is reported in column 3 of Table II.

The carbon monoxide $CT^{0.3}$ value associated with a probability of 1 in 10^6 of the initial behavioral response occurring, is determined by extrapolation. The value predicted by each of the models as the dose of CO corresponding to this very low risk, is listed in column 4 of Table II.

Quantal Dose Response Curve for the Loss of Escape

The data obtained by Winslow (1981) which relate loss of escape behavior as a function of carbon monoxide concentration and exposure duration are reprinted in Table III. Again, the $CT^{0.3}$ value provides a better description of the dose of CO, since the $CT^{0.3}$

TABLE II

Analysis of One-Hit, Probit, and Weibull Models
Fitted to Initial Behavioral Change Data[1]

Model	Equation of Best-Fit Curve	Chi Square	P value*	Carbon Monoxide $CT^{0.3}$ Associated with Probability of Response of 10^{-6}
One-Hit	$P(D) = 1 - EXP\left[-0.3336(D/1000)\right]$	210.36	$p < 0.001$	0.003 ppm-minutes
Probit	$P(D) = \Phi\ (-22.513 + 8.152\ LOG\ D)$	18.16	$0.30 < p < 0.50$	578 ppm-minutes
Weibull	$P(D) = 1 - EXP-\left[-0.00174 + 0.00769(D/1000)^{4.993}\right]$	10.09	$0.80 < p < 0.90$	742 ppm-minutes

[1]Original data compiled by Winslow (1981)

*The P value is the probability of obtaining this value of chi square or greater, if the underlying distribution of the observed curve is the expected curve. Probabilities of 0.05 or larger are generally considered to indicate a satisfactory fit. The greater the P value, the better the fit (Croxton, 1959). The number of degrees of freedom is 16.

TABLE III

Summary of Motivated Behavior Changes Observed During Acute CO Exposures [1]

Experiment #	Loss of Escape CT (ppm-min)	Time to Loss of Escape (min)	Average [CO] at Loss of Escape (ppm)	Loss of Escape $CT^{0.3}$ (ppm-min)
M-24	7203	2.2	3274	4148
OB-3	11913	5.5	2166	3612
M-19	11866	5.5	2157	3597
OB-9	12924	6.5	1988	3486
OB-5	14387	8.2	1755	3299
M-20	11187	5.8	1929	3269
M-21	29286	24.0	1220	3165
OB-2	13361	7.9	1691	3144
M-12	9079	4.6	1974	3120
OB-7	15267	10.3	1482	2983
OB-6	9503	5.3	1793	2957
M-18	10428	6.4	1629	2843
OB-8	13820	9.7	1425	2817
M-16	20138	17.1	1178	2761
M-8	13241	9.6	1379	2718
M-11	20497	18.6	1102	2649
M-17	11347	8.2	1384	2602
M-15	22074	23.2	951	2442
M-6	9500	7.2	1319	2385
M-5	9000	6.9	1304	2328
M-7	3925	2.5	1570	2067
OB-1	14915	17.0	877	2052
M-13	15891	19.7	807	1973
M-22	5433	4.5	1207	1895
M-23	6832	6.8	1005	1786
M-14	9349	11.7	799	1671
*				

[1] Data reprinted from Winslow, 1981.

* Experiment #M-25 and M-26 were omitted since times to initial behavioral change and to loss of escape were not consistently reported, precluding the determination of $CT^{0.3}$ values.

values corresponding to loss of escape are more nearly constant than are the CT values.

Figure 22 represents the frequency histogram derived from the data in Table III. The number of mice displaying the quantal response, loss of escape, for each successive carbon monoxide $CT^{0.3}$ increment of 200 ppm-minutes is plotted. The quantal dose-response curve resulting from the cumulative summation of this frequency histogram is shown in Figure 23. The data points of Figure 23 appear in tabular form in Appendix 6.

One-Hit, Probit, and Weibull Models Fitted to Loss of Escape Data

The best-fit curves of the one-hit, probit, and Weibull models applied to the loss of escape data (Figure 23), are graphed in Figures 24, 25, and 26. The parameters of the one-hit and Weibull models were determined using the PARAFIT program; to prevent program overflow, the $CT^{0.3}$ values for CO were divided by 1000 before fitting these functions. The probit model was fitted by means of a maximum likelihood estimation procedure suggested by Bliss (1938); the calculations required by this method are listed in Appendix 7.

The equations which best describe the best-fit curve for each model are given in Table IV. The expected possibilities of response, calculated from the model curve equations as a function of carbon monoxide $CT^{0.3}$, are given for the three models in Appendix 8, 9, and 10. The chi-square values, indicating the goodness of fit of the model curves to the loss of escape observations are included in Table

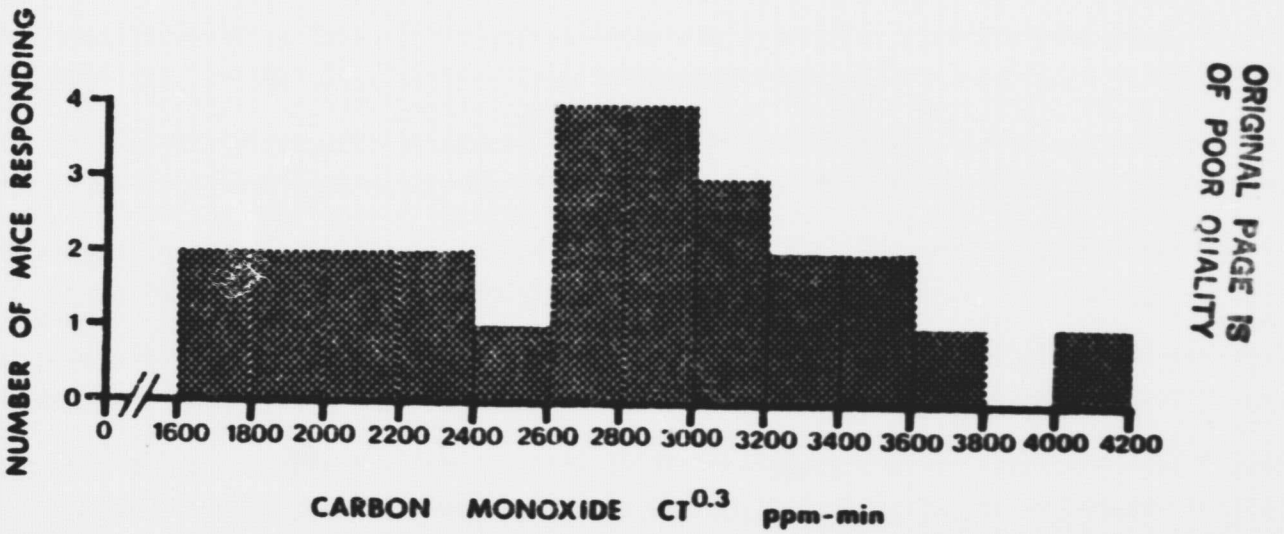


Figure 22

Relationship between Carbon Monoxide $CT^{0.3}$ and Loss of Escape Response

The frequency histogram showing the relationship between the dose of carbon monoxide ($CT^{0.3}$) and loss of the escape response is shown. Mice were continuously exposed to various concentrations of CO until loss of escape behavior occurred. The $CT^{0.3}$ value at which each animal responded is recorded in Table II. All animals responding between $CT^{0.3}$ increments of 200 ppm-minutes were grouped together. Original data compiled by Winslow (1981).

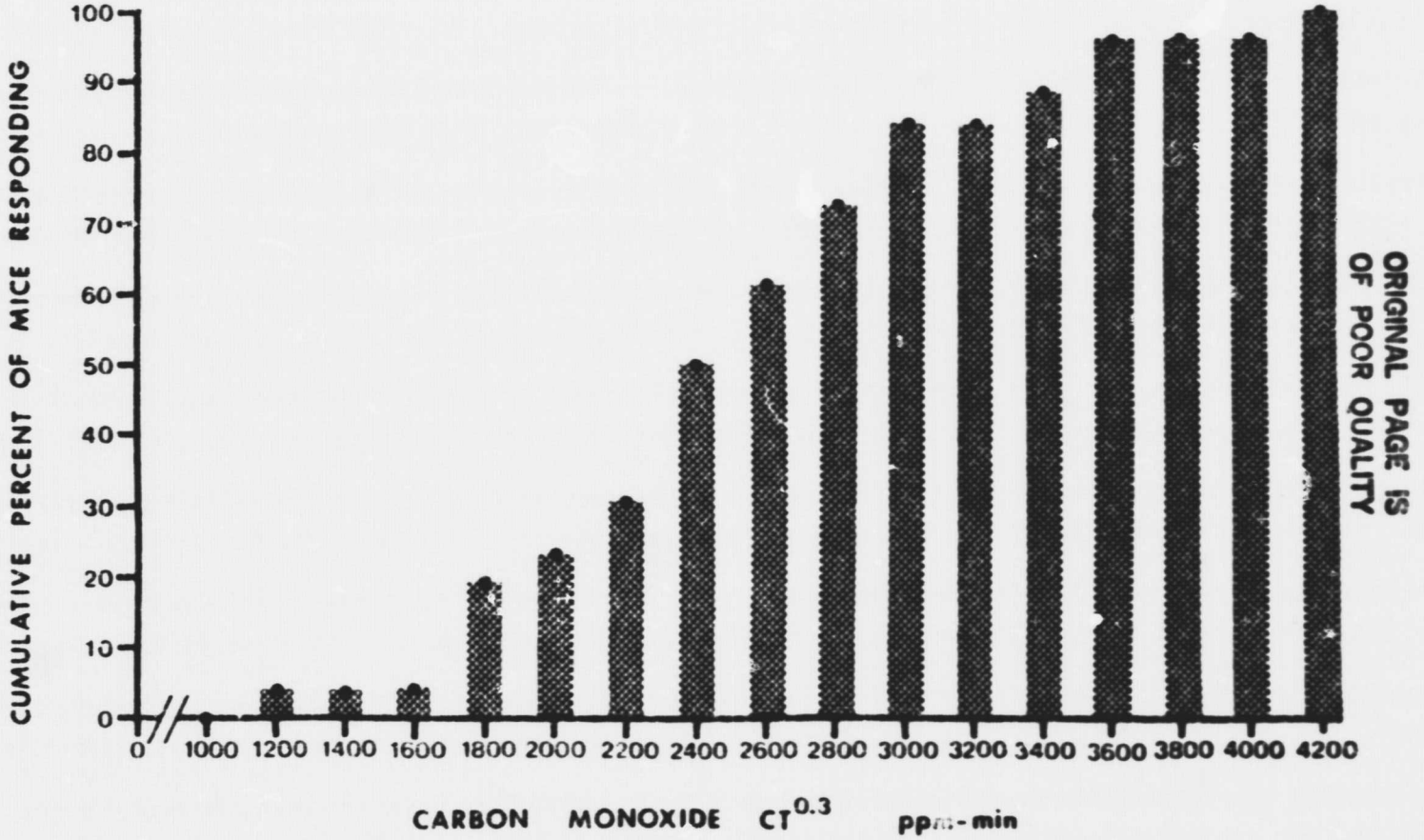
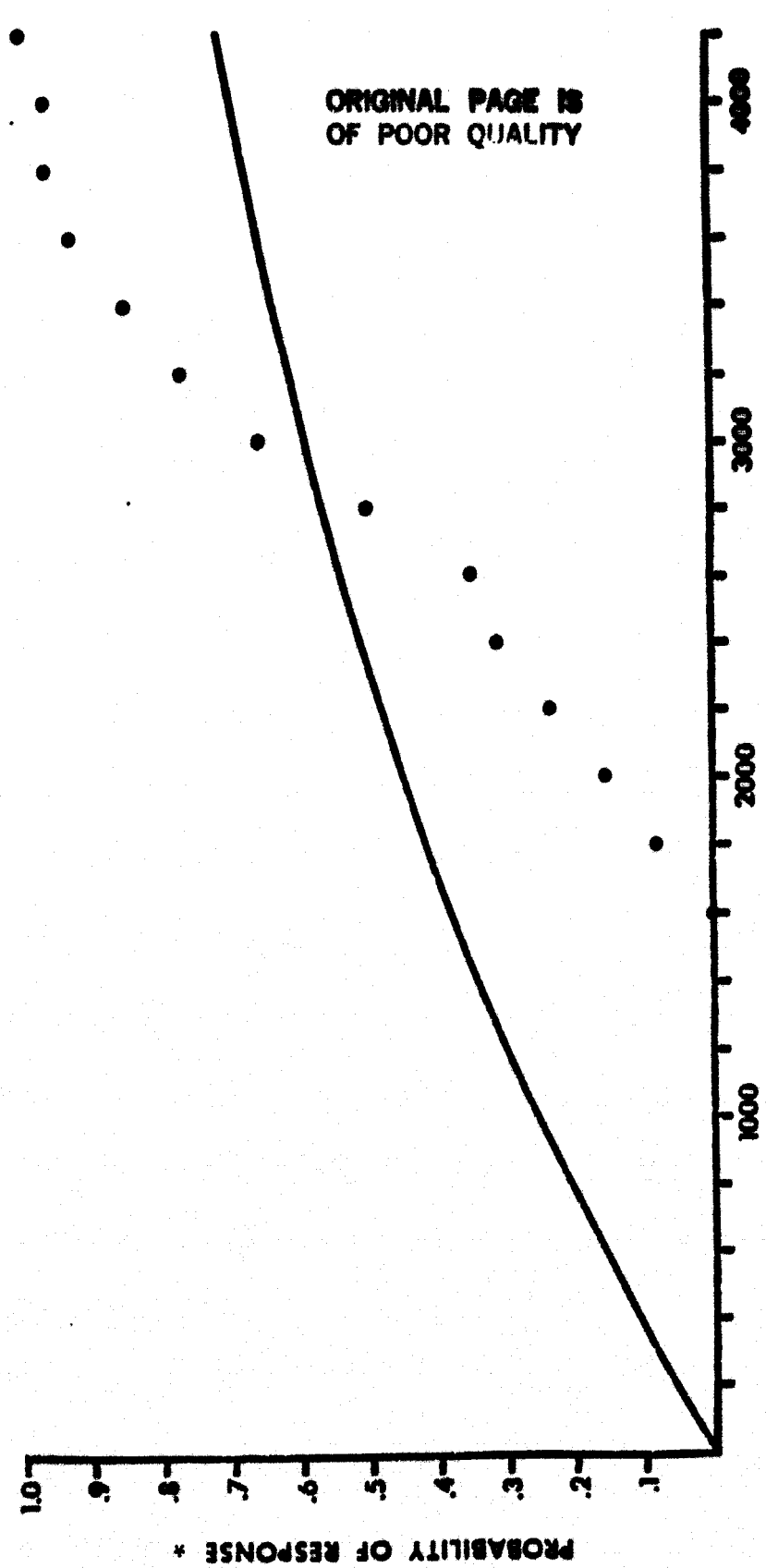


