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# HUMAN HEALTH STUDIES OF CARBON MONOXIDE (CO)

# UNDER CONDITIONS OF MILITARY WEAPONS SYSTEMS

# CREWMAN EXPOSURES

Subtitle:  Protocol 1:  Formation of COHb

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The present experiment was performed as a first step to evaluate the accuracy of prediction of carboxyhemoglobin (COHb) formation due to quasi steady state carbon monoxide (CO) exposure by use of the Coburn-Forster-Kane equation (CFKE) and related models. Thirteen healthy young males were exposed to CO in room air for 120 minutes. While they were being exposed to CO, they

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either rested or performed bicycle exercise at one of two moderate work levels (25 or 40 Watts). Also during exposure to CO, blood samples were drawn every five min to assess the COHb level. Many of the variables of the CFKE were measured in individual subjects rather than using published norms. The CFKE was then used to predict COHb formation and the results were compared to observed values.

Based on the data collected in the present study, the CFKE appeared to predict COHb formation accurately but a possibly important source of artifact was discovered. The artifact was due to small but noticeable increments in COHb which were formed during repeated measurements of the lung diffusion coefficient for CO. Even so, the artifact accounted for less than 0.5% COHb. Since the error was so small, the evidence for the accuracy of the CFKE, for the present set of circumstances, seems good.

In an appendix a sensitivity analysis of the CFKE is given. In this analysis, the sensitivity of the outcome of the prediction to variations in each of the variables in the equation is provided. From the results of the analysis, it is possible to judge how critical each of the variables are and when (during uptake) the variables are most critical.

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## EXECUTIVE SUMMARY

The health effects of carbon monoxide (CO) exposure are usually taken to be related to the formation of carboxyhemoglobin (COHb). The measurement of COHb usually requires blood samples. It would be more convenient to predict COHb formation from knowledge of CO exposure. The Coburn-Forster-Kane equation (CFKE) is a differential equation which was developed to describe COHb formation due to endogenous and low level exogenous steady state CO exposure. The CFKE has not been tested for accuracy, however, in cases of higher level exogenous exposure or in cases of transient (non steady state) exposures.

The present experiment was performed as the first step in a series of experiments to evaluate the CFKE for accuracy in prediction of COHb formation due to moderate quasi steady state CO exposure. Thirteen healthy young male subjects were exposed to 100 ppm CO in room air for 120 minutes while blood was drawn at five-min intervals to obtain COHb measurements. While exposed to CO, subjects either rested or systematically exercised on a bicycle ergometer at two moderate exercise levels (25 or 40 Watts).

The parameters of the CFKE include a number of physiological variables as well as the physical variables which determine exposure. It was desired to estimate as many of the parameters as possible from physiological and physical measurements rather than using published norms for the parameters. In this way not only would the equation's accuracy be most fairly tested, but the importance of each parameter could be empirically assessed.

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The results of the experiment indicated that the CFKE predicts well for the conditions used in the present experiment, but unfortunately, a possibly important source of artifact was discovered during the experiment which might narrow the applicability of the findings. One of the physiological variables, the diffusion coefficient of the lung for CO ($D_LCO$) was repeatedly measured during the experiment by a method which entailed breathing a small amount of CO isotope ($C^{18}O$) which also produced COHb. It was not anticipated that rebreathing $C^{18}O$ would produce an appreciable amount of COHb but inspection of data revealed that the assumption was wrong. Since no measures of $C^{18}O$ exposures had been provided in the experiment, it was not possible to accurately estimate total CO exposure and thus unambiguously test the CFKE. The error due to $C^{18}O$ exposure appears to be smaller than 0.5% COHb in the final analysis, however.

Exploratory analyses revealed that $D_LCO$ was sufficiently stable to have been measured on another day than the experiment. It was also shown that it is necessary to consider the level of exercise at which the $D_LCO$ variable will be used in order to accurately estimate its value.

In an appendix a sensitivity analysis of the CFKE is given. In this analysis, the sensitivity of the outcome of the prediction to variations in each of the variables in the equation is provided. From the results of the analysis, it is possible to judge how critical each of the variables are and when (during uptake) the variables are most critical. A time graph for each variable is shown for several versions of the CFKE.

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# TABLE OF ABBREVIATIONS

| | |
|---|---|
| A | Constant associated with work level in a MIL handbook equation for estimation of carboxyhemoglobin |
| ANOVA | Analysis of variance |
| B | See A |
| BTP | Body temperature and pressure |
| BTPS | Body temperature and pressure, saturated with $H_2O$ vapor |
| CFKE | Coburn-Forster-Kane equation |
| $C^{18}O$ | carbon monoxide isotope |
| $C_2H_2$ | Acetylene |
| CO | Carbon monoxide |
| $CO_2$ | Carbon dioxide |
| COHb | Carboxyhemoglobin |
| df | Degrees of freedom |
| $D_LCO$ | Lung diffusion coefficient for CO |
| e | Base of natural logarithm |
| F | Statistic of the Fisher's F test |
| f | Frequency |
| $f-V_E$ | Frequency of breathing |
| $F_ICO$ | Fraction of CO in inspired air |
| Hb | Hemoglobin |
| HCT | Hematocrit |
| He | Helium |
| M | Haldane constant |
| MHb | Methemoglobin |
| n | Number of observations (or subjects) |

| | |
|---|---|
| $N_2$ | Nitrogen |
| $O_2$ | Oxygen |
| $O_2Hb$ | Oxyhemoglobin |
| $p$ | Probability |
| $P_B$ | Barometric pressure (ambient) |
| $P_D$ | Barometric pressure (dry) |
| $P_cO_2$ | Average pulmonary capillary oxygen partial pressure |
| $P_ICO$ | Partial pressure of CO in inhaled (tracheal) air |
| $P_{H_2O}(T)$ | Saturation pressure of water vapor at temperature T |
| $r$ | Correlation coefficient |
| $R^2$ | Squared correlation coefficient |
| S.D. | Standard deviation |
| STP | Standard temperature and pressure |
| STPD | Standard temperature and pressure, dry |
| $t$ | Time |
| $T$ | Temperature ($^\circ C$) |
| THb | Total hemoglobin |
| $\dot{V}_A$ | Alveolar ventilation rate |
| $V_b$ | Blood volume |
| $V_{BAG}$ | Rebreathing bag volume |
| $V_C$ | Volume of capillary blood |
| $\dot{V}_{CO}$ | Endogenous CO production rate |
| $\dot{V}_{CO_2}$ | Carbon dioxide production rate |
| $V_D$ | Dead space |
| $V_E$ | Expiratory volume |
| $\dot{V}_E$ | Minute ventilation rate |
| $V_I$ | Inspiratory volume |

| | |
|---|---|
| $V_{O_2}$ | Oxygen consumption rate |
| $V_t$ | Tidal volume |
| $V_{SYS}$ | Rebreathing system volume |
| $Z$ | Impedance |

# GLOSSARY

Alpha - The probability value below which a result of a statistical test will be considered significant.  The probability of a type I inferential error

Alveolar Membrane - Membrane forming an alveolar sac, separating the lungs from blood vessels.

Carbon Monoxide - A clear, colorless, poisonous gas which is the product of inccmplete combustion.

Carboxyhemoglobin - Hemoglobin to which carbon monoxide has bound

CFKE - The Coburn-Forster-Kane equation for prediction of carboxyhemoglobin formation.

Endogenous - Produced or conducted inside the body  e.g. Endogenous CO production.

Exogenous - Produced or conducted outside the body.

Haldane Constant - Coefficient of relative affinity of CO to hemoglobin relative to oxygen.

Hemoglobin - The oxygen-carrying red pigment of the red blood corpuscles.

Minute Ventilation - The total ventilation rate in liters per minute.

Oxyhemoglobin - Hemoglobin to which oxygen has bound.

Quasi-Steady State - Approaching steady state (See Steady State).

Significance Test - A statistical method of deciding whether a result could have been due to chance.

Steady State - A condition during which all variables or compartments have reached equilibrium and no further changes occur.

Type I Error - A statistical inferential error of deciding that a result was significant when in the population the result does not hold.

# INTRODUCTION

It is widely accepted that the deleterious effects of exposure of humans to carbon monoxide (CO) is due to a hypoxic condition that develops as carboxyhemoglobin (COHb) forms (Coburn, 1979). Since the affinity for hemoglobin (Hb) of CO is much higher than the affinity of oxygen ($O_2$) for Hb, the result of exposure to CO is a preferential formation of COHb and a concomitant reduction of oxyhemoglobin ($O_2$Hb). COHb has been adopted frequently as a measure of CO exposure and as the relevant measure of health effects of such exposure (MIL-STD-1472C, US EPA, 1979). Since the measurement of COHb requires the drawing of a blood sample, and since such invasive procedures are not possible in many cases in which COHb must be known, it is of considerable importance to be able to estimate the formation of COHb from CO exposure indices and from other physiological and environmental conditions. The latter variables are either measurable in a noninvasive manner or can be estimated from environmental conditions and work specifications.

## COHb Prediction - Empirical Methods

The effects of carbon monoxide on the $O_2$ carrying capacity of the blood have been of interest since before Haldane and Smith's (1896) work. Initial efforts at quantification of the relative affinities of hemoglobin for $O_2$ and CO (e.g., Douglas et.al. 1912) showed that the ratio of $O_2$Hb to COHb was proportional to the ratio of their respective partial pressures. Empirical mathematical models were used to describe the $O_2$Hb and COHb saturation curves as rectangular hyperbolas. Much of the study of CO and its relationship with hemoglobin until the mid-

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1940's continued to be centered on determination of the relative affinity constant (also called the Haldane constant, K or M), e.g., Sendroy et al. (1929), and on the $O_2$ and CO dissociation curves (e.g., Roughton and Darling 1944).

Formal models which attempted to relate CO uptake to physiological and environmental variables did not appear until the mid-1940's. Forbes et al. (1945) proposed a model, in the form of an equation, for the rate of CO uptake. The model was based on empirical deductions about the temporal behavior of COHb in human experiments. The authors generated a nomograph from the model by which the change in COHb could be obtained at various times, levels of ventilation, and ambient concentrations of CO. The model was inadequately tested. Forbes et al. (1945) were the first to explicitly state that endogenous CO, the CO transfer factor for the lung, ambient $O_2$ and CO, blood volume, and physical activity (through both ventilation and pulmonary perfusion), should be examined for their contributions to COHb formation.

Pace, et al (1946) introduced a simple linear model for the concentration of COHb in the blood as a function of ambient CO concentration, total respired volume, and blood volume. The model was found to apply for periods of time which corresponded to about one-third of the equilibrium value of COHb. From the composite data on 32 subjects, a linear regression model was fitted to cover the ranges of observed COHb.

Hatch (1952), in an attempt to show the coupling between COHb formation and pulmonary variables introduced a complex model

which separates the transfer factor for the lung into a circulatory rate component, a ventilation rate component, and a diffusional resistance component. Based on the uptake nomograph of Forbes et al. (1945), he concluded that CO is absorbed as any other inert gas; that the uptake rate is diffusion and ventilation-limited, is independent of pulmonary circulation; and that the uptake rate varies directly with ambient concentration. Of particular interest is a calculation showing the relative effects of ventilation on diffusional area and alveolar membrane thickness which suggests that uptake rate should not be directly proportional to ventilation rate.

## COHb Prediction - Theoretical Models

In the mid-1950's, attention was returned to mathematical models in which the equations have counterparts in the physiological system. Among the first of these analogs was the one proposed by Forster et al. (1954 a,b). Concentration was primarily on the general problem of gas exchange in the lungs and the effects of nonuniformity of ventilation. The resulting equation was a comprehensive expression of COHb formation in response to both environmental and physiological variables. A number of important assumptions were made (e.g., ventilation is continuous, all sink compartments are in equilibrium, and blood circulation time is negligible compared with observation time). In addition, some of the variables were impractical to measure.