Figure 23

Quantal Dose-Response Curve for Loss of Escape Data

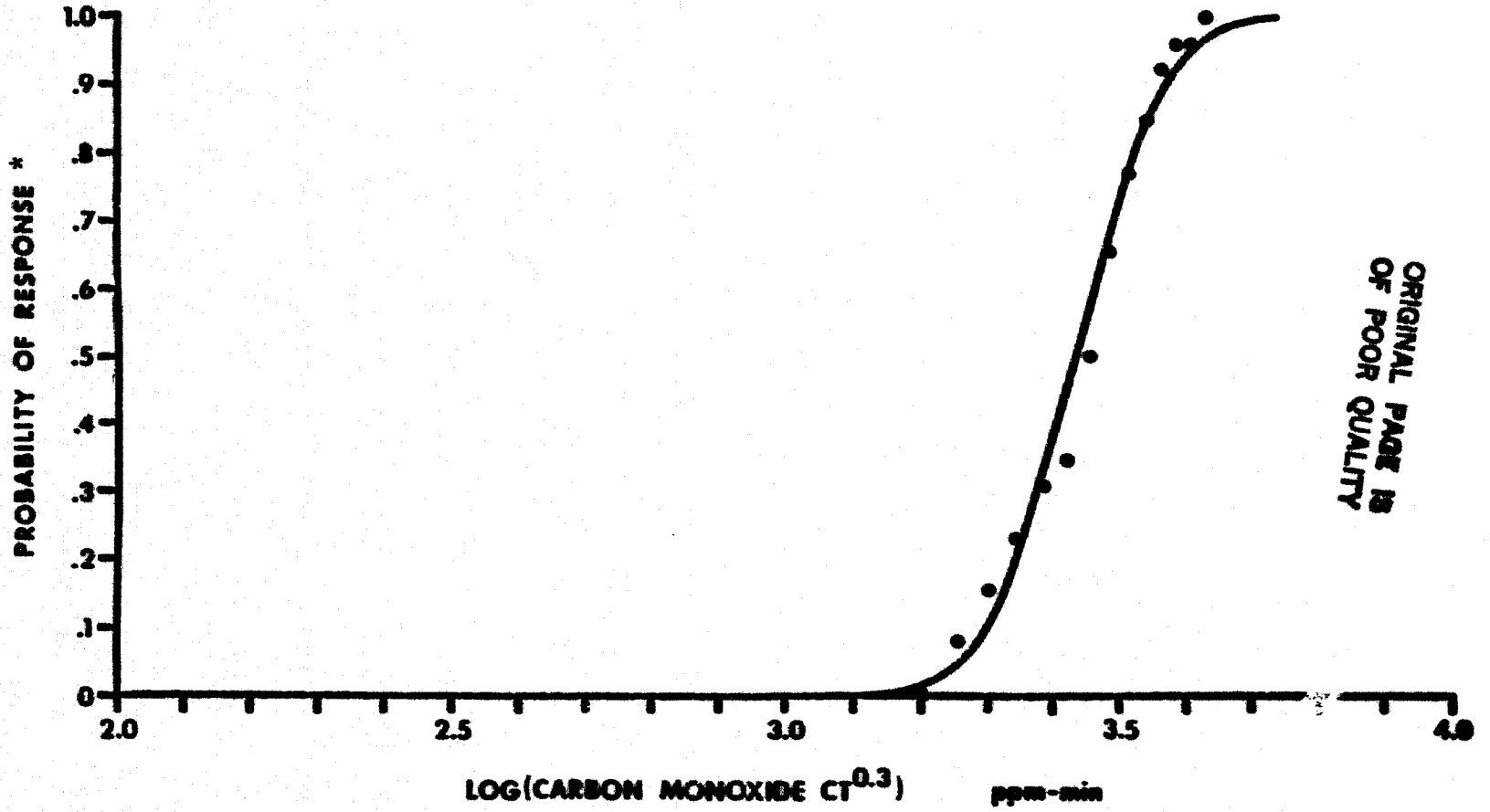
The quantal dose-response curve representing the summation of the frequency histogram in Figure 17 is shown. Every bar of Figure 17 is added to all prededing bars as the $CT^{0.3}$ increases in increments of 200 ppm-minutes. The total number of animals responding at or below a given $CT^{0.3}$ value, is plotted as a percent of total animals tested (n = 26). Original data compiled by Winslow (1981).



*The response is loss of escape. Original data compiled by Winslow (1981).

Figure 24

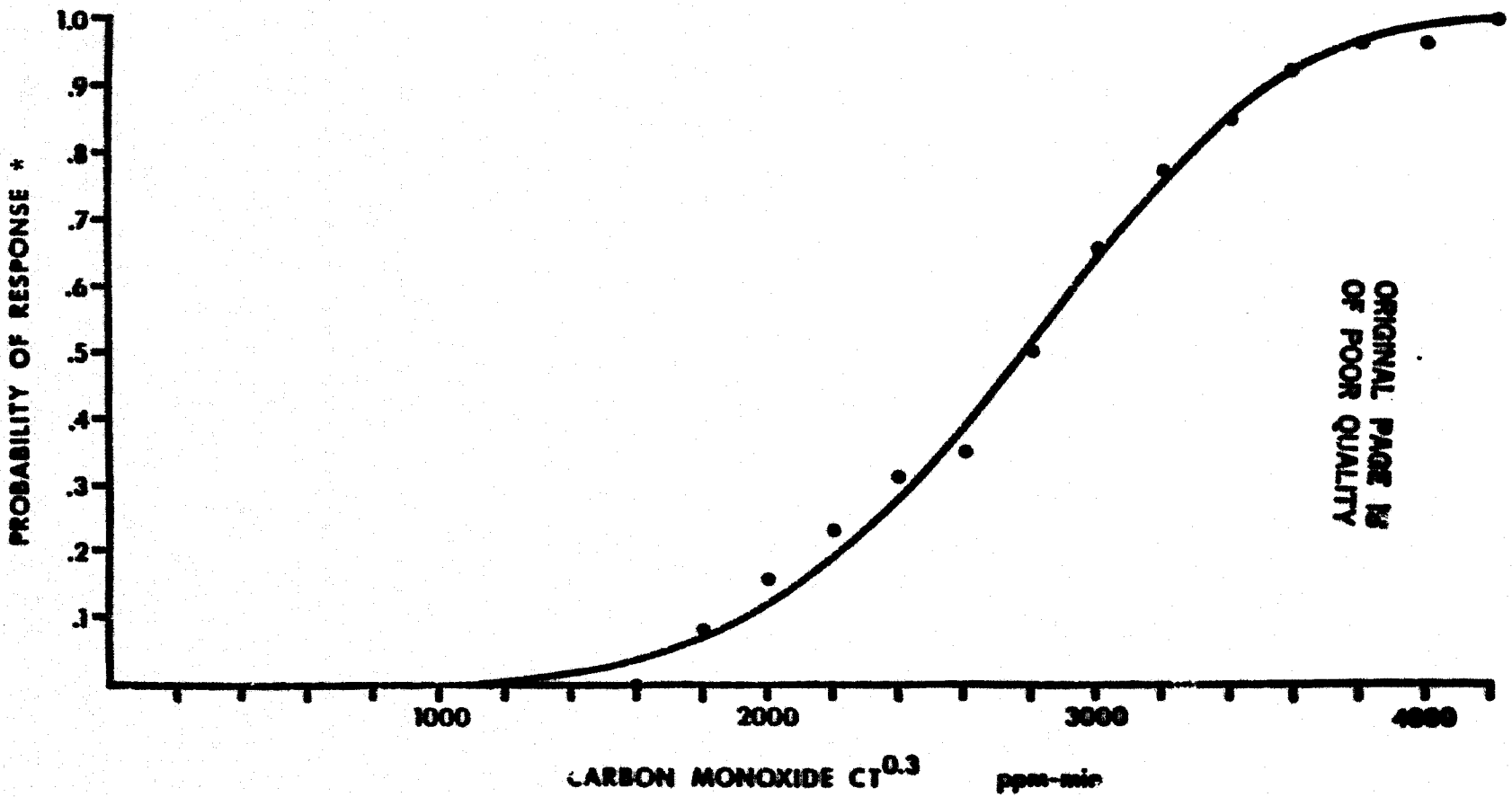
One-Hit Model Fitted to Loss of Escape Data.



*The response is loss of escape. Original data compiled by Winslow (1981).

Figure 25

Probit Model Fitted to Loss of Escape Data.



*The response is loss of escape. Original data compiled by Winslow (1981).

Figure 26

Weibull Model Fitted to Loss of Escape Data.