A highly simplified exponential model was proposed by Goldsmith et al. (1963) for treating both a slowly varying diurnal ambient CO and transient bursts which simulated smoking. The model used no physiological variables directly, but relied on

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the use of an equilibrium value for COHb which was obtained by unspecified means. The model was not tested against human exposures with measurement of both ambient CO and mixed venous COHb, but rather two experiments were used to empirically determine three coefficient values for the equation.

The Coburn-Forster-Kane equation (CFKE) model (Coburn et al. 1965) was developed to describe the venous blood concentration of COHb in response to typically nominal ambient concentrations of CO and to both typical and abnormal rates of endogenous production of CO. The primary application of the model was to estimate endogenous production rates of CO by using the venous COHb as an index. This model is of special interest since it has been extensively used by others to predict COHb formation.

The CFKE is a two-compartment system (the ambient CO environment and the tissue storage) with an exchange mechanism (the lungs) and an internal CO source. A nonlinear first-order differential equation describing the rate of change of COHb was derived using a number of important and explicitly stated assumptions which are worth repeating here:

1) The entire body stores are always equilibrated with blood COHb.

2) The partial pressure of CO is the same in all alveoli.

3) The lung washout time is not significant.

4) Inspiratory and expiratory minute volumes are equal.

5) CO exchange takes place only in the lungs.

Their use of the equation to describe perturbations of COHb saturation in the 0-10% range makes use of another important

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assumption: $O_2Hb$ remains constant. The primary advantage of this assumption is to allow the differential equation to be integrated to yield a closed form solution.

The assumptions used in the CFKE restrict its use to quasi-steady state changes in the coefficients and to relatively low saturations of COHb. The term "quasi-steady state" means that the rates of change of either system or input variables are slow with respect to the dominant time constant of the system. There is no mechanism built into the model to account for the limiting capacity of hemoglobin for CO (or for $O_2$), hence, the restriction of applicability to low values of saturation. The model was evaluated by the authors and found to be a useful tool for screening for abnormal endogenous CO production, but no claims were made for accuracy beyond this application.

## Tests of COHb Prediction Methods

Peterson (1970) exposed subjects to CO and fitted a quadratic regression expression to the data. Over a range of 0-33% COHb saturation, the equation was claimed to have a standard error of the estimate of about 1% COHb for 104 data points, but the range of time over which these measurements applied was not clearly stated.

Peterson and Stewart (1970) exposed resting subjects to a wide range of ambient CO concentrations (1-1000 ppm), measured exposure time and blood COHb, and tested both a pair of regression equations and a form of the CFKE (using assumed values for all coefficients) with the data. Under the experimental conditions, the CFKE yielded results closer to those measured

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than did the empirical expressions.

Stewart et al. (1973) based a logarithmic regression equation for concentrations from 1000 to 35,600 ppm on data from six subjects. Exposure times ranging from 0.75 to 10 minutes giving an integrated exposure from 10,000 ppm-min to 30,750 ppm-min were used and the exhaled CO was collected to calculate uptake. The model yielded high accuracy, but it is of limited utility in either experiments or field studies where the total absorbed quantity of CO is not known.

The CFKE was tested by Peterson and Stewart (1975) with a minimum set of measurements and an iterative solution. Regression formulae from the literature were used to select $P_CO_2$, lung CO diffusion coefficient ($D_LCO$), and blood volume ($V_b$). Initial blood COHb, total hemoglobin concentration, and ventilation were measured. The Haldane coefficient, M, and the endogenous CO production rate, $\dot{V}_{CO}$, were assigned values from the literature. Solution to the nonlinear CFKE equation was a numerical method that required iteration on $O_2$Hb starting from a trial value of COHb. Both a "continuous" solution which used only the initial value at each blood COHb measurement were used to calculate COHb measurement as a function to time. Subjects were exercised for 45 minutes and blood samples were taken at unspecified intervals (pre-exposure, entry to exposure chamber, during exercise, end of exercise). The conclusion reached was that the CFKE predicted concentrations for both men and women under both sedentary and exercise conditions.

Ott and Mage (1978) suggested returning to a simple first-order linear solution for the CFKE, but fit to data from the

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literature. This approach claimed as advantages that the model has an asymptotic value (which Peterson and Stewart's does not) and that it has utility for temporally-varying values of ambient CO concentration. Ambient CO monitoring data for a full year were applied to the proposed model and it was demonstrated that the 2% COHb level on which the Federal standards are based can be exceeded without violating the Federal exposure standard. The implication is that an exposure based on a uniformly weighted window average could produce COHb levels in excess of the target 2% maximum was further denoted by Venkatram and Louch (1979).

In a technical memorandum (Steinberg and Nielsen 1977) a modified form of the CFKE was proposed as a means of estimating blood COHb in response to environmental CO concentration with work load as a parameter. Rather than propose a new equation with additional assumptions about regression relations among variables, Steinberg and Nielsen chose to divide the CFKE coefficients into five ranges which correspond to alveolar ventilation ($\dot{V}_A$) and $D_LCO$ values representative of five levels of work. The remaining variables were assumed constant, and the resulting equation was intended to be applied to mission segments (a segment is a period for which the CO exposure and work load are relatively constant). This modified CFKE is the recommended means (MIL-HDBK-759A) of calculating blood COHb to determine if limit values in MIL-STD-472C are exceeded.

In yet another manipulation of the CFK model, Joumard et al. (1981) replaced $D_LCO$ with three regression equations (for men, women, and children) which have as input, height and weight;

calculated $\dot{V}_{CO}$ as a function of blood volume and hemoglobin concentration; and referred $\dot{V}_A$ to total body energy expenditure rate (using regression equations for each component: basal metabolic rate; muscular power; and specific dynamic action of food). Experimental verification of one or more of the models was made with sedentary and walking subjects. No significant difference between calculated and measured COHb were found after two hours. No statistical analyses were presented.

COHb prediction has been studied in other, less directly related contexts. The reader may find the following references of interest: (Lilienthal and Pine 1946, Pace et al. 1950, McIlvane et al. 1969, Long 1970, Weir and Viano 1977, Marcus 1980 a,b, Goldsmith 1980, Bernard and Duker 1981, Biller and Richmond 1982).

## Need For Additional Testing

The above review demonstrates that although there exist a number of COHb prediction algorithms which appear to work well, none of these methods has been tested over a wide range of important parameters. To be able to estimate COHb from environmental exposure to CO, a need exists to assure that such predictions can be made over a wide range of concentrations of CO, levels of exercise and durations of CO exposure.

The leading candidate for a useful method of COHb prediction is the CFKE. The CFKE has physical referents which can in many cases be independently measured. It is a deterministic differential equation. It has been tested more widely than other methods of prediction. In the circumstances under which tests

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have been made, the CFKE appears to work well. Such tests were, however, frequently less than definitive since many of the physical parameters of the CFKE were not determined very well for individual subjects but were estimated from published data giving means of groups.

The present experiment was designed to be the first of a series of studies to empirically validate the CFKE as a prediction algorithm for COHb. As a first step it was decided to use a restricted set of parameters under which the CFKE should work well according to the literature. The first experiment was designed to (a) measure CFKE parameters on individual subjects rather than make estimates from the literature (b) quantify exposure parameters and (c) measure COHb. The objectives of the first study were to (a) work out measurement problems for the various parameters (b) assure that the CFKE worked well in a well-documented situation when parameters were measured on individuals rather than from the literature and (c) to provide an initial data base from which more extensive experiments could be designed.

## Analysis of the CFKE

The model selected for COHb prediction in this study is the Coburn-Forster-Kane equation (CFKE). Originally, Coburn et al. (1965) used a nonlinear first-order differential equation,

$$\frac{d\ CO}{dt} = \dot{V}_{CO} - \frac{(COHb)(P_cO_2)}{(O_2Hb)M} \cdot \frac{1}{1/D_LCO + P_D/\dot{V}_A} + \frac{P_ICO}{1/D_LCO + P_D/\dot{V}_A} \qquad [1]$$

in which;

$\dfrac{d\ CO}{dt}$ = Rate of change of body stores of CO, ml/min

$\dot{V}_{CO}$ = Endogenous rate of CO production, ml/min (STPD)

COHb = Concentration of carboxyhemoglobin in the blood, ml gas/ml blood (STPD)

$P_cO_2$ = Average pulmonary capillary oxygen partial pressure, mmHg

$O_2Hb$ = Average concentration of oxyhemoglobin in the pulmonary capillary blood, ml gas/ml blood (STPD)

M = Haldane coefficient (dimensionless)

$P_ICO$ = Partial pressure of CO in the trachea, mmHg

$D_LCO$ = Lung transfer factor for carbon monoxide, ml gas $\cdot$ min$^{-1}$ $\cdot$ mm Hg$^{-1}$ (STPD)

$P_D$ = Alveolar gas pressure, dry, mmHg (=$P_B$ - $PH_2O(T)$)

$\dot{V}_A$ = Alveolar ventilation, ml/min (STPD)

$P_B$ = Barometric pressure, mmHg

$P_{H_2O}(T)$ = Saturation pressure of water vapor at temperature T, mmHg

Equation [1], with $O_2$ held constant, will be referred to as the "linearized CFKE". A further substitution was made to reflect the assumption that CO is associated primarily with hemoglobin, such that

$$CO = (COHb) \cdot V_b$$

in which;

$V_b$ = "Effective" blood volume, ml (no further definition

of "effective" was offered).

Equation [1] then becomes an expression for rate of change of carboxyhemoglobin concentration:

$$\frac{d(COHb)}{dt} = \frac{\dot{V}_{CO}}{V_b} - \frac{(COHb)(P_CO_2)}{(O_2Hb)(M)(V_b)} \cdot \frac{1}{1/D_LCO+P_D/\dot{V}_A} + \frac{P_ICO}{(1/D_LCO+P_D/\dot{V}_A)V_b} \quad [2]$$

The nonlinearity of [2] is apparent only when $O_2Hb$ is recognized as a variable which is dependent on COHb. Substitution of a function,

$$O_2Hb = f(COHb)$$

makes the equation nonintegrable in closed form. The nonlinearity is not severe, even as saturation values of COHb are reached, and a solution may be obtained by a wide variety of numerical methods. Equation [2] will be called the nonlinear form of the CFKE.

For many of the applications of interest, the nonlinear CFKE [2] is appropriate. In any case, it is a more general form of [1]. For this reason all evaluations of the CFKE will refer to [2]. Clearly if [2] were shown to be valid then [1] would implicitly have been validated as well. The particular solutions to [2] will be computed using a fourth-order Runge-Kutta

integration algorithm since [2] has no analytic solution.

The equation used to determine compliance with MIL-STD-1472C is a form of the CFKE which stratifies alveolar ventilation and the lung transfer factor for CO according to five levels of physical activity. Furthermore, typical values are assumed for all other variables in the CFK model. The equation as currently applied is

$$\%COHb(t) = \%COHB(0)\ ^{-t/A} + 218(1-\ ^{-t/A})(1/B + ppmCO/1316) \qquad [3]$$

in which %COHb = percent saturation of hemoglobin with CO

ppmCO = ambient concentration of CO, in parts per million

A,B = constants associated with work level as shown below

| Work Effort Scale | Work Effort Description | A | B |
|---|---|---|---|
| 1 | Sedentary | 365 | 939 |
| 2 | | 211 | 1623 |
| 3 | Light Work | 155 | 2211 |
| 4 | | 119 | 2874 |
| 5 | Heavy Work | 97 | 3536 |

The equation is applied by segmenting the observation period each time that work level or the exposure level of CO changes. An initial value of %COHb(0) = 1% is used, the values of A and B which are consistent with the first work level are substituted into the equation, and a %COHb is calculated for the end of the first segment. This value is then used as %COHb(0) for the second segment, and so on. The value for time (t) is not the total elapsed time of the scenario, but is the length of the segment over which %COHb is being calculated.

Since no new theory is involved it will be considered in this work that [3] will be validated if (a) the CFKE were shown to be valid and (b) the substitutions into [3] are appropriate.

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Thus no separate test of [3] is needed.

## Independent Variables

Haldane Coefficient. The Haldane coefficient (M) in the CFKE expresses the affinity of Hb for CO relative to $O_2$. Numerous values appear in the literature ranging from 210-250 (Joels et al. 1958, Joumard et al. 1981, Longo 1970, Rodkey et al 1969, Sendroy et al. 1929). Fetal value for M has been given as 181 (Astrand and Rodahl 1977). No independent means of estimating M was available so an arbitrary value of 245 was selected.

Blood Volume. "Effective blood volume", ($V_b$ as used in the original CFKE is not further defined by the authors, but the numerical value used in their calculations suggests simply "blood volume" was used. Carbon monoxide is known to bind with other heme molecules in the body, notably myoglobin, cytochrome $a_3$ and cytochrome $P_{450}$ (Argade, et al. 1984; Marden, 1982; Forster, 1970) which may account for as much as 15% of the total body stores (Coburn, 1970). The primary effect of an underestimate of $V_b$ would be to reduce the calculated time constant for equilibration with a consequent overprediction of COHb by the model during a rising transient response.

In this study red cell volume was chosen as a measure, following which blood volume was calculated because it was expected to have a smaller fluctuation over the time period of the experiments than would total blood volume. The red cell volume was obtained by removing approximately 30 ml of blood from an antecubital vein, incubating it for 10-30 minutes with <200 microCuries $Na_2^{51}CrO_4$ and reinjecting an accurately known volume

of the labeled cells. Approximately 30 minutes later, a blood sample was taken from the contralateral vein, the radioactivity levels of the injectate and sample were determined, and the inferred dilution was used to calculate the total volume of red blood cells. Hematocrit (HCT) value was obtained each time a blood sample was withdrawn.

Each blood sample during the experiment removed approximately 3 ml of whole blood, or 3(HCT/100)ml of red blood cells, hence, the value of $V_b$ was recalculated at each sample time to compensate for the total amount of blood removed during the experiment. Replacement of red blood cells in response to the small loss of blood (75-80 ml over 2 hours) was not anticipated to occur.

CO Transfer Factor for Lungs. The rate of diffusion of CO from alveolar gas to the blood or vice versa ($D_LCO$) is a function of the available exchange surface area, the diffusion properties of the alveolar/capillary barrier and the differential driving pressure across the barrier. These terms are generally coalesced into a single term, called the diffusion factor or transfer factor of the lung. $D_LCO$ is generally estimated using either a single breath-holding technique or a multi-breath rebreathing technique. A multi-breath technique was used in this study, but with the addition of gases which allow simultaneous estimation of other variables such as cardiac output, oxygen consumption rate, and lung capillary volume. The subject breathed ambient gas through a mouthpiece and valve. Breathing was paced by a metronome at approximately 40/min. At the end of the measurement, a valve switched the subject's gas supply to a bag

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which contained a volume that was approximately twice the subject's resting tidal volume (e.g., 1.5 liters). Rebreathing at the paced rate continued for 20-30 seconds while the bag contents were continuously sampled by a mass spectrometer. The bag temperature was also sensed by a thermistor in the flow stream.

For the measurement of $D_LCO$, the bag gases of interest were He and $C^{18}O$. The heavy CO was used because (a) its transfer coefficient presumably may be obtained independently of the presence of $C^{16}O$ from either endogenous or exogenous sources, and (b) $C^{16}O$ is indistinguishable from $N_2$ in the mass spectrometer. Helium was used to determine when mixing of the lung and bag gases was complete. Because He is relatively insoluble in tissue, when its concentration becomes constant, it was evenly distributed in the respired volume. Once this condition was met, loss of $C^{18}O$ from the system was presumably only into the pulmonary capillary blood.

Figure 1 shows the typical behavior of He and $C^{18}O$ concentrations during a rebreathing maneuver. The ordinate is the sampled gas concentration on a logarithmic scale. Note that after a few breaths, the peak-to-peak excursions of CO concentration became of constant height, which in log coordinates means that a constant fraction of the CO disappeared per unit time.

Calculation of $D_LCO$ typically was made by identifying the time at which concentration of He was constant (within a specified band), moving forward to some fiduciary point on the CO

concentration curve (e.g., the fourth minimum after He stabilized), and fitting a straight line to the remaining CO data (peaks, valleys, or all). For single isolated determinations of $D_L CO$, the equation used was (Cotes, 1975):

$$D_L C^{16}O = - \frac{V_{SYS}}{P_D \cdot t} \cdot \ln \frac{(C^{18}O)(t_2)}{(C^{18}O)(t_3)} \cdot (30/28)^{1/2} \qquad [4]$$

in which;

$V_{SYS}$ = total volume of lungs and rebreathing bag, (ml STPD)

$P_D$ = dry barometric pressure in lung/bag system

$(P_B - P_{H_2O}(T(bag)))$, (mmHg)

t = time interval between CO determinations

$(t_3-t_2)$, min, $t_3 > t_2$

$(C^{18}O)(t)$ = concentration of alveolar $C^{18}O$ at time,

t, (% fraction, or mmhg)

30/28 = ratio of molecular weights, $(C^{18}O/C^{16}O)$

When multiple determinations were made with intervals which were insufficient for the partial pressure of $C^{18}O$ to decay to zero, then the driving pressure between lung gas and blood was no longer simply alveolar partial pressure (or concentration), but the difference between alveolar and blood partial pressures. The corrected equation was then

$$D_L C^{16}O = - \frac{V_{SYS}}{P_D \cdot t} \cdot \ln \frac{(C^{18}O)(t_2) - (C^{18}O)_c}{[C^{18}O)(t_3) - (C^{18}O)_c]} \cdot (30/28)^{1/2} \qquad [5]$$

in which $(C^{18}O)_c$ is the concentration of $C^{18}O$ in equilibrium with COHb in the pulmonary capillary blood.

In the present work, $D_L CO$ was determined multiple times over a two-hour period on both the "air" day and the "CO" day. On the

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air day, a final blood COHb corresponding to as much as 5% saturation resulted from the rebreathing maneuvers. This corresponds to an equilibrium concentration of $C^{18}O$ in the range of 30 ppm. Bag concentrations which were initially on the order of 3000 ppm generally decayed to approximately 200 ppm at the times corresponding to $t_3$ thus the "back pressure" may have provided as much as 15% error to the denominator of the log expression. In effect the corrected equation [5] was used where the value for $(C^{18}O)_c$ was obtained from the measured blood COHb and a curve fit to saturation data (WHO, 1979). A least-squares cubic fit to the tabular data is

$(C^{18}O)_c = 0.00106 (COHb)^3 + 0.0462(COHb)^2 + 5.68(COHb) - 0.02$  [6]

where COHb is in % saturation, and $(C^{18}O)_c$ is in ppm.

While this correction was relatively straightforward for young, healthy, normal subjects with a low resting level of COHb, the problem on a "CO" day became complex. While it was true that the use of $C^{18}O$ to measure a CO transfer factor would suffer no interference from $C^{16}O$ if one were dealing only with physical solubility of gases in plasma, the fact that $C^{18}O$ competed with $C^{16}O$ for hemoglobin means that $C^{16}O$ did indeed interfere with the $D_LCO$ measurement through a reduction in the effective solubility of $C^{18}O$. Therefore, the $D_LCO$ calculation on the "CO" day used an alveolar CO corrected for the equilibrium pressure which corresponded to the measured COHb.

Because respiration is not a continuous function with respect to breath-to-breath variation of CO concentration, the exact form of the exponential decay to be used for $D_LCO$

calculation (or of $PI_{CO}$ during rebreathing) was not obvious. A straight line (in the semi-log coordinates) could be fit to the relative minima, the relative maxima, or to all the data in the region where a single exponential appeared to apply. The slopes of these lines would all have been the same if the blood concentration of CO were zero, but one is still faced with defining the driving pressure for a breath as the relative minimum, the relative maximum, or some average of the two. When the COHb was not zero, the same complication arose and in addition, the slopes of the three curve fittings were not identical.

As a means of dealing with this problem, a constant value was subtracted from the rebreathing CO concentration corresponding to the partial pressure of CO in equilibrium with the value of COHb obtained immediately prior to the rebreathing maneuver. A straight line approximation was made to the difference data over the range for which a single exponential appeared to apply.

Calculation of $D_L CO$ then reduced to

$$D_L C^{16}O = V_{SYS} \cdot A_1 \cdot (30/28)^{1/2} / P_D \qquad [7]$$

in which the variable $A_1$ is the slope of the difference data $(\ln(C^{18}O(t) - (C^{18}O)_c))$. As with a few other variables in the CFK equation, averaging of data was used in estimating $D_L CO$.

Inspired Partial Pressure of CO. CO was delivered to the subject through both the chamber ambient air (during exposure) and through the closed rebreathing system (during $D_L CO$ testing). Determination of the partial pressure ($P_I CO$) for the two sources was quite different. In the chamber air, the fraction of CO,

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$F_ICO^*$ in ppm (quantities with an asterisk refer to an ambient air measurement) was obtained from an infrared analyzer via a sampling tube near the chamber air supply vent. $P_ICO^*$ in the chamber was calculated as the product of $F_ICO^*$ and barometric pressure, $P_B$ in mmHg, obtained 3 or 4 times during the experiment.

$$P_ICO^* = F_ICO^* \cdot P_B \cdot 10^{-6} \text{mmHg} \qquad [8]$$

During rebreathing, gas composition in the subject/bag system was determined with a mass spectrometer which removed gas at a rate of 60 ml/min. The mass spectrometer supplied voltages in the range of 0-10 volts for each channel ($O_2$, $N_2$, $CO_2$, $C_2H_2$, $C^{18}O$) which were converted by a 12-bit analog to digital (A/D) converter under control of a microprocessor.

The value of $P_ICO$ which is used in the CFK model is intended to be that which enters the alveoli, and thus $P_ICO^*$ must be corrected for water vapor saturation.

$$P_ICO^* = (P_B - P_{H_2O}(T)) \cdot F_ICO^* 10^{-6} \text{mmHg} \qquad [9]$$

in which $P_{H_2O}(T)$ is calculated for body temperature.

Calculation of $P_ICO$ during the rebreathing maneuvers was complex, because the concentration of CO in the inspired air varied over a range of more than 10:1 from beginning to end. In addition, the equivalent bag/lung system temperature also changed, so that the correction factors for temperature and water vapor pressure used to obtain STPD values also varied. A simple algorithm for accurately dealing with this situation was not available. The rate at which changes were taking place was comparable to the respiratory period.

An algorithm was devised for the above calculation after several others were tried and discarded. The final choice was an algorithm which used the inspired $C^{18}O$ peaks and their corresponding sample times as estimates of peak alveolar concentration. The peaks were computed by taking the $C^{18}O$ minima multiplying them by the MAX/MIN ratios. At worst, it was assumed that the peak data would represent an upper bound on the estimate of $C^{18}O$ exposure. This method was found to produce estimates of COHb formation on the AIR DAY which compared favorably with the observed COHb.

Average Pulmonary Capillary Oxygen Pressure. A forced end expiratory gas sample was presumed to be representative of alveolar air average pulmonary capillary oxygen pressure ($P_CO_2$). In these experiments, the value obtained via the mass spectrometer was the minimum value observed at end expiration, during rebreathing determinations of $D_LCO$. It is not clear whether the rebreathing maneuver results in a good approximation of an alveolar air sample, as the breathing is paced rapidly (40 per min.) and the bag volume is relatively small (approximately 1.5 liters).

Average Pulmonary Oxyhemoglobin Concentration. $O_2Hb$ is another variable which, as a single measurable quantity, does not exist. The appropriate point for sampling within the capillary, even if such transduction were possible, is not obvious. At best, measurements of arterial and mixed venous values might be used to calculate an average value.