TABLE IV

Analysis of One-Hit, Probit, and Weibull Models
Fitted to Loss of Escape Data[1]

Model	Equation of Best-Fit Curve	Chi Square	P value*	Carbon Monoxide $CT^{0.3}$ Associated with Probability of Response of 10^{-6}
One-Hit	$P(D) = 1 - EXP[-0.2968(D/1000)]$	169.32	$p < 0.001$	0.003 ppm-minutes
Probit	$P(D) = \Phi\ (-28.179 + 9.683\ LOG\ D)$	9.48	$0.70 < p < 0.75$	813 ppm-minutes
Weibull	$P(D) = 1 - EXP-[-0.00856 + 0.00441(D/1000)^{4.969}]$	6.27	$0.90 < p < 0.95$	1143 ppm-minutes

[1]Original data compiled by Winslow (1981)

*The P value is the probability of obtaining this value of chi square or greater, if the underlying distribution of the observed curve is the expected curve. Probabilities of 0.05 or larger are generally considered to indicate a satisfactory fit. The greater the P value, the better the fit (Croxton, 1959). The number of degrees of freedom is 13.

IV. The table also lists the extrapolated values of the dose of CO at which a very low risk (1 in 10^6) of loss of escape is expected.

Rat Physiological Data

Effects of CO on Serum Enzyme Activities: Graded Response

Table V summarizes the mean serum activity levels of lactate dehydrogenase (LDH), alpha-hydroxybutyrate dehydrogenase (HBDH), and creatine phospnokinase (CPK), in rats two hours after 20-minute exposures to various concentrations of CO in air. The control group of rats consists of seven previously untreated rats and seven sham-treated rats. Sham-treated rats were restrained in the animal exposure chamber for 20 minutes, during which time they inhaled room air. No statistically significant difference was observed between mean enzyme levels of untreated and sham-treated rats (two-tailed T test, 95% confidence level).

Figures 27, 28, and 29 represent the graded response curves generated from the data in Table V. The intensity of response (the amount of enzyme present in serum) varies as a function of the dose of CO, expressed as a $CT^{0.3}$ value. The $CT^{0.3}$ relationship was chosen to express the dose of CO in order to maintain continuity with the mouse behavioral data. However, for the purpose of measuring the physiological responses of rats to CO, the exposure duration was always 20 minutes; since T is constant, $CT^{0.3}$ is directly proportional to CT.

TABLE V

Summary of acute effects of CO on serum enzyme activities

Experiment	CO Exposure CT (ppm-min)	Exposure Duration T (min)	Average CO Concentration C (ppm)	CO Exposure $CT^{0.3}$ (ppm-min)	Mean Serum Enzyme Activity ± 1 S.D. (mIU*/ml serum)		
					LDH	HBDH	CPK
Control	0	20	0	0	23.6 ± 11.6	23.7 ± 11.2	61.3 ± 22.1
C-4	24,032	20	1202	2.95×10^3	41.9 ± 17.3	46.7 ± 18.7	146.4 ± 129.4
C-5	52,253	20	2613	6.42×10^3	85.3 ± 53.3	95.9 ± 47.8	413.7 ± 333.3
C-6	83,953	20	4198	10.31×10^3	106.7 ± 52.3	101.7 ± 35.7	648.8 ± 496.7
C-1, C-2	101,425	20	5071	12.46×10^3	159.2 ± 95.5	185.2 ± 82.5	723.0 ± 292.6

* International Unit = the amount of enzyme which converts one micromole of substrate in one minute under standard conditions

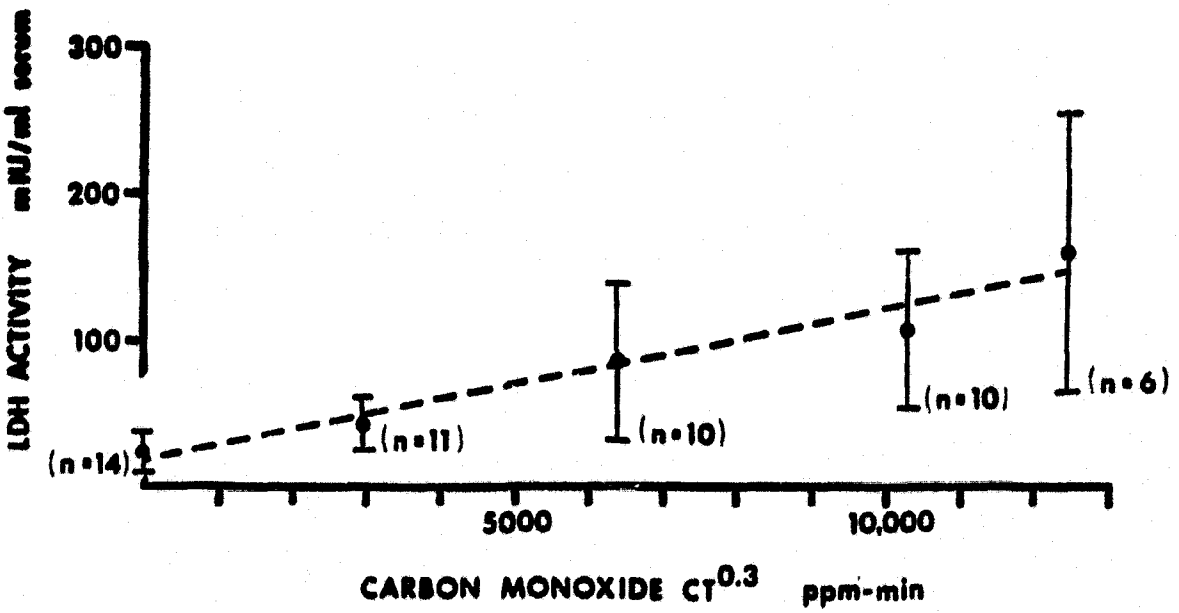


Figure 27

Graded Response Curve for Serum LDH Activity as a Function of Carbon Monoxide $CT^{0.3}$

Serum lactate dehydrogenase activity in rats, two hours after a 20 minute exposure to various concentrations of CO in air. Values plotted are mean $\pm$ 1 standard deviation; n = number of rats in sample.

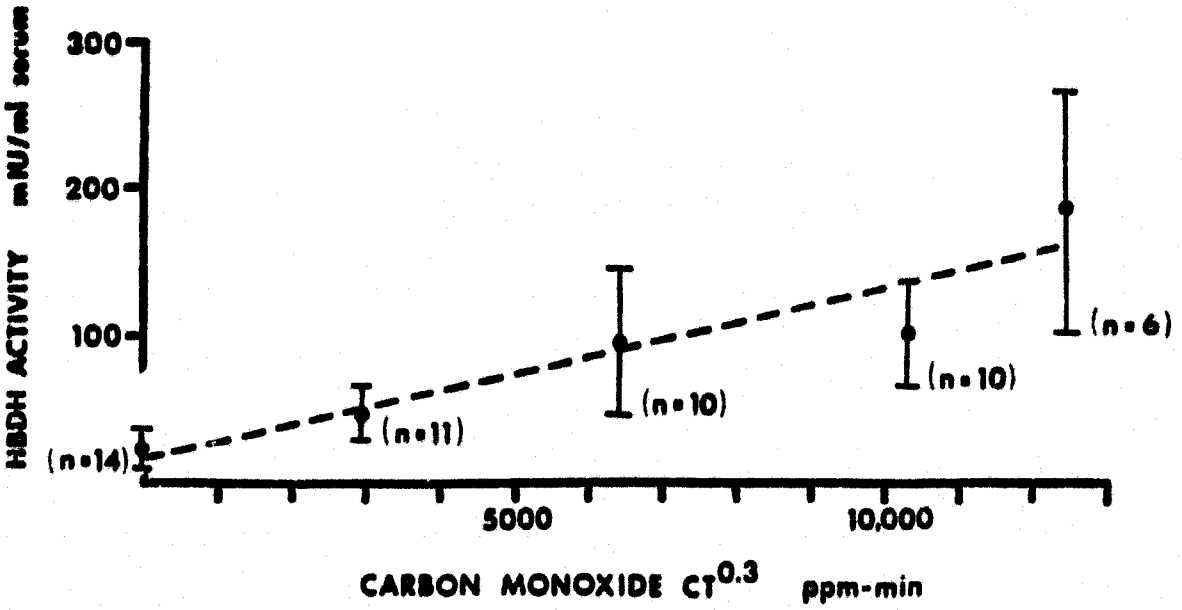


Figure 28

Graded Response Curve for Serum HBDH Activity as a Function of Carbon Monoxide $CT^{0.3}$

Serum α-hydroxybutyrate dehydrogenase activity in rats, two hours after a 20 minute exposure to various concentrations of CO in air. Values plotted are mean $\pm$ 1 standard deviation; n = number of rats in sample.

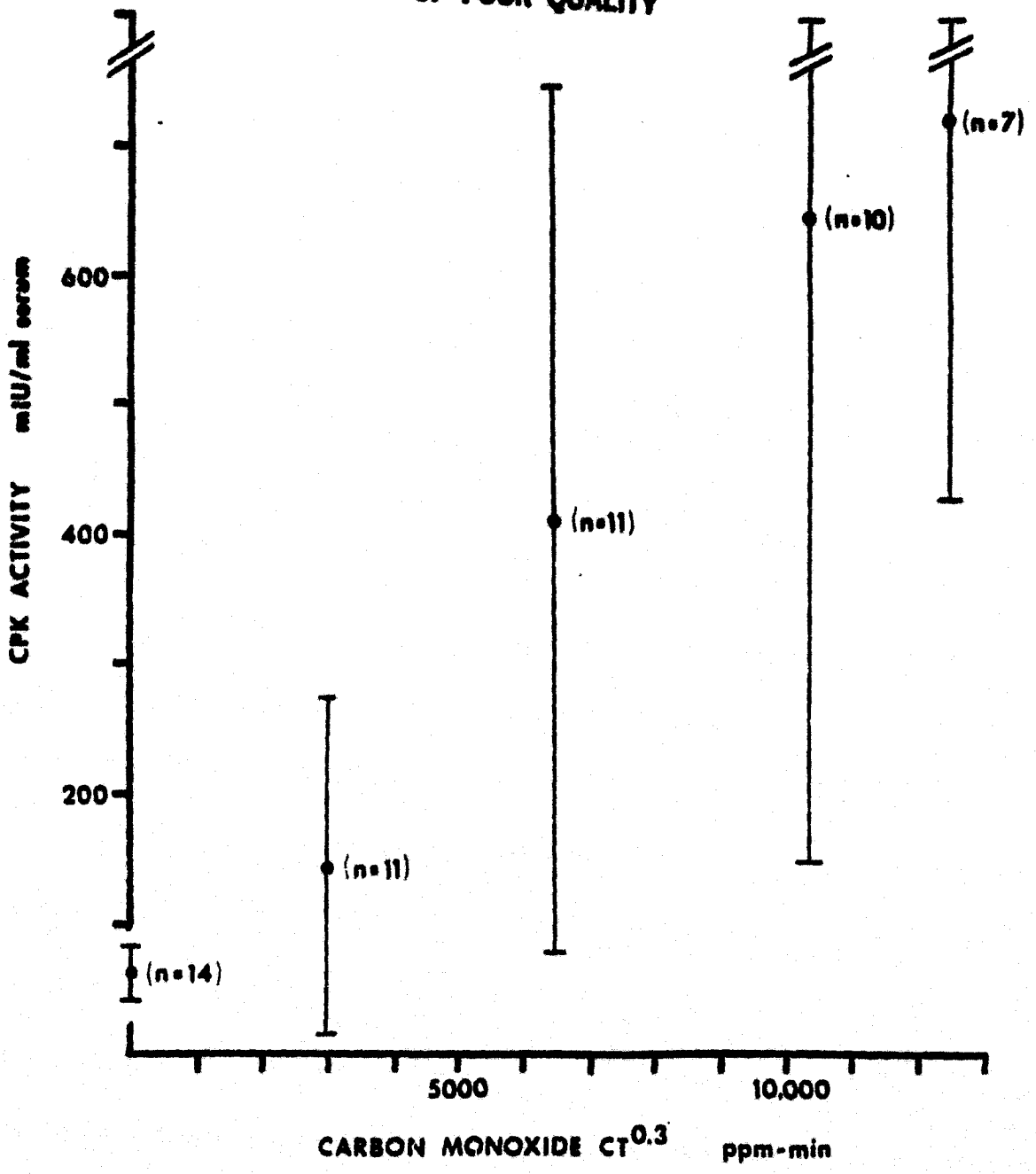


Figure 29

Graded Response Curve for Serum CPK Activity as a Function of Carbon Monoxide $CT^{0.3}$

Serum creatine phosphokinase activity in rats, two hours after a 20 minute exposure to various concentrations of CO in air. Values plotted are mean $\pm$ 1 standard deviation; n = number of rats in sample.