In this study, concentration was treated differently in the linearized and nonlinear CFKE. In the former

$$O_2Hb = k(THb(100-\%MHb-\%COHb) \times 1.39 \times 10^{-4} \qquad [10]$$

in which k is a fixed number <1 (typically 0.97).

THb is the measured total hemoglobin concentration and 1.39
x $10^{-2}$ is a conversion factor to yield $O_2Hb$ in ml gas/ml blood.
In the nonlinear CFK equation

$$O_2Hb = k(THb(100 - \%MHb)/(100 - \%COHb) \qquad [11]$$

in which COHb is the calculated value (dependent variable) and k
is as in [10] above.

Alveolar Ventilation. Measurement of alveolar ventilation
($\dot{V}_A$) is complicated by several factors:

(1) Alveolar ventilation is not directly measurable and must
     be inferred from operations on expiratory minute
     ventilation ($\dot{V}_E$).

(2) Reasonably accurate means of obtaining minute
     ventilation alters respiratory rate and depth, e.g., the
     use of mouthpieces or masks.

(3) More innocuous estimation methods of $\dot{V}_E$ (e.g. inductive
     plethysmography, magnetometry, electrical impedance
     spirometry) are sensitive to variables other than gas
     volume motion and thus exhibit large variance when
     posture is not rigidly controlled.

$\dot{V}_E$ signals from an inductive plethysmograph (Respitrace)
were recorded continuously on a stripchart recorder for later
conversion to digital form using a graphics digitizer pad.   A
scale factor was developed to relate  the 1-liter syringe volume
recorded with the spirometer to pen excursions for the
Respitrace.   The Respitrace record was "recalibrated" each time

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the subject executed a rebreathing maneuver. The peaks and valleys of paced respiration recorded by both spirometer and Respitrace were digitized for 10 corresponding cycles, the total times for both records were obtained (which should be identical), and a "calibration" factor was calculated. $\dot{V}_A$ was then calculated as:

$$\dot{V}_A = (\dot{V}_E - V_D) \cdot f \cdot k_{tp} \text{ ml/min, BTPS} \qquad [12]$$

in which;

$\dot{V}_E$ = minute ventilation in ml/min, BTPS

$V_D$ = physiological dead space in ml, BTPS

$f$ = breathing frequency, min$^{-1}$

$k_t$ = 273/(273 + $T_M$)

$k_p$ = ($P_B$ - $P_{H_2O}(T)$)/760

$T_M$ = temperature, degrees Centigrade, at which
        volume or flow is measured

$P_B$ = ambient atmospheric pressure, mmHg

$P_{H_2O}(T)$ = saturation pressure of water vapor at
        temperature T.

The value used for dead space is an anatomic dead space which was calculated from subject height. The dead space ventilation was calculated as a product of dead space volume and respiratory frequency. The latter value was obtained from digitizing the Respitrace stripchart recording of 10 breaths which were selected near the time of each scheduled blood withdrawal. Considerable operator judgment was required in

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selecting the segments of data to be digitized, particularly when the subject was at rest. Both depth and rate of respiration were variable and in some cases could not be distinguished from artifacts.

Saturation Pressure of Water Vapor. Saturation pressure of water vapor, $P_{H_2O}(T)$, is a function of temperature of the gas mixture of which it is a component. In this experimental work, $P_{H_2O}(T)$ can range from 20 to 50 mmHg (corresponding to a temperature range of 22°C to 38° C). The usual correction from BTPS to STPD assumes a body temperature of 37°C and a resulting $P_{H_2O}(T)$ of 47 mmHg. Because a portion of this work uses a rebreathing system, in which both the temperature and water vapor pressure are functions of time, a least-squares curve fit to a second-order polynomial was used to derive a continuous expression for $P_{H_2O}(T)$ vs. temperature. The resulting function is;

$$P_{H_2O}(T) = 31.33 - 1.73 \, T_M + 0.058 \, T_M^2 \text{ mmHg} \quad\quad [13]$$

in which $T_M$ is the measured temperature in °C. This function reproduces the values at integer values of temperature between 25°C and 42°C within 0.05 mmHg.

COHb and THb. COHb was calculated from the percent COHb saturation in blood samples

$$COHb(0) = \%COHb(0) \cdot THb \cdot 10^{-2} \cdot (1.39 \cdot 10^{-2}) \text{ml gas/ml blood} \quad [14]$$

Total hemoglobin concentration, THb, and %COHb are obtained from the IL282 CO-Oximeter. The multiplier, $10^{-2}$, converts %COHb(0) to a fraction, and the term, $1.39 \times 10^{-2}$, converts hemoglobin concentration in g/dl to the equivalent volume of bound gas (CO

or $O_2$) in ml gas/ml blood (STPD).

## Subjects

Forty male nonsmoking subjects, aged 19-32 years, were recruited for this study. The majority of subjects were students of the local universities with some participation from non-university personnel from surrounding communities. The volunteers underwent initial medical screening which comprised physical examination, medical history, routine blood chemical screening (using the Technicon SMA 20) and complete blood count with differential. If the above tests were within normal limits, and specifically if the subject had a normal cardiovascular and pulmonary status, the subjects underwent exercise stress testing. Following physical evaluation and initial screening, 17 subjects had withdrawn from further participation; thus, only 23 subjects were stress tested. The Standard Bruce exercise stress protocol was employed including 12-lead ECG and blood pressure measurements every 1.5 minutes, under supervision of a physician experienced in cardiac stress testing. If no abnormality was detected, subjects were scheduled for blood volume determination at the end for a subsequent training session. At this stage four more subjects decided not to continue with further testing and exposures.

A total of 19 subjects completed all the tests and exposures. The initial group of six subjects participated in a pilot study, while the remainder (13 subjects) formed the main study group. They were all healthy, young black or white men, nonsmokers, with no history of cardiovascular or pulmonary disease.

## Procedure

All subjects who completed the study were exposed on consecutive days (Thursday and Friday morning). During the initial pilot study (six subjects), the chamber atmosphere was only air on the first day and air with 100 ppm CO on the second day. Other methodological alterations were made during the pilot phase, thus rendering the six pilot subjects non-comparable to the main study group as well as among themselves. In the main study (13 subjects), the first day was randomly assigned to air only or air and 100 ppm CO, with the second day as the alternate choice. No effort was made to conduct the study in a blind or double-blind manner. The pilot study was carried out between December 1982 and April 1983, and the main study was halted at the end of December 1983.

Prior to the first exposure day, each subject had completed all screening tests and had undergone a blood volume determination. Upon arrival on the first exposure day, the subject was given a physical examination, a symptom interview, and was again briefed on the experimental procedures.

The subject, then supine and dressed in a jumpsuit, had an indwelling catheter inserted into the left antecubital vein, and two samples of blood were removed for blood gas analysis. During subject preparation, the investigators calibrated the chamber and monitoring instrumentation. When the subject arrived at the exposure chamber, he had the three-lead ECG telemetry system connected, and a pair of Respitrace transducer belts and a blood pressure cuff attached. Half of the main study subjects were

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also fitted with 12-lead ECG electrodes and circumferential electrodes for systolic time interval measurement.

The exercise bicycle, rebreathing assembly, and lead dress were adjusted for minimum subject discomfort. Several "dry runs" were then made with the ergometer and rebreathing mouthpiece to allow the work loads for the desired range of ventilation to be determined. Rebreathing maneuvers using only air were also performed to ensure the subject understood the procedure.

Simultaneous recordings of ventilation estimated by both the Respitrace and the spirometer were made with the subject in the normal cycling and rebreathing position, and a final adjustment of the Respitrace channel was made. The subject then performed four or five rebreathing maneuvers, using the special gas mixture. Upon satisfactory completion of the maneuvers, he was disconnected from all systems (except the ECG telemetry), and was allowed to rest outside the chamber for approximately one hour while additional preparations were made and the chamber atmosphere was established.

Immediately before entering the chamber for the exposure, a pre-exposure blood sample was taken to establish the initial value of blood COHb that was to be used in all model calculations. The subject then entered the chamber, was reconnected to the measurement system, and was seated on the ergometer.

Figure 2 shows the target events for the duration of the experiment. Blood samples were taken at approximately five-minute intervals, exercise levels changed at fifteen-minute intervals, and rebreathing maneuvers were scheduled at fifteen-

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minute intervals to lie within the last third of each exercise level. Exercise work load was either 25 or 40 Watts.

At the completion of the exercise, the subject rested in the chamber for 30 minutes while the data sampling routines continued. At the end of the two-hour experiment, the subject left the chamber. If his blood COHb was greater than 10%, he was given 100% $O_2$ to breathe from a demand valve for 10-30 minutes. At the end of the $O_2$ breathing, blood COHb was again measured. In all subjects, the post-$O_2$ value of COHb was below 10%. Upon achievement of a level below 10%, heart rate and blood pressure were recorded, the intravenous catheter was removed, and the subject was released.

## Quantitative Methods

Missing Data. As is inevitable in a procedure as complicated as the present experiment, some of the data values were missing due to equipment failure, recording errors, etc. The missing data were estimated and substituted into the data set by the procedures discussed in the following paragraphs.

Data in the experiment can be considered to be a time series. Therefore missing data could be estimated by averaging across adjacent values in the same experimental condition. If the missing element was in the body of the data array (i.e., not the first or last element), then the substituted value was the average of the data values immediately before and after the missing element. If the missing element was first in the array, then the second value is substituted. If the missing value is the last, then the next to the last value is substituted.

Occasionally more than one adjacent element was missing. If two adjacent elements were missing, the first missing element was filled with the value immediately before it, and the second missing element was filled with the average of the values immediately before and after the missing pair. If three or more missing elements occurred at the end of the data set, no estimates were made and the data set was rejected. Finally, if two or more adjacent missing variables occurred at the beginning of a data set, or three or more adjacent missing values occurred in the body of a data set, then the data set was rejected.

Pairing Time Series Data. Variables in the experiment were sampled at various rates, thus there were no values for some of the slowly sampled variables which corresponded to each value of the more rapidly sampled variables. There were three types of algorithms used to expand the slowly-sampled data so that values existed for pairing with the most rapidly sampled case.

Algorithm 1 used the actual data value when available. The remaining blank data elements were filled with the mean of the actual sample values before and after the blank segment. The program values interpolated using algorithm 1 are: THb, $O_2Hb$, COHb, MHb, $V_D$, $\dot{V}_E$, $\dot{V}_A$, $P_B$, ambient temperature, body temperature, $PH_2O$, and $P_ICO$.

Algorithm 2 took the actual data sample and copied it to each blank data element until the next actual data sample was reached. Hence the interpolated data were held constant between actual sample records. The variables interpolated using this algorithm are: $V_b$ and $\dot{V}_{CO}$.

Algorithm 3 used a description of the experiment protocol to

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hold data values constant across each work level segment. The average value of the actual data samples was determined for each segment and used to fill the associated blank data elements. The variables interpolated using algorithm 3 are: $V_{O_2}$, $D_LCO$ and $P_cO_2$.

Statistics. The principal hypothesis of the experiment was about the ability of CFKE (and related models) to predict COHb formation. To test this hypothesis, the predicted COHb was calculated as a time function for each of the 24 blood samples collected during exposure by use of the CFKE. Thus there was a predicted and an observed COHb time function for the air day and the CO day for each subject. Even though other models than the CFKE were tried, they were all strict subsets of the CFKE and thus did not require formal testing. Note that for the air day and the CO day as well, the effects of the CO exposure during the rebreathing maneuver were considered (see "Inspired Partial Pressure of CO, $P_ICO$").

Statistical tests of the data were performed on the differences between predicted and observed COHb. The air and CO days were tested separately by the repeated measures approach to analysis of variance (Kirk 1968). Since two tests were performed on data from the same subject in the same experiment and the experimentwise type I error rate was to be controlled at alpha=0.05, the individual tests were evaluated at alpha=0.025 each.