Effects of CO on Serum Enzyme Activities: Quantal Response

In order to transform the graded response curves of Figures 27, 28, and 29 into quantal dose-response relationships, a response criteria was established. The criteria chosen was an increase in serum enzyme activity greater than or equal to the observed mean control value + three standard deviations (Table V). Thus, an individual rat would have shown a response to CO if it had a post-exposure, serum enzyme activity greater than or equal to a value of 58.4 mIU/ml for LDH, 57.3 mIU/ml for HBDH, or 127.6 mIU/ml for CPK. The reasons for this choice of criteria are the following. Presumably, differences in serum enzyme activities of control rats are the result of normal biological variability and not profound differences in health. The range of serum enzyme activities observed in the control sample will thus be defined as normal, and values outside this range will be considered a significant response. If serum enzyme activities in the healthy rat population are distributed normally, then only 0.2% of the means of equal-sized samples from this population would be expected to have enzyme activities which fall outside the range of values given by the mean $\pm$ three standard deviations. Also, none of the observed serum enzyme levels of individuals in the control sample fell outside the range designated by the mean $\pm$ three standard deviations; this was not the case if the mean $\pm$ one standard deviation or the mean $\pm$ two standard deviations was substituted for the response criteria.

Tables VI, VII, and VIII summarize the results of quantal analysis of the serum enzyme data using the response criteria. The enzyme data is now expressed in terms of the percent of the population responding,

TABLE VI

Quantal Analysis of Increases in Serum LDH Activity
Occurring in Response to Carbon Monoxide

Experiment	Carbon Monoxide $CT^{0.3}$ (ppm-min)	Number of Rats responding* / Number of rats tested	Probability of Response
C-4	2.95×10^3	3/11	0.273
C-5	6.42×10^3	7/10	0.700
C-6	10.31×10^3	9/10	0.900
C-1, C-2	12.46×10^3	6/6	1.000

* Response is defined as a serum LDH activity greater than or equal to the mean control value + 3 standard deviations

TABLE VII

Quantal Analysis of Increases in Serum HBDH Activity
Occurring in Response to Carbon Monoxide

Experiment	Carbon Monoxide $CT^{0.3}$ (ppm-min)	Number of rats responding* / Number of rats tested	Probability of response
C-4	2.95×10^3	3/11	0.273
C-5	6.42×10^3	9/10	0.900
C-6	10.31×10^3	10/10	1.000
C-1, C-2	12.46×10^3	6/6	1.000

* Response is defined as a serum HBDH activity greater than or equal to the mean control value + 3 standard deviations

TABLE VIII

Quantal Analysis of Increases in Serum CPK Activity
Occurring in Response to Carbon Monoxide

Experiment	Carbon Monoxide $CT^{0.3}$ (ppm-min)	Number of rats responding* / Number of rats tested	Probability of response
C-4	2.95×10^3	4/11	0.364
C-5	6.42×10^3	11/11	1.000
C-6	10.31×10^3	10/10	1.000
C-1, C-2	12.46×10^3	7/7	1.000

* Response is defined as a serum CPK activity greater than or equal to the mean control value + 3 standard deviations

or the probability of response, as a function of the carbon monoxide $CT^{0.3}$, and can thus be applied to the theoretical, dose-response models.

One-Hit, Probit, and Weibull Models Fitted to Serum Enzyme Data

The best-fit curves of the one-hit, probit, and Weibull models to the quantal dose-response data for serum LDH (Table VI) are plotted in Figures 30, 31, and 32, respectively. Figures 33, 34, and 35 represent the best-fit curves of these functions to the quantal dose-response data for HBDH (Table VII). However, it did not seem appropriate to apply these models to the quantal data for CPK (Table VIII). With only one intermediate response rate observed (all other response rates were 100%), the error in estimating the critical parameters in the model equations is potentially very large.

The equations which describe the best-fit curve of each model to the quantal enzyme data are given in Table IX for LDH, and in Table X for HBDH. Tables IX and X provide a summary of the goodness of fit of each model, and the predicted carbon monoxide $CT^{0.3}$ expected to produce a response rate of 1 in 10^6. Values of expected versus observed response rates for each model fitted to both LDH and HBDH data, are given in Appendix 11, 12, 13, 14, 15, and 16. Calculations involved in the fitting of the probit model to the LDH and HBDH data are found in Appendix 17 and 18.

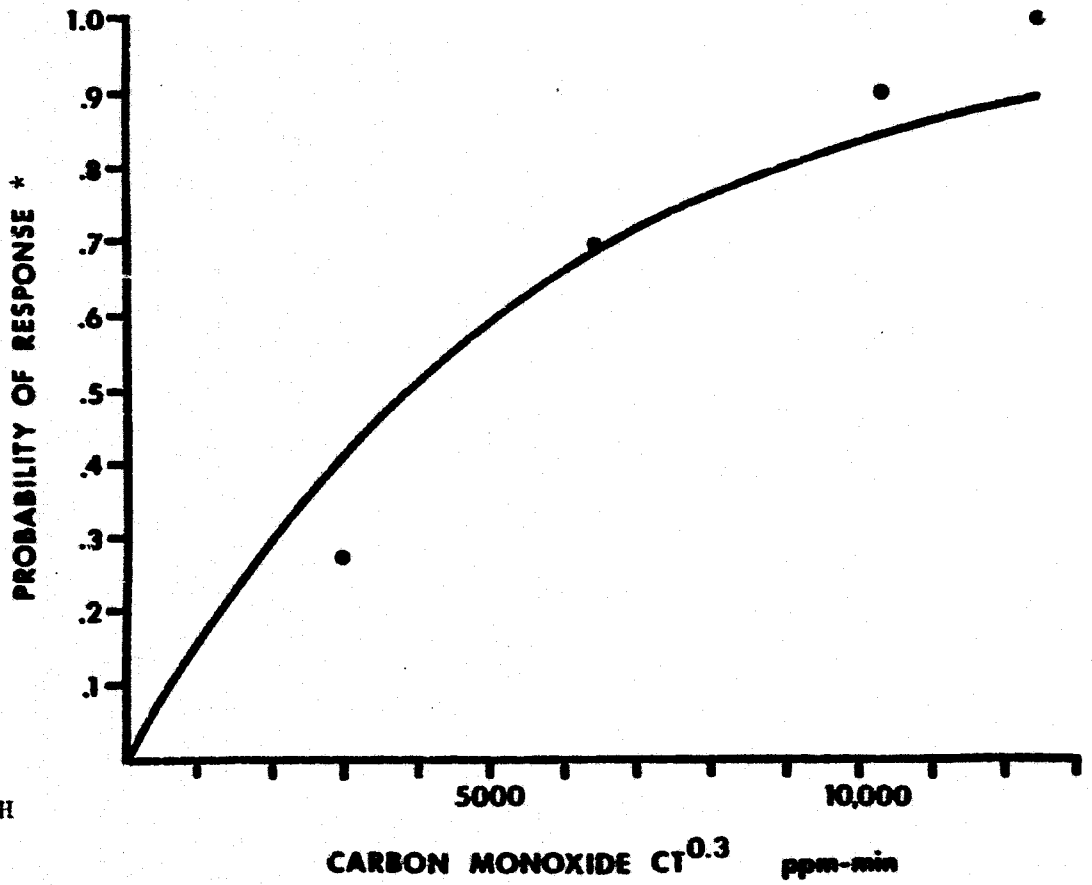
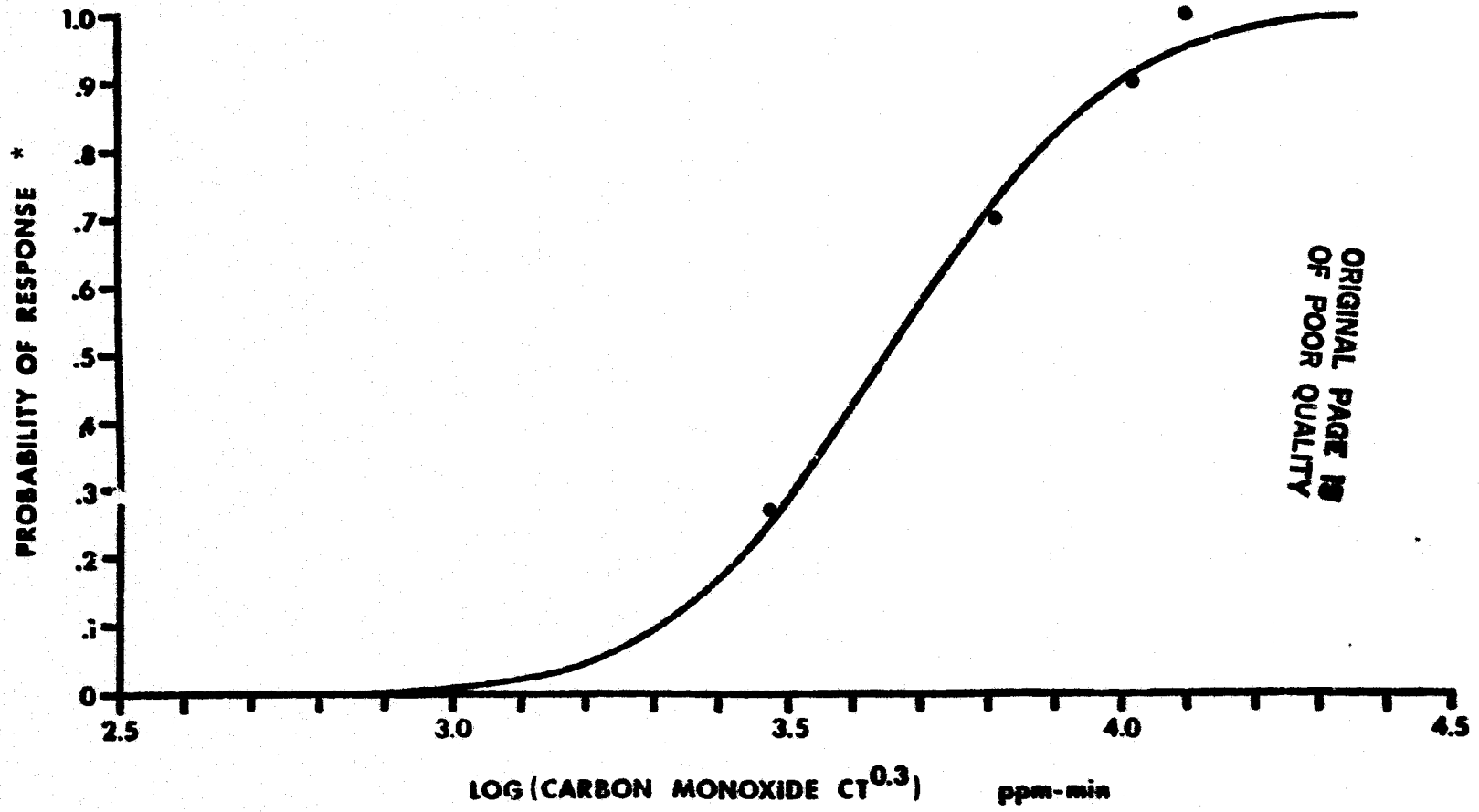


Figure 30

One-Hit Model Fitted to LDH Serum Enzyme Data.