Exploratory analysis was also performed on $D_LCO$ measurements. Special attention was given this variable because it is quite costly to measure and is an important variable in the

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CFKE. A multifactor repeated measures analysis of variance was employed to test the effects of CO exposure, time, exercise and all of their interactions upon $D_L CO$.

Various descriptive statistics (means, measures of variation and correlation) were used to describe the measures used in the CFKE. It should be ^mphasized that the descriptive data were not tested for stat    ·     significance in order to avoid inflation of experimentwise ype I error rate. With only 13 subjects in the main experiment, test power would be severely compromised if alpha were distributed over many significance tests.

## RESULTS

The means, standard deviations and ranges for the physical and hematological characteristics of the thirteen subjects in the main study are given in table 1.

### COHb Prediction

Figure 3 is a graph of the COHb values at each of the times in the experiment when blood was drawn. Both air and CO days are shown. Prediction errors (model prediction minus measured COHb) are plotted for the nonlinear CFKE in figure 4. Prediction errors were computed for individual subjects and then averaged. Standard deviations were pooled across all measures to produce the estimated standard deviation shown on the graphs.

Table 2 gives the results of two ANOVAs, one for air day and one for exposure day. The ANOVAs are tests of the significance of errors of prediction from the nonlinear CFKE. The only significant errors of prediction occurred on the air day in which there was a significant linear trend in errors. Inspection of figure 4 and table 2 reveals that the nonlinear CFKE consistently underpredicted COHb formation on air day and that the trend worsened linearly over time. No statistically significant prediction errors occurred on exposure day.

### Exploratory Analyses

Other Models. Similar analyses of prediction error were conducted for the linear CFKE and the MIL-HDBK-759A model. Since these are both strict subsets of the nonlinear CFKE, these analyses are only mentioned here. The results were similar to those from the nonlinear CFKE, as would be expected.

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Diffusion of the Lungs for CO. The means and standard errors of $D_L CO$ measurements broken down for the two experimental days (air and exposure) the three levels of exercise (rest, level 3 and level 4) and time during exposure (first vs. second hour) are given in table 3. A Geisser-Greenhouse corrected ANOVA of the $D_L CO$ data on Table 3 is given in Table 4. Only exercise level was observed to have a "significant" effect. Table 5 gives the simple effects of exercise on $D_L CO$ as the means of $D_L CO$ at each level of exercise averaged across all other variables. It is observed that $D_L CO$ increases as a function of exercise.

Hemoglobin. The values for $O_2 Hb$ for each blood sample are plotted in figure 5. Comparison of figure 5 with figure 3 confirms that $O_2 Hb$ is the compliment of COHb. Mean MHb did not vary detectably throughout the experiment (grand mean = 0.358%, S.D. = $\pm 0.137$, range = 0.03 - 0.90).

Respiration. Figure 6 is a plot of $\dot{V}_E$ values across the time of the experiment for both air and CO days. $\dot{V}_E$ is seen to increase during exercise. Figure 7 is a corresponding plot for $f\text{-}\dot{V}_E$. As discussed in Methods, $\dot{V}_E$, as measured by Respitrace, is a highly erratic procedure and is influenced by body posture and electrode movement. Errors in $\dot{V}_E$ due to such variables were quantified by pre-post calibration procedures. Such errors had a mean absolute value of 0.131.

Since $\dot{V}_A$ was calculated from $\dot{V}_E$ and $V_D$, the latter of which is estimated from $f\text{-}\dot{V}_E$ and subject height, $\dot{V}_A$ will also be poorly estimated.

The value of $PCO_2$ was not influenced by COHb and had a mean value of 108.6 mmHg, S.D. = $\pm 7.43$, range = 64.5 - 130.1.

# DISCUSSION

## CFKE Prediction

It would appear from table 2 and figure 4 that the CFKE is a good predictor of COHb formation on the CO day, since no statistically significant departure from predicted values was observed. This conclusion must, however, be tempered by other findings and observations. An indication that some problem exists is that on the air day, the COHb formation (due to $C^{18}O$ transients during the rebreathing maneuver) was significantly more than predicted by CFKE, and the tendency for underprediction worsened linearly with time.

The method for estimation of CO exposure due to the rebreathing maneuver, as discussed in the methods section, was a post hoc invention, after it was recognized that such exposure was not trivial. The method is by no means a unique solution. It is possible therefore that the underestimation of COHb formation on the air day was the product of a poorly quantified CO dose.

The CFKE was devised for nearly "steady state" conditions. The CO exposure during rebreathing maneuvers was a transient exposure. It is not clear that the CFKE would not over or under predict under transient exposure conditions. Thus, the underprediction of COHb formation on the air day may have been due, in some unknown proportion, to problems in the CFKE with the transient case.

The underprediction of COHb formation on the air day presents a problem with interpretation of the CO day data. The same method was used on the CO day to estimate COHb formation due to transient CO exposure in addition to COHb formation due to the quasi-static exposure to CO. If the method for estimation of COHb due to transient exposure underpredicted on the air day, it may well have done so on the CO day. In that case the good prediction on the CO day may have been due to the sum of two prediction errors; a slight overprediction of COHb formation by the CFKE during quasi-static exposure to CO, and an underprediction of COHb formation due to transient $C^{18}O$ exposure, for whatever reason. Such errors could have offset each other and have produced the "accurate" estimates on the CO day.

While the above argument is plausible, there is no way to evaluate the probability that compensatory error did occur. In fact, without measurement of transient exposure and evidence that the CFKE works well in the transient case, there is no way to evaluate the errors of estimation. To be sure, the error of prediction on the air day was small, even though statistically significant. Thus, even if compensatory errors occurred, their magnitude would probably have accounted for less than 0.5% COHb. There is no way, however, to generalize such speculations to higher or lower levels of COHb formation due to quasi-static exposure with a superimposed $C^{18}O$ transient exposure. Until further studies are performed, the issue of CFKE accuracy must remain in question.

## Respiratory Variables

Measurement of $\dot{V}_E$ is particularly critical to the evaluation of the CFKE since $\dot{V}_A$ is an important variable in the CFKE and since $\dot{V}_A$ must be estimated from $\dot{V}_E$. In the present study, an inductive impedance pneumogram was used to measure $\dot{V}_E$. This was to avoid alterations in respiratory patterns which are typically induced by mask or mouthpiece pneumography. The errors in pre-post measurement calibration, however, were quite large. The result of such errors would be to raise the variability of $\dot{V}_E$ and any other variables derived from $\dot{V}_E$. The variability of $\dot{V}_E$ and $f-V_E$ is reflected in figures 6 and 7.

Increased variability in $\dot{V}_E$ would have increased the error variance in estimates of COHb formation by the CFKE. This effect would have decreased the sensitivity of the experiment. Since the COHb prediction error was in fact found to be statistically significant, the experiment was apparently fairly sensitive. Therefore, despite the fact of increased variance in $\dot{V}_E$, the conclusions from the study remain valid. Increased variance in $\dot{V}_E$ remains, however, the limitation in the sensitivity of the study.

The instability in the calibration of the inductive pneumogram was apparently due to changes in the subject's posture during the measurement of $\dot{V}_E$ and to changes in electrode position. While the method allows undisturbed respiratory patterns, it leaves much accuracy to be desired.

## Lung Diffusion for CO

The diffusion coefficient of the lung for CO, $D_LCO$ was measured repeatedly during the experiment by the rebreathing method. Such frequent measurement was performed because the stability of $D_LCO$ across time and across experimental manipulations was in question. The latter issue has been resolved in this study.

As demonstrated in the exploratory analysis of $D_LCO$, the repeatability of the measure is quite good, even across days. It appears, therefore, that the measure need not have been repeatedly made in the study nor even on the same day as the experiment. In future experiments the problem of transient CO exposure due to the rebreathing maneuver, can be entirely avoided. $D_LCO$ can be measured on a day previous to the experiment so that all exposure on the experimental day will be due to the quantified experimental manipulation.

The only variable that affected $D_LCO$ was that of exercise. The exercise level is likely to have increased alveolar perfusion and thereby have increased $D_LCO$. Therefore, if $D_LCO$ is to be measured on an occasion, other than during the experiment, then the measurement must be made at the same exercise level or levels as will be used in the experiment. In that way, the appropriate $D_LCO$ value can be used.

## Miscellaneous Exploratory Results

All measurements of subject characteristics and hematological variables appear to be within normal limits. $O_2Hb$ and COHb are complimentary, as would be expected. No other remarkable result was found in exploratory analyses.

## Conclusions

1.  COHb formation seems to be well predicted by CFKE in the quasi-steady-state case but the conclusion is tempered by the problem of an unknown amount of $C^{18}O$ exposure during the rebreathing maneuver. Thus the present experiment is not a general test of the accuracy of the CFKE.

2.  $D_LCO$ need not be measured during the experiment or even on the same day as the experiment.

3.  Inductive pneumography was the least accurate measure and thereby limited the sensitivity of the experiment.

## TABLE 1

### CHARACTERISTICS OF SUBJECTS IN MAIN STUDY (n=13)

|  | Mean | S.D. | Range |
|---|---|---|---|
| Age (years) | 23.60 | 3.40 | 19.6-31.3 |
| Height (cm) | 179.30 | 4.80 | 174.0-190.0 |
| Weight (kg) | 72.20 | 9.80 | 55.3-87.4 |
| THb (gm%) | 15.40 | 1.00 | 11.4-17.0 |
| $V_b$ (liters) | 5.047 | 0.603 | 3.51-6.05 |
| $V_{rbc}$ (liters) | 1.998 | 0.241 | 1546.0-2323.0 |
| HCT (%) | 44.5 | 2.517 | 34.0-48.0 |

Abbreviations:

cm = centimeters

gm = grams

HCT = hematocrit

kg = kilograms

THb = total hemoglobin concentration

$V_b$ = blood volume

$V_{rbc}$ = red blood cell volume

# TABLE 2

## TESTS OF SIGNIFICANCE FOR PREDICTION ERRORS OF THE NONLINEAR CFKE

| SOURCE | AIR DAY | | | CO DAY | | |
|---|---|---|---|---|---|---|
| | F | df | p | F | df | p |
| OVERALL MEAN | 29.18 | 1,6 | 0.002* | 0.407 | 1,9 | 0.540 |
| LINEAR | 51.96 | 1,6 | <0.001* | 0.477 | 1,9 | 0.507 |
| QUADRATIC | 5.44 | 1,6 | 0.059 | 1.379 | 1,9 | 0.270 |
| CUBIC | 4.74 | 1,6 | 0.072 | 1.045 | 1,9 | 0.333 |

*$p < $ alpha.    Alpha = 0.025 (per ANOVA)

Alpha = 0.05 (Experimentwise)

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# TABLE 3

## MEANS AND STANDARD DEVIATIONS OF $D_LCO$ FOR VARIOUS TIMES DURING EXPERIMENT

### (n=8)

### Hour 1

|      | Rest        | Exercise 3  | Exercise 4  |
|------|-------------|-------------|-------------|
| Air  | 32.9 ±2.86  | 37.5 ±3.48  | 36.9 ±5.69  |
| CO   | 33.8 ±5.29  | 38.1 ±5.97  | 40.0 ±5.80  |

### Hour 2

|      | Rest        | Exercise 3  | Exercise 4  |
|------|-------------|-------------|-------------|
| Air  | 33.5 ±3.03  | 37.1 ±2.55  | 38.7 ±2.86  |
| CO   | 33.9 ±6.28  | 38.7 ±5.06  | 40.7 ±5.83  |

# TABLE 4

## EXPLORATORY UNIVARIATE REPEATED MEASURES ANALYSIS OF $D_L CO$

### (n=8)

| Effect | df | F | $p^*$ |
|---|---|---|---|
| CO | 1,7 | 1.14 | 0.32 |
| HOUR | 1,7 | 0.71 | 0.43 |
| EXERCISE | 1.23, 8.59* | 45.76 | <0.0001 |
| CO x HOUR | 1,7 | 0.07 | 0.80 |
| CO x EXERCISE | 1.37, 9.60* | 2.57 | 0.14 |
| HOUR x EXERCISE | 1.16, 8.11* | 0.31 | 0.62 |
| CO x HOUR x EXERCISE | 1.59, 11.10* | 0.57 | 0.54 |

*Geisser-Greenhouse corrected

TABLE 5

MEANS OF $D_L CO$ AT EACH LEVEL OF EXERCISE

| Rest | Exercise 3 | Exercise 4 |
|------|-----------|-----------|
| 33.5 | 37.9 | 39.1 |

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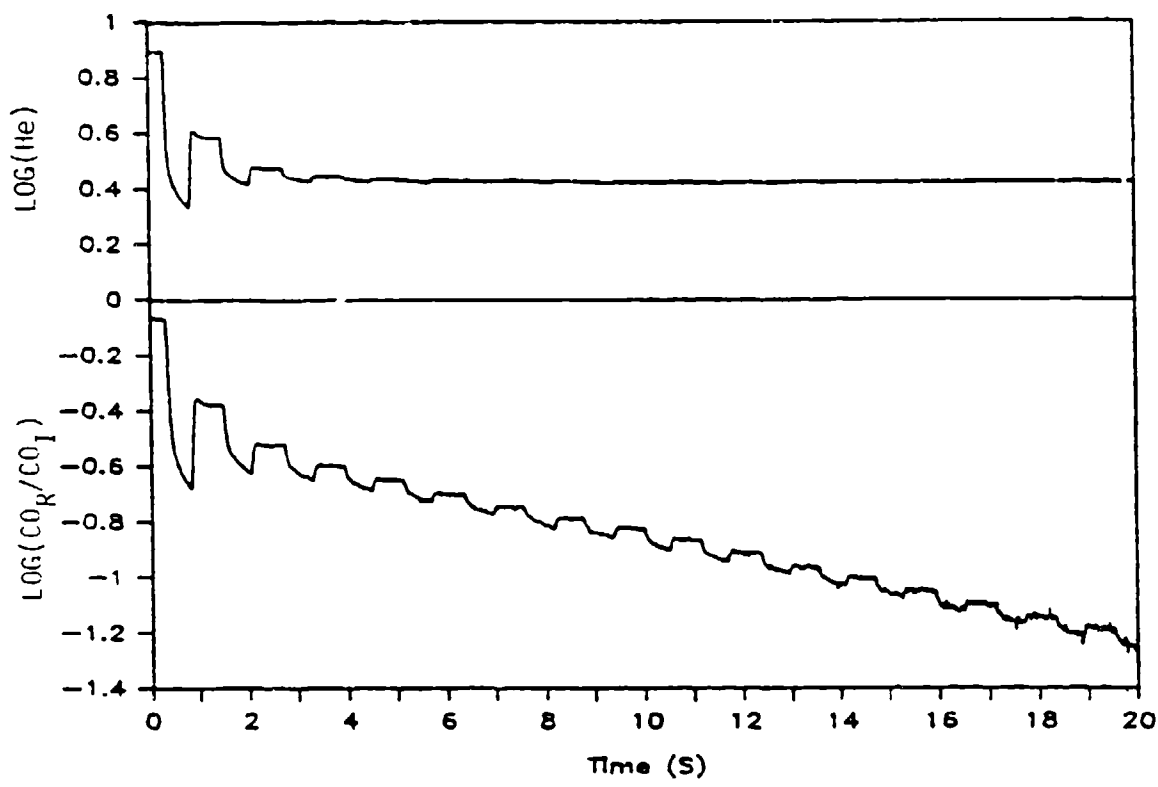
Figure 1. Concentrations vs. time for He and $C^{18}O$ during a rebreathing maneuver. Note that the $C^{18}O$ has been normalized to the initial bag concentration to allow simultaneous viewing with He. $CO_R = C^{18}O$ during rebreathing, $CO_I$ = Initial bag concentration of $C^{18}$, both measured in ppm. He measured in %.