*The response is an LDH activity ≥ mean control value + 3 S.D.



*The response is an LDH activity $\geq$ mean control value + 3 S.D.

Figure 31

Probit Model Fitted to LDH Serum Enzyme Data.

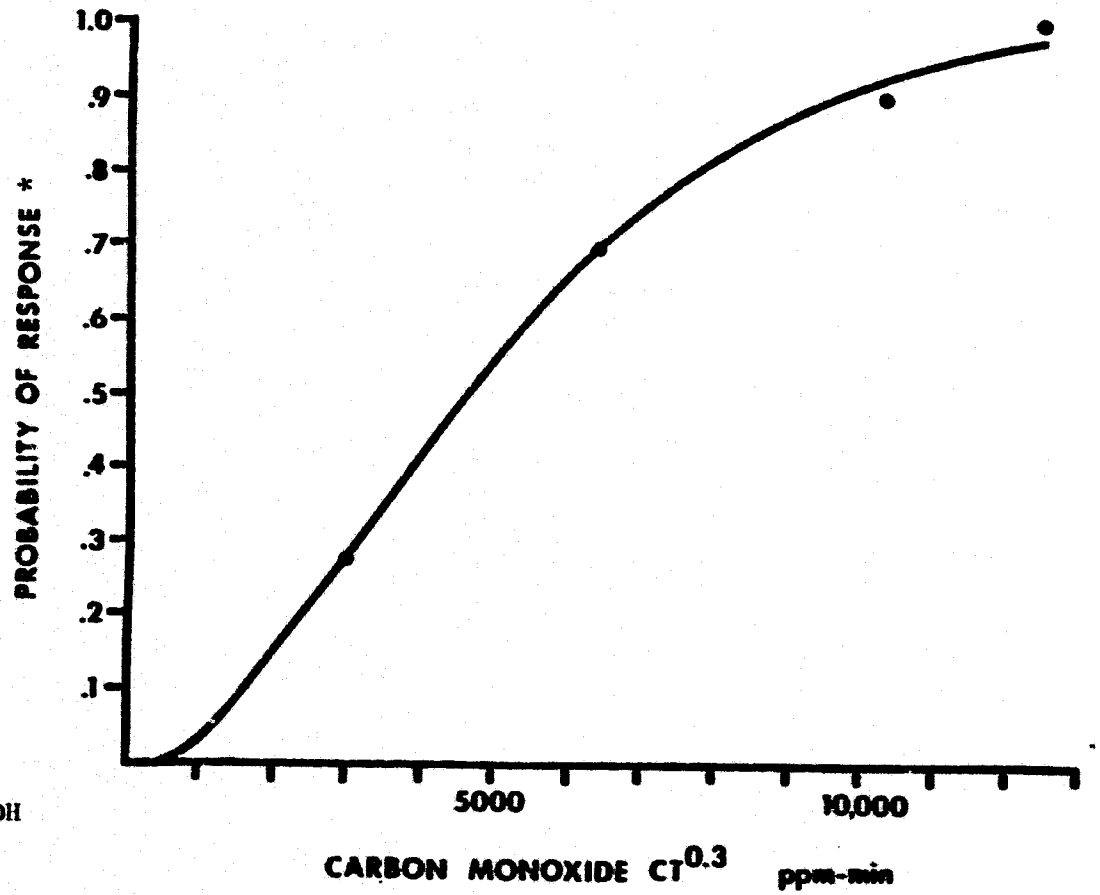


Figure 32

Weibull Model Fitted to LDH Serum Enzyme Data.

*The response is an LDH activity ≥ mean control value + 3 S.D.

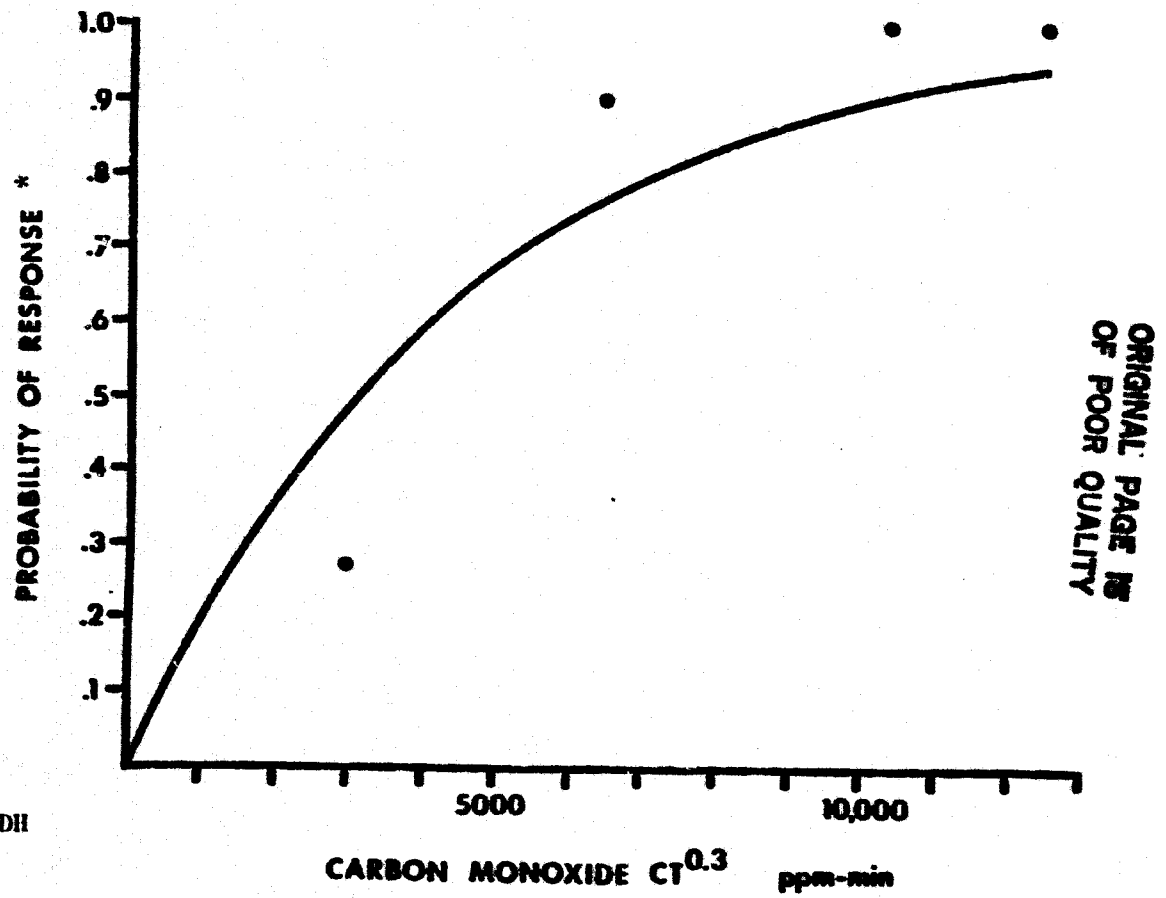
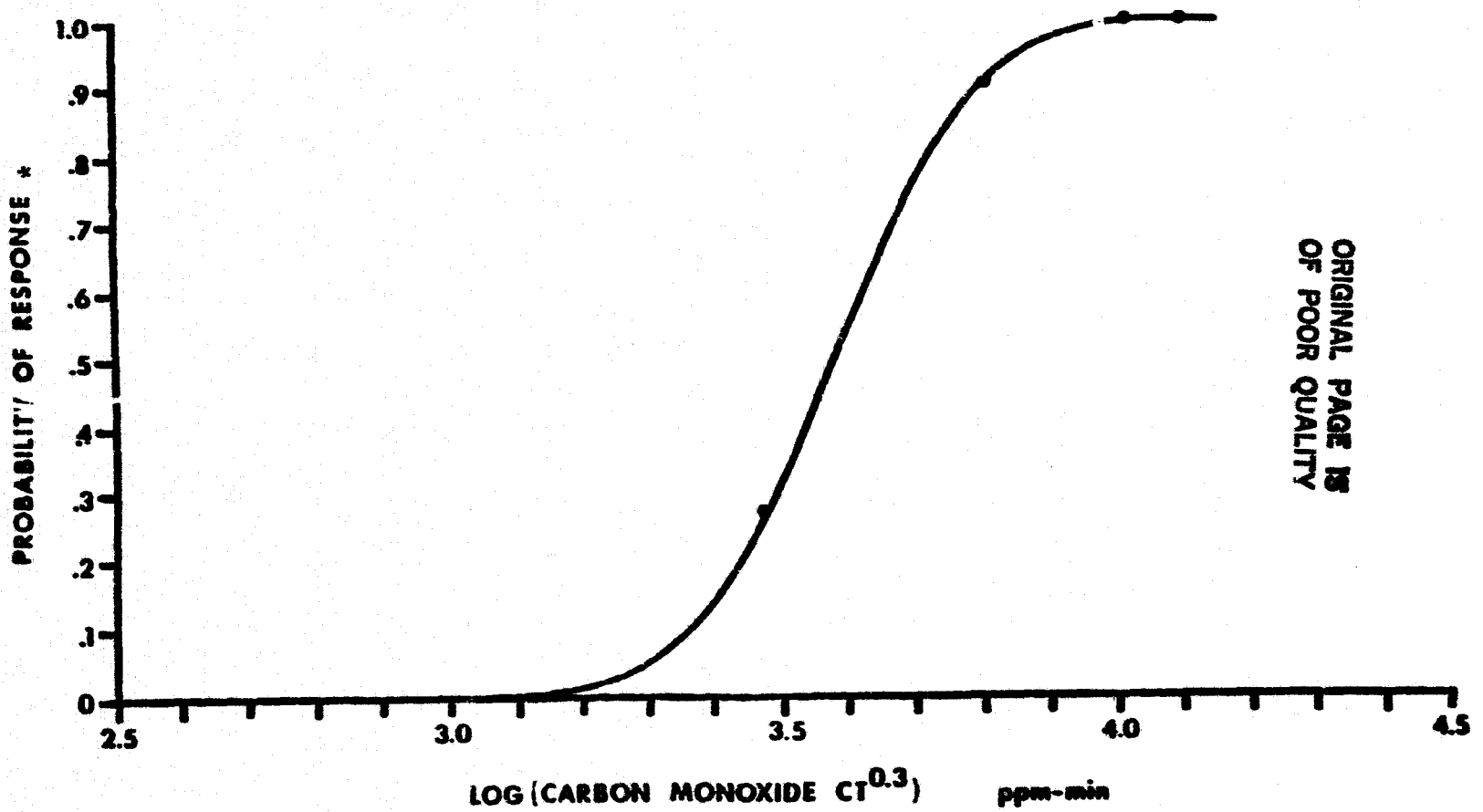


Figure 33

One-Hit Model Fitted to HBDH Serum Enzyme Data.

*The response is an HBDH activity $\geq$ mean control value + 3 S.D.



*The response is an HBDH activity $\geq$ mean control value + 3 S.D.

Figure 34

Probit Model Fitted to HBDH Serum Enzyme Data

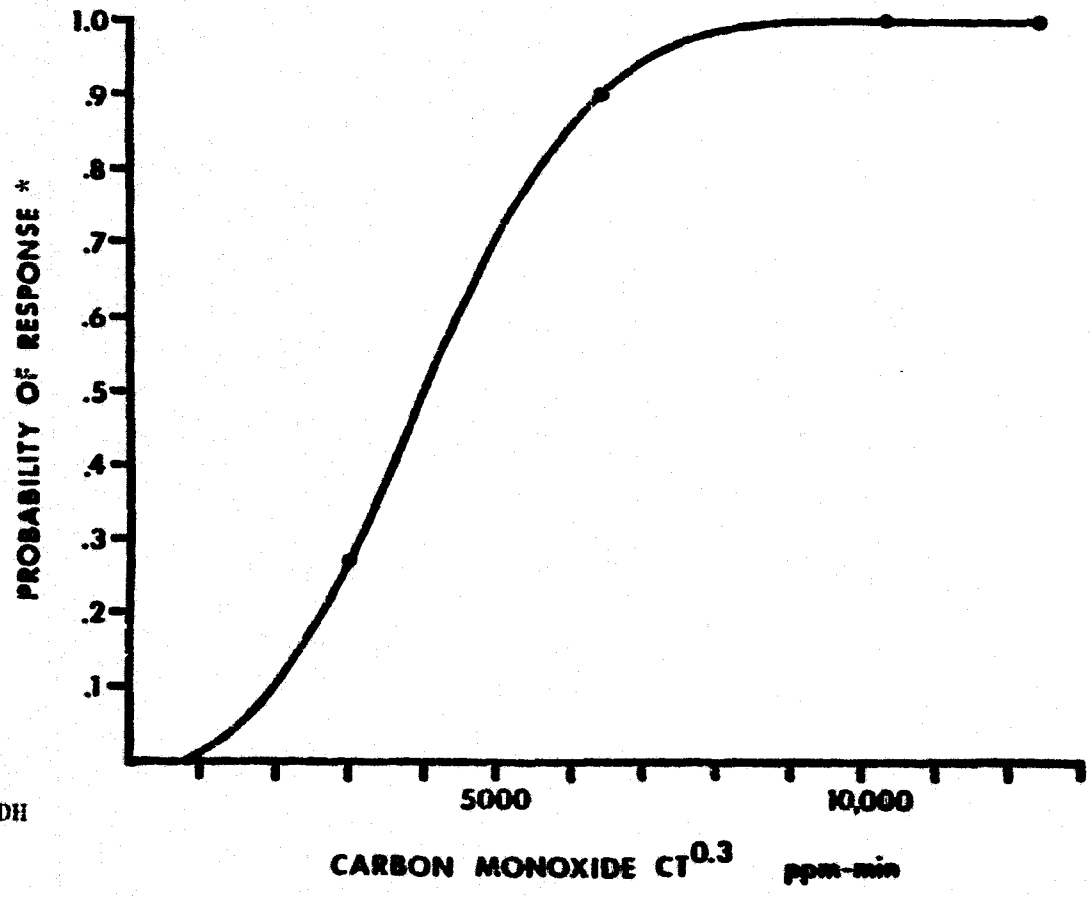


Figure 35

Weibull Model Fitted to HBDH Serum Enzyme Data.

*The response is an HBDH activity ≥ mean control value + 3 S.D.

TABLE IX

Analysis of One-Hit, Probit, and Weibull Models
Fitted to LDH Serum Enzyme Data

Model	Equation of Best-Fit Curve	Chi Square	P value*	Carbon Monoxide $CT^{0.3}$ Associated with Probability of Response of 10^{-6}
One-Hit	$P(D) = 1 - EXP-[-0.1815(D/1000)]$	6.43	$0.05 < p < 0.10$	0.006 ppm-minutes
Probit	$P(D) = \Phi\ (-8.625 + 3.738\ LOG\ D)$	0.462	$0.90 < p < 0.95$	203 ppm-minutes
Weibull	$P(D) = 1 - EXP-[-0.023 + 0.062(D/1000)^{1.598}]$	0.168	$0.98 < p < 0.99$	537 ppm-minutes

*The P value is the probability of obtaining this value of chi square or greater, if the underlying distribution of the observed curve is the expected curve. Probabilities of 0.05 or larger are generally considered to indicate a satisfactory fit. The greater the P value, the better the fit (Croxton, 1959). The number of degrees of freedom is 3.

TABLE X

Analysis of One-Hit, Probit, and Weibull Models
Fitted to HBDH Serum Enzyme Data

Model	Equation of Best-Fit Curve	Chi Square	P value*	Carbon Monoxide $CT^{0.3}$ Associated with Probability of Response of 10^{-6}
One-Hit	$P(D) = 1 - EXP[-0.2287(D/1000)]$	13.	$0.001 < p < 0.005$	0.004 ppm-minutes
Probit	$P(D) = \Phi(-16.268 + 5.946 \text{ LOG } D)$	0.045	$0.995 < p$	544 ppm-minutes
Weibull	$P(D) = 1 - EXP-[-0.0039 + 0.0203(D/1000)^{2.549}]$	0.001	$0.995 < p$	523 ppm-minutes

*The P value is the probability of obtaining this value of chi square or greater, if the underlying distribution of the observed curve is the expected curve. Probabilities of 0.05 or larger are generally considered to indicate a satisfactory fit. The greater the P value, the better the fit (Croxton, 1959). The number of degrees of freedom is 3.

Electrocardiographic and Respiratory Changes in Response to CO

Not enought replicate records were obtained to establish a dose-response relationship for electrocardiographic or respiratory changes as a function of CO. However, the following changes in these physiological parameters were observed in individual rats. At a $CT^{0.3}$ for CO of 2950, an increase in heart rate of 18% relative to control was observed, and a decrease in respiratory rate of 14% was observed. At a $CT^{0.3}$ for CO of 6420, a heart rate depression of 16%, and a respiratory rate depression of 19% was observed. At a $CT^{0.3}$ for CO of 10,310, a depression of 18% in the heart rate and a depression of 40% in the respiratory rate occurred. At a $CT^{0.3}$ for CO of 12,460, heart rate depressions of 50 and 40% were observed, and a respiratory depression of 77% occurred. Additionally, at a $CT^{0.3}$ of 10,310, 8.3% lethality was observed, and at a $CT^{0.3}$ of 12,460, 14.8% lethality occurred.

Chapter 5

DISCUSSION

As stated previously, the risk estimate procedure entails the following;

i. selection of an appropriate experimental bioassay

ii. selection of a theoretical dose-response model(s), and estimation of its parameters from data

iii. extrapolation of experimental results to very low probabilities of response outside the experimentally observable range of response, based on the best-fit equations of the theoretical model(s)

iv. extrapolation of predicted response rates in animals at low dose levels to man, by the application of interspecies conversion factors.

Each of these steps in the risk estimate process will now be addressed in turn, as they pertain to the estimation of a VSD (virtually safe dose) for man, based on the behavioral and physiological responses of rodents to CO.

The behavioral and physiological assays which were chosen and tested with CO, incorporate a number of important features relevant to risk estimation in their experimental designs. The assays provided quantitative, dose-response data from two mammalian species and both sexes at multiple dosages of CO. Most importantly, these assays utilized a number of sublethal toxic endpoints, thus providing a range of toxic sensitivities.

The theoretical dose-response models chosen to be fitted to the dose-response data generated from these assays, are representative of the two general classes of theoretical models, tolerance distribution and hit-theory models. The probit model, based on the assumption that toxic thresholds are distributed normally in a population, has been more widely used than other tolerance distribution models. The one-hit model is the simplest of hit-theory models, while the Weibull model is one of a number of generalizations of the one-hit model. All of the hit-theory models are derived from the assumption of no-threshold, but the Weibull model fits data with an apparent threshold quite well (Carlborg, 1981a). Additionally, this selection of theoretical models represents a range of conservatism. Since the rapidity with which each model generally approaches zero response differs, the predicted VSDs derived from the models will also differ. The one-hit model is most conservative in this respect; the probit model and Weibull model are considerably less conservative.

It is apparent from Tables II, IV, IX, and X (Results) that the Weibull and probit models consistently provide good fits to both the behavioral and physiological dose-response data, as determined by a chi-square test. The one-hit model however, gives only a very poor fit to any of the data sets, and will therefore not be considered any further for the purpose of estimating human risk.

The VSD is by general consensus agreed to be the dose corresponding to a response rate of 1 in 10^6, and is obtained by extrapolation of the best-fit, theoretical dose-response curve below the experimentally observable range of responses. By comparison, the VSDs for

rodents derived from the probit model were more conservative than the VSDs derived from the Weibull model for all but one of the toxic responses of interest (see Tables II, IV, IX, and X). In order to complete the risk estimate process by extrapolating from these estimated VSDs for rodents to predicted VSDs for man, the biological factors which mediate toxicity must be considered.

Interspecies Variables that Affect Toxicity

Of the many biological processes which influence the ultimate expression of a toxic response, absorption, metabolism (whether detoxification or bioactivation), and elimination are generally very important for acute, inhalation intoxications.

During an inhalation exposure, the amount of CO which can be absorbed by the blood depends primarily on the amount of blood available, the diffusion capacity of the respiratory membrane for CO, and the amount of CO available in the lungs during that time (Pace et al., 1946). The amount of blood available, or the total blood volume, varies directly with body weight in mammalian species (Sjöstrand, 1962). The amount of CO available in the lungs is a function of respiratory rate and tidal volume. The resting minute respiratory volume (respiratory rate times tidal volume) for mammalian species is approximately proportional to a power of body weight (Guyton, 1947). An experimental estimate of the respiratory diffusing capacity for CO in rodents (mice) has recently been reported (Depledge et al., 1981)

and when adjusted for body weight, is the same order of magnitude as the values observed for humans (Coburn et al., 1965).

To estimate the relative rates of uptake of CO between two mammalian species, Haldane (1895) therefore used the following empirical relationship:

$$\text{ratio of rates of CO absorption} \propto \frac{\text{ratio of body surface areas}}{\text{ratio of body weights}}$$

Since body surface area in mammals is proportional to the 2/3 power of body weight, this equation reduces to:

$$\text{ratio of rates of CO absorption} \propto (\text{ratio of body weights})^{-1/3}$$

This relationship reflects the dependence of the rate of CO uptake on blood volume and minute respiratory volume, both of which vary with body weight. By substituting into this equation, the average weight of a mouse (.0375 kg) or rat (.250 kg) used in these studies, or a reference weight of 70 kg for humans (ICRP, 1975), the following interspecies conversions for the rates of CO absorption are obtained. A mouse will absorb CO approximately 12 times faster than a human. A rat will absorb CO approximately 6.5 times faster than a human.

Once absorbed, metabolic conversion of CO via oxidation of CO_2 is generally considered to occur in only negligible amounts in mammals, although potential CO metabolism during acute CO intoxication is not well studied. During prolonged exposure (4 days) to low concentrations (700-900 ppm) of CO, the slow disappearance of CO from the blood via

oxidation has been observed in mice (Root, 1965), and has been similarly reported to occur in man (Forbes et al., 1945). In humans, following inhalation of large, single doses of radiolabelled CO, Tobias and associates (1945) observed that less than 0.1% of the amount of CO eliminated from the blood during the first hour post-exposure was oxidized to CO_2.

The major route of elimination of CO from the body for all mammalian species is gas exchange via the lungs. Carbon monoxide is released from the lungs by diffusion across the respiratory membrane once the partial pressure of CO in physical solution in the blood exceeds the partial pressure of CO present in the alveoli (Forbes et al., 1945). Thus, the same factors which govern the uptake of CO in the blood are responsible as well for its removal. It is interesting to note that the average carboxyhemoglobin (COHb) half-life of 250 minutes for man (Root, 1965) is approximately 11 times greater than the average COHb half-life of 23 minutes for mice (Winslow, 1981). This eleven-fold faster elimination of CO in mice relative to man is consistent with the twelve-fold faster uptake of CO in mice estimated previously.