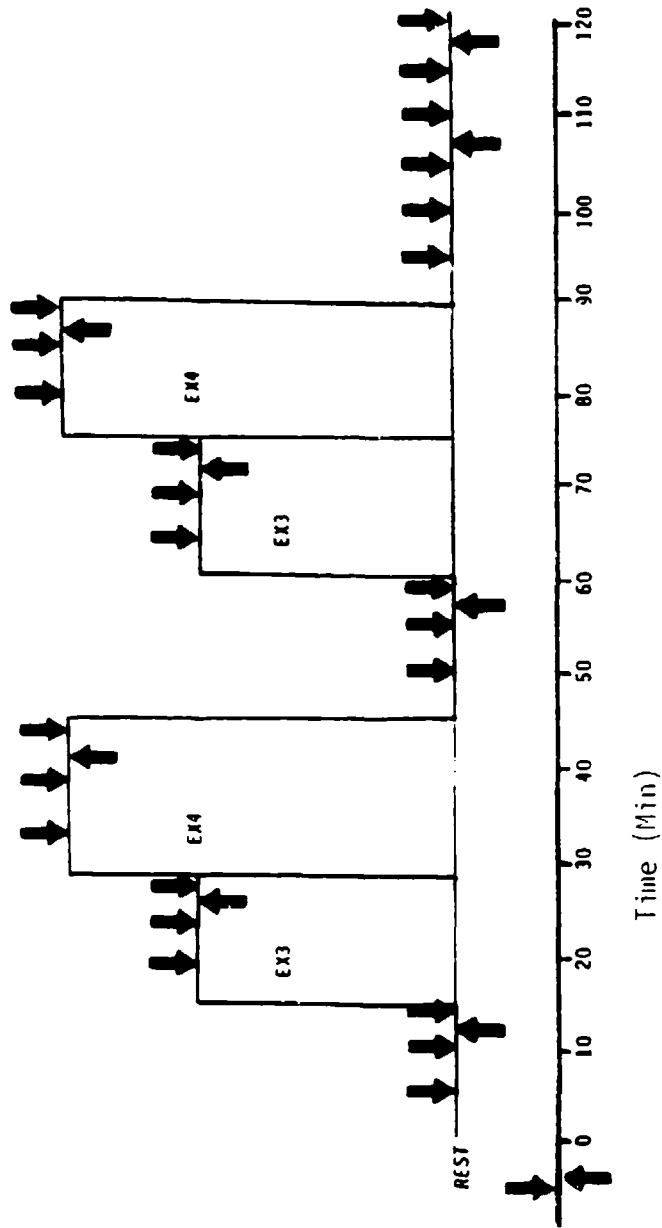
Figure 2. Schedule of events during the experiment. Bars labeled EX3 and EX4 refer to exercise periods. EX3 was 20 Watts, EX4 was 40 Watts.
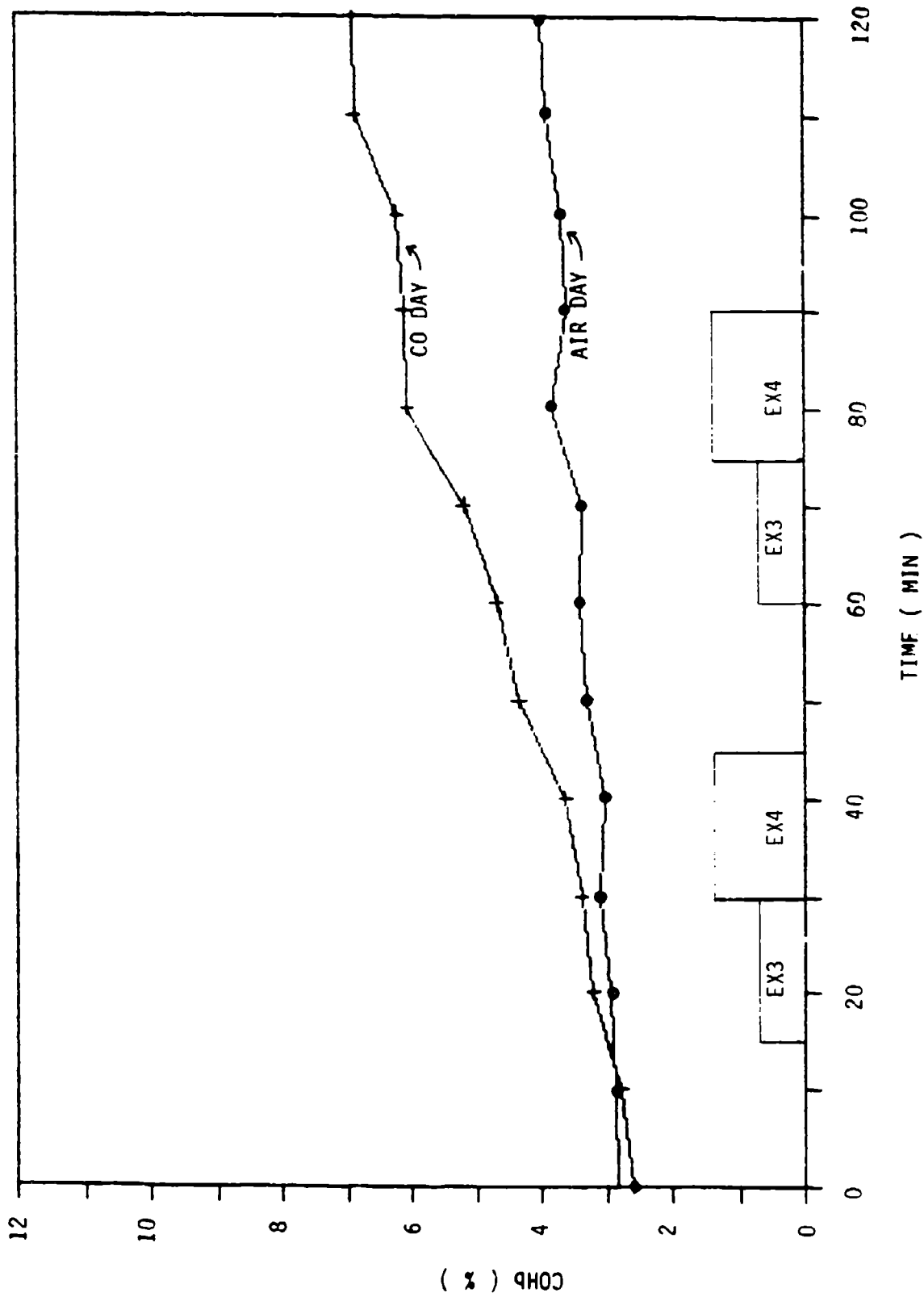
Figure 3. Graphs of mean COHb levels for air and CO days. See figure 2 for definition of symbols.
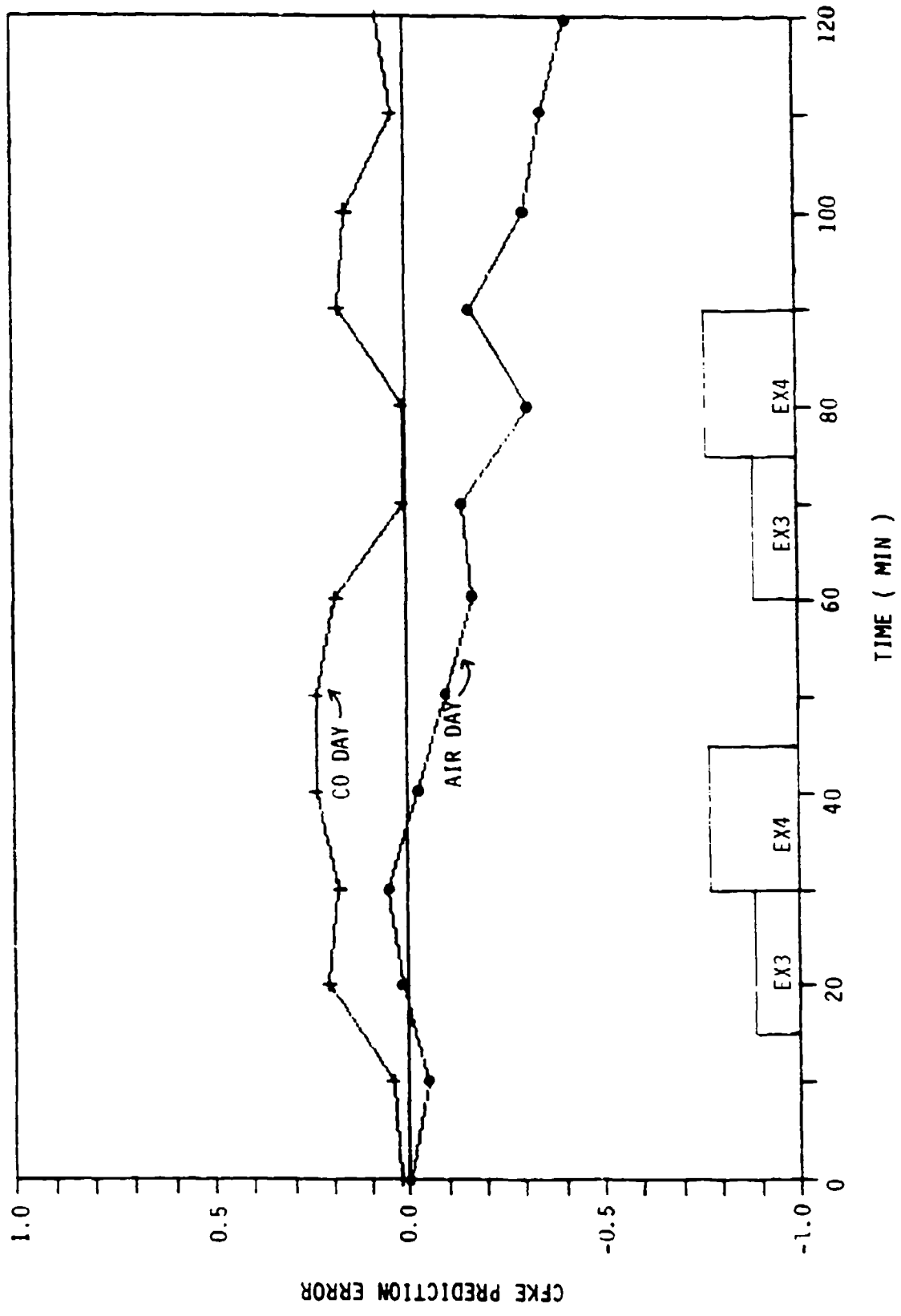
Figure 4. Graphs of mean CFKE prediction errors for air and CO days (predicted minus measured COHb). See Figure 2 for definition of symbols.
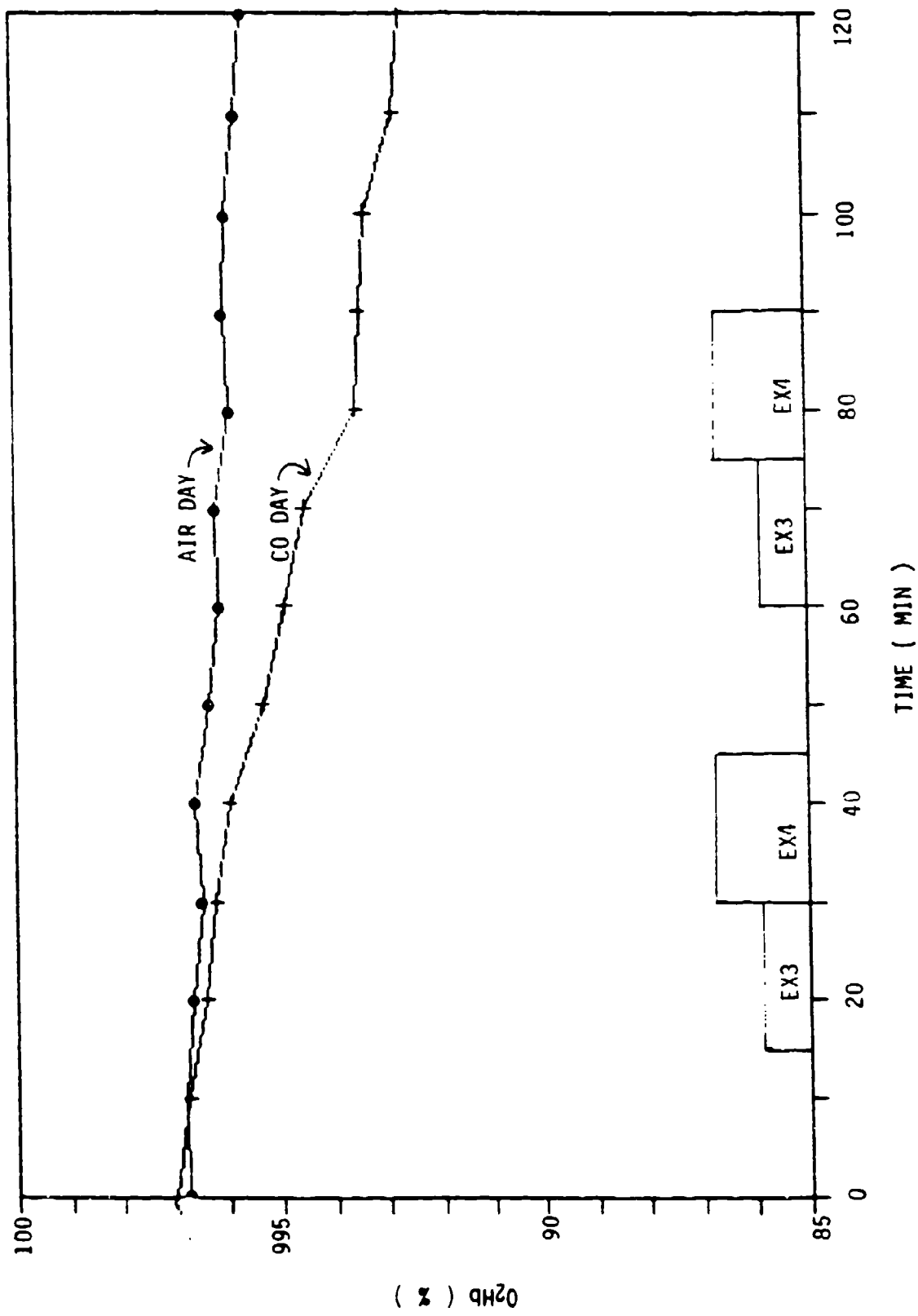
Figure 5. Graphs of mean $O_2$ levels on air and CO days. See Figure 2 for definition of symbols.
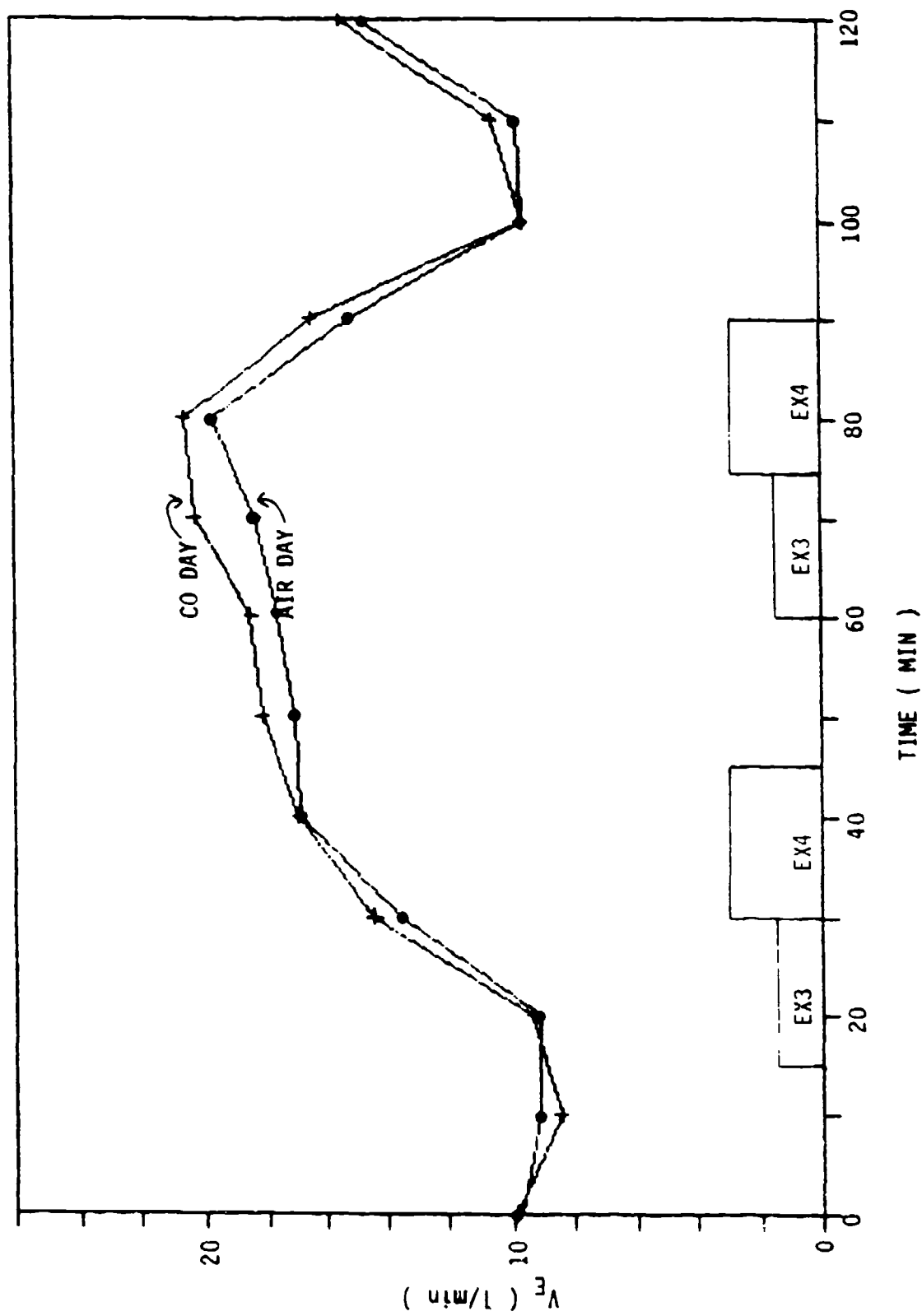
Figure 6. Graphs of mean $\dot{V}_E$ for air and CO days. See Figure 2 for definition of symbols.
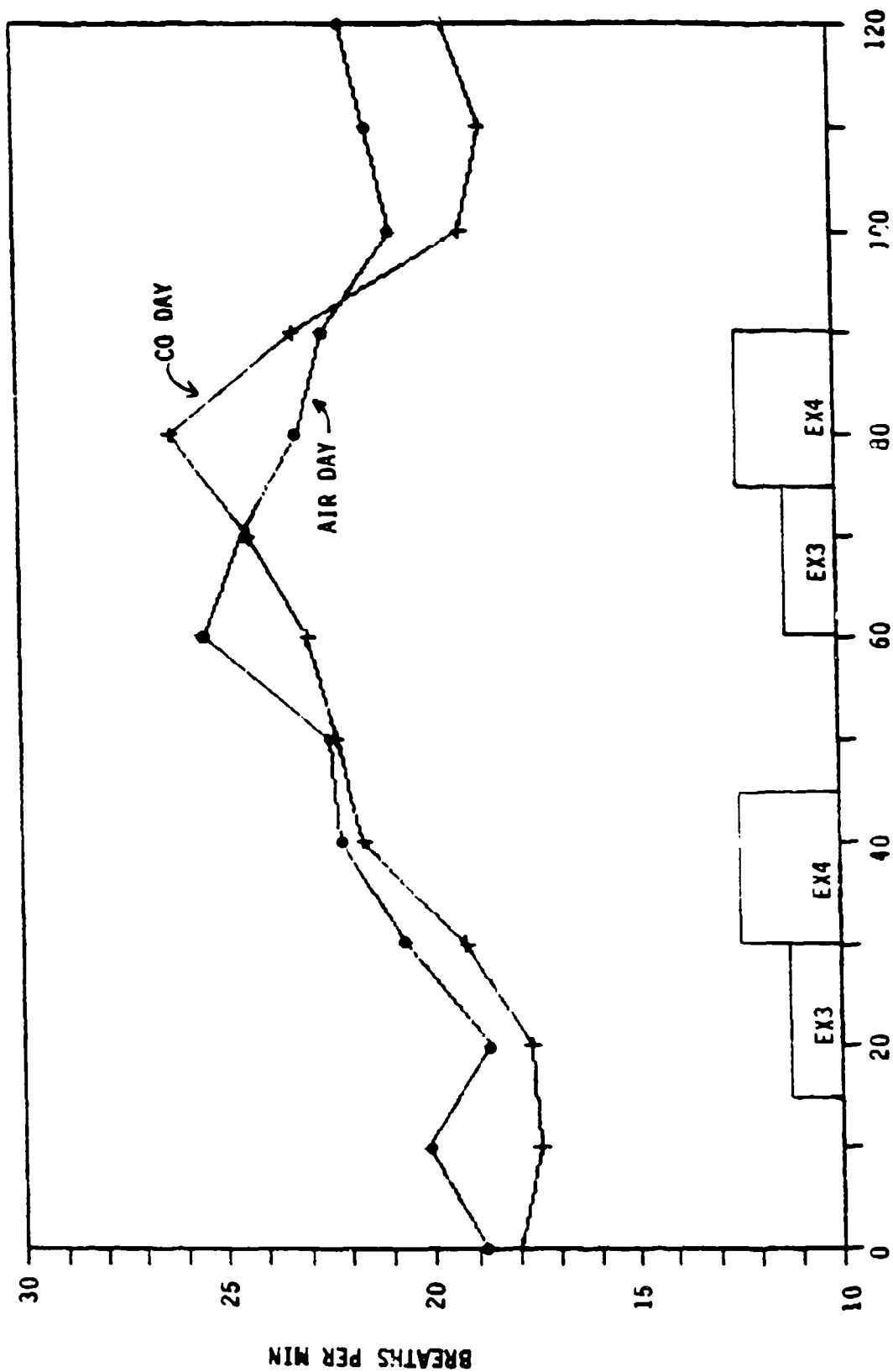
Figure 7. Graphs of mean f-V$_E$ for Air and Co days. See Figure 2 for symbols.