Estimation of a Virtually Safe Dose of Carbon Monoxide for Man

Based on the preceding discussion, it is apparent that a conversion factor to account for interspecies differences in rates of CO absorption should be included in the risk estimate process. Since metabolism of CO, at the low rates generally reported for mammals, is

expected to be minimal during very brief exposure durations such as were used in these experiments, no conversion factors for metabolism will be applied. Additionally, elimination of CO from the blood should not occur to any substantial degree during the course of the acute exposure while CO is being continually supplied in the inspired air. Under these conditions, the amount of CO in solution in the blood should eventually come to an equilibrium which would not diminish significantly until the CO supply in the inspired air was decreased or stopped. In the case of the mouse behavioral assay, both toxic endpoints develop during the course of exposure while elimination of CO from the blood is not an important consideration. However, even though the serum enzyme responses are measured two hours post-exposure, during which time a large portion of CO is undoubtedly eliminated, they are presumed to be an index of the irreversible cellular damage initiated during the acute exposure period. The appearance of these enzymes in extracellular fluids following CO exposure is a time-dependent phenomenon (Penney and Maziarka, 1976) and serum activity levels continue to increase for several hours post-exposure, despite large decreases in blood CO concentration during the same time period. Therefore, no conversion factor for elimination will be applied in the risk estimate to follow.

The interspecies conversion factors for relative CO,absorption rates represent a time factor, and as such, must be raised to the 0.3 power to maintain consistency with the $CT^{0.3}$ units in which the rodent VSD estimates are expressed. The 12 times faster uptake of CO in mice versus man becomes a factor of 2.1; the 6.5 times faster

uptake of CO in rats versus man becomes a factor of 1.8. Since the faster rates of absorption of CO in the rodent species imply greater sensitivity at equivalent CO exposures, these factors must be multiplied by the appropriate rodent VSDs to arrive at estimates of VSDs for man. The resulting predicted VSDs for man based on each of the toxic responses of interest are summarized in Table XI.

Additional safety factors to allow for interindividual variability in CO uptake and sensitivity would necessarily be considered for the purpose of establishing regulatory guidelines. However, for the purpose of examining how well human risk estimates extrapolated from behavioral and physiological responses in rodents correlate with available data on human responses to CO, no arbitrary safety factors were included in the predicted human VSDs.

Comparison of Predicted Human VSDs with Acute Responses to CO in Man

Only a few quantitative studies of the acute effects of CO in humans are avilable at the range of CO concentrations in which the rodent tests were conducted (i.e., 700 ppm and greater). It is important to consider only similar acute CO exposures in man since there is evidence that the rate of CO saturation in the blood affects toxicity. Plevová and Frantík (1974) compared the motor performance of rats at equivalent COHb levels which were reached under different exposure conditions. Rats exposed to 700 ppm of CO for 30 minutes developed a COHb level of 19.6% and displayed a decrement in motor performance of 60%, while rats exposed for 24 hours to 200 ppm of CO developed a

TABLE XI

Estimated Human VSDs for Single, Acute CO Exposures

Toxic response	Predicted VSDs for man estimated from toxic responses in rodents fitted to:	
	Weibull model $CT^{0.3}$ (ppm-min)	Probit model $CT^{0.3}$ (ppm-min)
Initial behavioral change	1558	1214
Loss of escape	2400	1707
Serum LDH activity > mean control value + 3 S.D.	967	365
Serum HBDH activity > mean control value + 3 S.D.	941	979

slightly higher COHb of 22.8%, but displayed a decrement in motor performance of only 33%. Although the COHb levels did not differ significantly in either case, the $CT^{0.3}$ values for exposures were different. The $CT^{0.3}$ value for CO associated with greater decrement in motor performance is 1942, while the lesser decrement was observed at a $CT^{0.3}$ of 1772. Seppänen and associates (1977) similarly suggest that the rate of COHb saturation influenced the sensitivity of a test of visual perception in humans.

The human studies reported in the literature which involved CO exposures of 700 ppm or greater are summarized in Table XII. The first five studies were conducted at average levels of CO equivalent to those tested in the mouse behavioral and rat physiological assays. One of the studies (Pace et el., 1946) was intended as a CO absorption study for the development of an equation for the rate of COHb formation. Unfortunately, no subjective or objective tests of toxicity were conducted or reported, although presumably the CO exposures tested were survivable under the experimental conditions given. The last two studies were conducted at exposure concentrations of CO up to 10 fold greater than were tested in the rodent assays. These exposures were of a necessarily short duration, usually less than 2 minutes.

It is apparent from the predicted VSDs for man reported in Table XI, that the rodent assays present a range of sensitivities to the effects of CO. Intuitively, one would expect to see a progression from cellular injury (enzyme release) to a moderate psychomotor change (initial behavioral change) to a severe psychomotor deficit (loss of escape), as the carbon monoxide $CT^{0.3}$ value increases. The VSDs

TABLE XII Summary of data for human response to acute CO exposures

Carbon Monoxide Exposure $CT^{0.5}$ (ppm-min)	Average CO (ppm)	Time (min)	Exposure Conditions	Number of Subjects	Response
2390 or less	700	60	Subjects at rest	28	[1] Marginally significant change in complex psychomotor task; suggestion of more incorrect responses with CO when task is unfamiliar, and of attentional lapses or gaps in performance with CO
2976	950	45	Subjects at rest	20	[2] Significant decrement in speed of psychomotor reaction to a visual stimulus
2732 - 3415	800 to 1000	60	Subjects at rest	6	[3] Increasingly severe frontal headaches; occasional Cheyne-Stokes' breathing
2497 - 4913	900 to 2180	15 to 30	Subjects at rest or walking on a level	24	[4] No subjective or objective measures of toxicity reported; CO absorption study
5046 - 11,259	1200 to 1500	11.5 to 120	Subject at rest or moderate activity	1	[5] Seven separate exposures of 1 subject; throbbing headache and hyperpnea when resting; weak limbs, staggering, indistinct hearing and vision, and confusion during moderate activity
1995 - 35,600	1000 to 35,600	.75 to 10	Subjects at rest	6	[6] Headache but no significant EEG or EOG abnormalities observed; no changes in blood chemistries
40,613 - 91,557	50,000	.5 to 1	Cardiac catheterized patients	26	[7] Significant increase in minute ventilation, cardiac output, and O_2 consumption; resembles adrenergic stimulation

References: [1]McFarland (1973); [2]Ramsey (1973); [3]Hendersen (1921); [4]Pace et al., (1946); [5]Haldane (1895); [6]Stewart et al., (1978); [7]Ayres et al., (1969)

predicted for man on the basis of the rodent data reflect this relationship; the VSD derived from changes in serum enzyme activities is lower than that for the initial behavioral change, which is in turn lower than the VSD estimated from loss of escape. A range of toxic sensitivities can also be observed in the human data reported in Table XII, although the toxic responses reported differ qualitatively from those measured in the rodent assays.

The highest VSD estimate for man ($CT^{0.3}$ = 2400) which was obtained using the Weibull curve for the loss of escape in mice (Table XI), is about the same as the $CT^{0.3}$ value at which McFarland (1973) reports a marginally significant change in a complex psychomotor task in humans (Table XII). The psychomotor task tested in McFarland's study consisted of two superimposed tasks; one required pressing an appropriate foot switch in response to one of two lights, and the other required pressing appropriate finger switches in response to one of six lights.

By comparison, the highest estimated VSD of 2400 ($CT^{0.3}$) might appear too conservative with respect to the last two human studies in Table II, in which $CT^{0.3}$ values for CO of from 35,600 to 61,577 were tolerated. However, there is an important difference in the exposure conditions for the mouse behavioral assay versus the exposure conditions for the majority of the human studies. In the former case, the mice are free-running and must perform coordinated motor tasks (pole-jumps) under stressful conditions (Winslow, 1981); in the latter, human subjects are usually at rest.

In mammals, the minute respiratory volume is sensitive to both stress and amount of activity or work. The minute volume may increase by three fold with light work (walking on a level) and up to five fold under conditions of heavy activity (slow jog trot) (Forbes et al., 1945). Since increasing the minute respiratory volume proportionally increases the rate of CO absorption (Pace et al., 1946, Forbes et al., 1945), subjects at rest would not absorb as much CO as active or stressed subjects at equivalent CO exposures. Therefore, subjects at rest could tolerate higher carbon monoxide $CT^{0.3}$ values without apparent toxicity. Escaping from a fire environment would undoubtedly involve some degree of stress and activity; the predicted VSD for CO should therefore be conservative enough to take this factor into account. Even greater conservatism would be afforded by using the probit model VSD for loss of escape, or the VSDs predicted on the basis of the initial behavioral change or enzyme release.

Since the VSDs have been expressed as $CT^{0.3}$ values, the concentration of CO which would be virtually safe for a given length of time can be calculated. For example, for the loss of escape VSD of 2400 ppm-min, one would predict that an exposure of 2400 ppm of CO for 1 minute would be virtually safe, as would an exposure of about 1480 ppm for about 5 minutes, 1200 ppm for 10 minutes, etcetera. Such calculations are necessary when considering the toxicity of combustion exposures where the amount and type of toxicant generated as well as how rapidly it is evolved varies.

Interestingly, Book (1982) has recently suggested a procedure for scaling the acute lethality of inhalation exposures of nitrogen

dioxide (NO_2) observed in animals to man. Book found that the exposure durations and NO_2 concentrations associated with lethality frequency data from 5 animal species, mouse, rat, guinea pig, rabbit, and dog, were best described by power functions ranging from $CT^{0.24}$ to $CT^{0.3}$. The average value chosen by Book, $CT^{0.28}$, to express this relationship for man is in good agreement with the $CT^{0.3}$ relationship established by Winslow (1981) for CO. Additionally, a linear relationship was observed for these 5 species between the average minute respiratory volume multiplied by the $CT^{0.28}$ value for NO_2 lethality for each species, and average body weight. On this basis, Book predicted the $CT^{0.28}$ value for NO_2 lethality in man would be 2.1 fold greater than that for mice, and 1.5 fold greater than that for rats. These values are in good agreement with the interspecies conversion factors of 2.1 for mice to man, and 1.8 for rats to man, used earlier in this discussion to predict carbon monoxide VSDs for man.

Applications of Risk Estimation for Combustion Toxicology

Estimation of the toxic hazard to man of a fire atmosphere clearly involves two aspects; whether the toxic exposure is survivable by virtue of being escapable, and whether survivors sustain significant sub-lethal injury.

Winslow (1981) has discussed the utility of the pole-jump response as an index of psychomotor functions important for survival in a fire atmosphere. The estimation of VSDs for man based on loss of escape

behavior in rodents, for individual combustion components such as CO, is one way of assessing the potential toxic risk to survival in complex fire atmospheres.

Obviously, survival in fire atmosphere does not imply that no toxic injury is sustained. From the predicted human VSDs given in Table XI, it is apparent that at CO exposures not expected to affect escape capability, significant cellular injury, measured by cellular enzyme release, and early psychomotor changes may occur. Whether the early psychomotor changes observed in mice would manifest themselves in humans as qualitatively equivalent behavioral responses is impossible to determine. Also, the relevance of these responses, (cellular enzyme release and initial behavioral changes) with respect to whether they represent significant, irreparable injury, is an area for further investigation.

Clearly, risk estimation is an ongoing process. As further information about the toxic mode of action of acute CO in humans or higher mammals becomes available, risk estimates must be modified and continually fine-tuned. Until such information becomes available, predictions of potential toxic hazard for man necessarily involve some assumptions about the equivalence of toxic responses betweeen mammalian species, that may later prove invalid.

Chapter 6

CONCLUSIONS

Quantal dose-response data for the acute toxic effects of carbon monoxide, obtained from a behavioral assay in mice (Winslow, 1981) and from a physiological assay in rats, were fitted to several theoretical models currently employed for risk estimation. By extrapolation of these models to low response rates (1 in 10^6), and the application of interspecies conversion factors, a virtually safe dose of CO for man for several toxic endpoints was estimated. Observations regarding the utility of the risk estimate approach for prediction of the acute toxic hazard of CO, are the following:

1. The probit and Weibull models consistently provided the best-fits to the dose-response data for CO. The probit model is based on the assumption that a distribution of toxic thresholds exists, while the Weibull model will fit data with an apparent threshold. Since the acute effects of CO are non-stochastic in nature, a threshold would be expected. In general, the probit model provided more conservative estimates of virtually safe doses than did the Weibull.
2. The one-hit model provided an extremely poor approximation of the dose-response data for CO. The model is much too conservative (i.e., it approaches zero response too slowly) to be of use for the prediction of reliable risk estimates for CO in man.

3. The behavioral and physiological endpoints utilized in the rodent assays represent a spectrum of toxic sensitivities. The VSDs estimated on the basis of these responses reflect this. Cellular injury and moderate behavioral changes may occur at virtually safe levels determined for escape capability.

4. By comparison with available data on human responses to CO at concentrations equivalent to those used in the rodent tests, it appears that even the highest predicted VSD in this study has been reported to produce only marginal change in a complex psychomotor task in man. The predicted VSDs however, may not be overly conservative in light of the fact that tests of human responses to CO were generally conducted while subjects are at rest. When subjects are active or stressed, as was the case for the mice in the behavioral assay, increased uptake of CO results in greater expression of the toxic effects of CO.

Several areas for further investigation suggest themselves to increase the reliability of the risk estimate procedure as it has been employed in this thesis. These include the following:

1. Testing of the acute effects of CO at similar exposure concentrations and durations in other mammalian animal species for responses that are qualitatively equivalent to the behavioral and physiological endpoints measured in these studies. Confidence in interspecies extrapolations of toxicity is increased when data from more than one species are available for comparison of the way in which the mean species $Cr^{0.3}$ associated

with a toxic response, relates to such variables such as minute volume, blood volume, and body weight, as Rook (1982) has done for NO_2.

2. Investigation in animals of the significance of the selected toxic endpoints, particularly cellular enzyme release; do they represent lasting alterations in structure or function, and if not, at what level do they become significant? It is apparent from the graded dose-response enzyme data in rats, that increasing levels of these enzymes are observed as carbon monoxide $CT^{0.3}$ increases to levels where significant compromise of physiological function and death may occur.

APPENDIX 1

Quantal Dose-Response Data : Initial Behavioral Change .

Carbon Monoxide $CT^{0.3}$ (ppm-min)	Probability of Response
1000	0.0000
1200	0.0385
1400	0.0385
1600	0.0385
1800	0.1928
2000	0.2308
2200	0.3077
2400	0.5000
2600	0.6154
2800	0.7308
3000	0.8462
3200	0.8462
3400	0.8846
3600	0.9615
3800	0.9615
4000	0.9615
4200	1.0000

APPENDIX 2

Probit Line Analysis[1] of Initial Behavioral Change Data

Log Dose (x)	Empirical Probit[2]	Expected Probit	Corrected Probit (y)	Weight (w)
3.000	-	1.912	1.613	0.286
3.079	3.238	2.554	4.173	1.456
3.146	3.238	3.098	3.260	4.004
3.204	3.238	3.570	3.311	7.592
3.255	4.130	3.984	4.142	11.232
3.301	4.264	4.358	4.268	14.222
3.342	4.499	4.691	4.505	15.990
3.380	5.000	5.000	5.001	16.562
3.415	5.292	5.284	5.292	16.068
3.447	5.616	5.544	5.614	14.872
3.477	6.019	5.788	5.998	13.156
3.505	6.019	6.015	6.019	11.232
3.531	6.200	6.226	6.199	9.360
3.556	6.774	6.430	6.696	7.592
3.580	6.774	6.625	6.757	5.994
3.602	6.774	6.803	6.774	4.680
3.623	-	6.974	7.396	3.588

[1] Method described by Bliss (1938)

[2] Obtained from Finney (1952)

APPENDIX 2 (continued)

Constants computed from the data:

$\Sigma w = 157.846$

$\Sigma wx = 537.654$

$\Sigma wy = 829.192$

$\bar{x} = 3.406$

$\bar{y} = 5.253$

$|wx^2| = 2.348$

$|wxy| = 19.142$

$b = 8.152$

APPENDIX 3

Expected Probabilities of Response from One-Hit Model Fitted to Initial Behavioral Change Data

Carbon Monoxide $CT^{0.3}$ (ppm-min)	Expected Probability of Response
1000	0.2817
1200	0.3299
1400	0.3711
1600	0.4136
1800	0.4515
2000	0.4869
2200	0.5200
2400	0.5510
2600	0.5799
2800	0.6071
3000	0.6324
3200	0.6561
3400	0.6783
3600	0.6991
3800	0.7185
4000	0.7367
4200	0.7537

APPENDIX 4

Expected Probabilities of Response from Probit Model Fitted to Initial Behavioral Change Data

Carbon Monoxide $CT^{0.3}$ (ppm-min)	Expected Probability of Response
1000	0.001
1200	0.008
1400	0.031
1600	0.082
1800	0.164
2000	0.273
2200	0.394
2400	0.516
2600	0.628
2800	0.721
3000	0.797
3200	0.855
3400	0.898
3600	0.930
3800	0.957
4000	0.968
4200	0.978

APPENDIX 5

Expected Probabilities of Response from Weibull Model Fitted to Initial Behavioral Change Data

Carbon Monoxide $CT^{0.3}$ (ppm-min)	Expected Probability of Response
1000	0.0059
1200	0.0172
1400	0.0388
1600	0.0756
1800	0.1332
2000	0.2158
2200	0.3246
2400	0.4549
2600	0.5958
2800	0.7308
3000	0.8432
3200	0.9225
3400	0.9686
3600	0 9900
3800	0.9976
4000	0.9996
4200	1.0000

APPENDIX 6

Quantal Dose-Response Data : Loss of Escape

Carbon Monoxide $CT^{0.3}$ (ppm-min)	Probability of Response
1600	0.0000
1800	0.0769
2000	0.1538
2200	0.2308
2400	0.3077
2600	0.3462
2800	0.5000
3000	0.6538
3200	0.7692
3400	0.8462
3600	0.9231
3800	0.9615
4000	0.9615
4200	1.0000

APPENDIX 7

Probit Line Analysis[1] of Loss of Escape Data

Log Dose (x)	Empirical Probit[2]	Expected Probit	Corrected Probit (y)	Weight (w)
3.204	-	2.550	2.190	1.450
3.255	3.525	3.079	3.876	3.893
3.301	3.981	3.557	4.123	7.510
3.342	4.264	3.982	4.306	11.227
3.380	4.449	4.376	4.503	14.369
3.415	4.604	4.740	4.607	16.136
3.447	5.000	5.072	5.000	16.510
3.477	5.396	5.383	5.396	15.694
3.505	5.736	5.673	5.734	14.027
3.531	6.019	5.943	6.016	11.915
3.556	6.426	6.203	6.397	9.628
3.580	6.774	6.452	6.704	7.424
3.602	6.774	6.680	6.766	5.555
3.623	-	6.898	7.138	4.014

[1]Method described by Bliss (1938)

[2]Obtained from Finney (1952)

APPENDIX 7 (continued)

Constants computed from the data:

$\Sigma w = 139.352$

$\Sigma wx = 481.382$

$\Sigma wy = 734.347$

$\bar{x} = 3.454$

$\bar{y} = 5.270$

$|wx^2| = 1.250$

$|wxy| = 12.108$

$b = 9.683$

APPENDIX B

Expected Probabilities of Response from One-Hit Model Fitted to Loss of Escape Data

Carbon Monoxide $CT^{0.3}$ (ppm-min)	Expected Probability of Response
1600	0.3780
1800	0.4139
2000	0.4477
2200	0.4795
2400	0.5095
2600	0.5378
2800	0.5644
3000	0.5895
3200	0.6132
3400	0.6355
3600	0.6565
3800	0.6763
4000	0.6949
4200	0.7125

APPENDIX 9

Expected Probabilities of Response from Probit Model Fitted to Loss of Escape Data

Carbon Monoxide $CT^{0.3}$ (ppm-min)	Expected Probability of Response
1600	0.016
1800	0.048
2000	0.112
2200	0.207
2400	0.326
2600	0.456
2800	0.579
3000	0.687
3200	0.776
3400	0.844
3600	0.895
3800	0.931
4000	0.955
4200	0.971

APPENDIX 10

Expected Probabilities of Response from Weibull Model Fitted to Loss of Escape Data

Carbon Monoxide $CT^{0.3}$ (ppm-min)	Expected Probability of Response
1600	0.0363
1800	0.0706
2000	0.1215
2200	0.1920
2400	0.2834
2600	0.3935
2800	0.5164
3000	0.6420
3200	0.7580
3400	0.8533
3600	0.9222
3800	0.9647
4000	0.9867
4200	0.9959

APPENDIX 11

Expected Probabilities of Response from One-Hit Model Fitted to LDH Serum Enzyme Data

Carbon Monoxide $CT^{0.3}$ (ppm-min)	Expected Probability of Response
2.95×10^3	0.491
6.42×10^3	0.770
10.31×10^3	0.905
12.46×10^3	0.942

APPENDIX 12

Expected Probabilities of Response from Probit Model Fitted to LDH Serum Enzyme Data

Carbon Monoxide $CT^{0.3}$ (ppm-min)	Expected Probability of Response
2.95×10^3	0.257
6.42×10^3	0.729
10.31×10^3	0.915
12.46×10^3	0.954

APPENDIX 13

Expected Probabilities of Response from Weibull Model Fitted to LDH Serum Enzyme Data

Carbon Monoxide $CT^{0.3}$ (ppm-min)	Expected Probability of Response
2.95×10^3	0.2784
6.42×10^3	0.6949
10.31×10^3	0.9224
12.46×10^3	0.9688

APPENDIX 14

Expected Probabilities of Response from One-Hit Model Fitted to HBDH Serum Enzyme Data

Carbon Monoxide $CT^{0.3}$ (ppm-min)	Expected Probability of Response
2.95×10^3	0.491
6.42×10^3	0.770
10.31×10^3	0.905
12.46×10^3	0.942

APPENDIX 15

Expected Probabilities of Response from Probit Model Fitted to HBDH Serum Enzyme Data

Carbon Monoxide $CT^{0.3}$ (ppm-min)	Expected Probability of Response
2.95×10^3	0.263
6.42×10^3	0.915
10.31×10^3	0.995
12.46×10^3	0.999

APPENDIX 16

Expected Probabilities of Response from Weibull Model Fitted to NBDH Serum Enzyme Data

Carbon Monoxide $CT^{0.3}$ (ppm-min)	Expected Probability of Response
2.95×10^3	0.2710
6.42×10^3	0.9016
10.31×10^3	0.9996
12.46×10^3	1.0000

APPENDIX 17

Probit Line Analysis[1] of LDH Serum Enzyme Data

Log Dose (x)	Empirical Probit[2]	Expected Probit	Corrected Probit (y)	Weight (w)
3.470	4.396	4.391	4.397	6.108
3.808	5.524	5.528	5.524	5.741
4.011	6.282	6.218	6.279	3.634
4.096	-	6.497	7.016	1.615

Constants computed from the data:

$\Sigma w = 17.098$

$\Sigma wx = 64.255$

$\Sigma wy = 92.713$

$\bar{x} = 3.758$

$\bar{y} = 5.422$

$[wx^2] = 0.941$

$[wxy] = 3.525$

$b = 3.738$

[1] Method described by Bliss (1938)

[2] Obtained from Finney (1952)

APPENDIX 18

Probit Line Analysis[1] of HBDH Serum Enzyme Data

Log Dose (x)	Empirical Probit[2]	Expected Probit	Corrected Probit (y)	Weight (w)
3.470	4.396	4.396	4.396	6.137
3.808	6.282	6.282	6.281	3.428
4.013	-	7.425	7.792	0.581
4.096	-	7.688	8.204	0.117

Constants computed from the data:

$\Sigma w = 10.263$

$\Sigma wx = 37.162$

$\Sigma wy = 54.002$

$\bar{x} = 3.621$

$\bar{y} = 5.262$

$[wx^2] = 0.367$

$[wxy] = 2.182$

$b = 5.946$

[1]Method described by Bliss (1938)

[2]Obtained from Finney (1952)

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