APPENDIX

SENSITIVITY ANALYSIS OF THE COBURN, FORSTER, KANE (CFK) MODEL

SENSITIVITY ANALYSIS OF THE COBURN, FORSTER, KANE (CFK) MODEL

## BACKGROUND

Numerous models for carboxyhemoglobin (COHb) formation have appeared since the early empirical work of Douglas, et al. (1912) which attempted to mathematically describe the association of hemoglobin with oxygen and carbon monoxide (CO). Later attempts such as those of Forbes, et al. (1945), Pace, et al. (1946), and Lilienthal and Pine (1946) were still empirical, i.e., consisted of fitting equations to data without proposing a physical model.

In 1954b, Forster, et al., proposed an equation for COHb as a function of a number physiological variables, and emphasized the exchange dynamics of CO in the lungs under conditions of breath-holding and steady-state breathing. Also considered was the rate of formation of COHb from CO dissolved in the plasma. This paper appears to be the first comprehensive attempt to create a mathematical model which is an analog of the physiological system for COHb formation, rather than a curve-fitting model.

Goldsmith, et al. (1963) proposed a single time constant, linear, exponential model to which they applied time-varying concentrations of ambient CO and physical activity. Calculations were made with analog and digital computers and compared with COHb samples taken from two subjects. Accuracy of the model was not investigated beyond noting a disparity between calculations and COHb data.

It was not until 1965, when Coburn, Forster, and Kane published a nonlinear model, that an attempt was made to describe COHb formation in terms of most of the known pertinent physiological variables. Of particular interest in this model was the inclusion of the endogenous rate of CO production. This model [A1] known as the CFK Model, has been widely applied, misapplied, abused, and criticized, often without acknowledgement of its creators'

caveats and statements of assumptions, approximations, and limitations.

$$\frac{d[COHb](t)}{dt} = \frac{\dot{V}_{CO}}{V_b} - \frac{[COHb](t) \; P_cO_2}{[O_2Hb] \; M} \cdot \frac{1}{1/D_LCO + (P_B - PH_2O)/\dot{V}_A}$$

[A1]

$$+ \frac{P_ICO}{1/D_LCO + (P_B - PH_2O)/\dot{V}_A}$$

The CFK mathematical model describes a straightforward, four-element physical system consisting of an exogenous CO source, a transfer interface, an endogenous CO source, and a storage compartment (Figure A1). The model was originally developed to study quasi-steady-state behavior of [COHb] in response to endogenous CO production and essentially constant, low concentrations of ambient CO.

In Coburn, et al.'s (1965) work, and in succeeding models (e.g., Peterson & Stewart, 1975; Weir & Viano, 1977; Venkatram & Louch, 1979; Marcus, 1980a and 1980b; Bernard & Duker, 1981; Joumard, et al., 1981), most of the emphasis has been on methods of estimating values for the equation's variables and on comparing calculated blood COHb with measured values. Very little explicit attention has been paid to the equations as entities separate from any physiological system. Some authors (Coburn, et al., 1965; Biller & Richmond, 1982) have made rudimentary sensitivity analyses of equations, but these have been numerical or graphical and usually treat only end point calculations of COHb.

Test Point—Blood [COHb]

Blood/Tissue Storage

Endogenous CO

Respiratory Dynamics
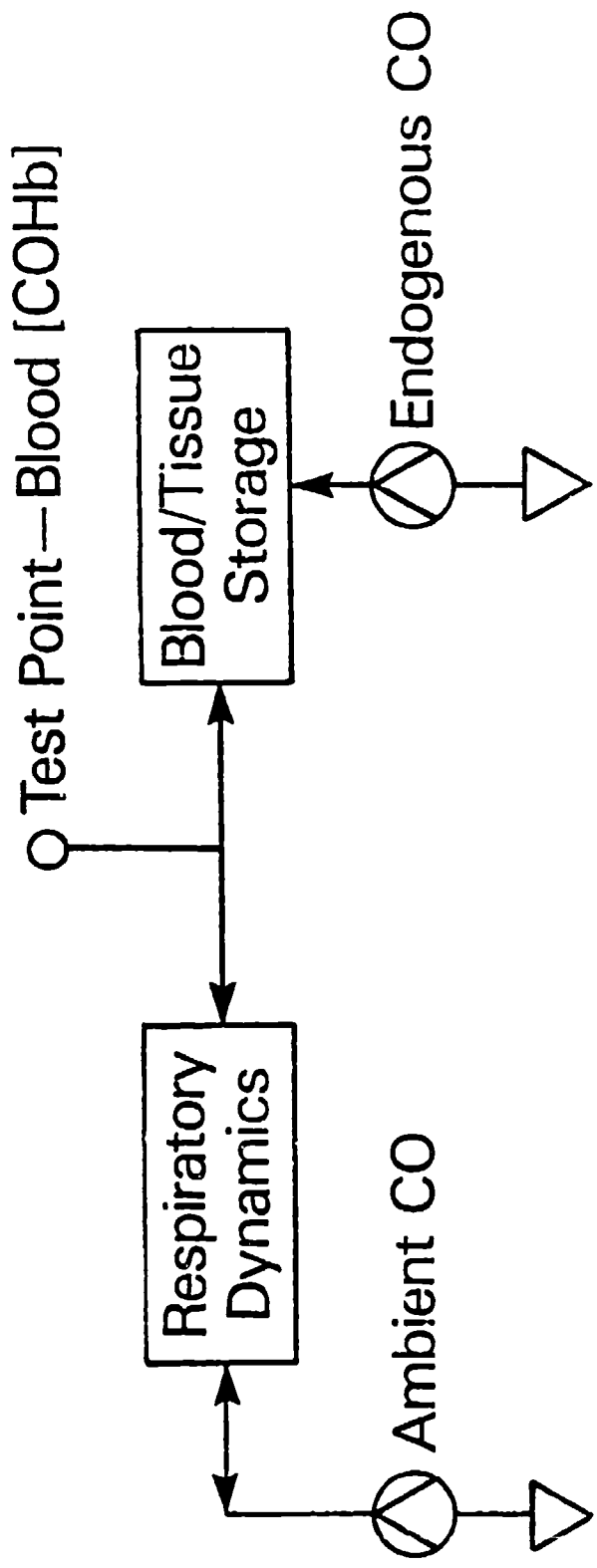
Ambient CO

Ambient CO: $P_{iCO}$
Respiratory Dynamics: $\dot{V}_A$, $P_b$, $P_{H_2O}$, $P_{CO_2}$, DL
Blood/Tissue Storage: $[O_2Hb]$, $V_b$, M
Endogenous CO: $\dot{V}_{CO}$

CFK PHYSICAL MODEL

Figure A1

There are two primary reasons why calculated COHb may not have the same value as that measured in an experimental subject. These reasons should be obvious, but are often lost in the modeling studies published to date. The first reason is that the model is an inaccurate description of the physiological system for the experimental conditions imposed. When calculations and data do not agree, this reason is the most common one cited. The second reason is that the data entered into the model are inaccurate. Unless the sensitivity of the model to errors in its component variables is known, then it is difficult to partition blame for the deviation between model behavior and system behavior.

## METHODS

As part of the experimental study of the temporal response of COHb to steady-state exposure to CO, but with variations in exercise, we have undertaken analytic treatments of several forms of the CFK equation. The intent was to develop sensitivity analyses for both the nonlinear and linearized forms of the equation to determine the effect of error in each variable on the calculated value of COHb.

The results of this study are presented in three parts:

1) Sensitivity to each explicit variable in the linearized CFK equation;

2) Sensitivity to each explicit variable in the nonlinear CFK equation; and

3) Sensitivity to the experimental variables in the nonlinear CFK equation.

The first two analyses are generated by assuming that all explicit variables are independent, even though in a typical experiment this will not be true. There are several justifications for our approach. First, we note that in the

literature, substitution of assumed values (rather than measurements) for the explicit variables is common. Our approach allows us to estimate the effect of errors in these assumed variables on calculated [COHb]. Second, our analysis also allows an initial estimate of which explicit variables need to be acquired with the most care, which in turn dictates which measurements must be made with the most care.

In the linearized CFK equation, a closed-form solution can be obtained, thus it is quite simple to derive expressions for the sensitivities to each variable. Because the nonlinear form of the CFK equation does not have a closed-form solution, numerical methods must be used. The same numerical methods can be applied to the linearized equation as a check of the method and step size used.

The third study required rewriting the CFK equation in terms of the measurements and assumed variables actually used in calculating [COHb]. This analysis is not complete, in that not all measurements are explicitly used. For example, the determinations of $\dot{V}_A$ and $D_L CO$ require averaging discrete data or curve fitting. A sensitivity analysis based on the individual measurements would be cumbersome and provide only marginal insight into the effect of the variable on calculated [COHb] (e.g., the effect of noise on a single data point in an averaged value of $F_I CO^*$ during rebreathing would be highly dependent on the number of data points used).

As part of an experimental study on the temporal response of COHb to steady-state exposure to CO, but with variations in exercise, we began with an analytic treatment of the linearized, integrated form of the CFK equation as shown at the top of Table A1.

Our approach was to take the partial derivative of [COHb](t):

$$Sx_i = \frac{\partial [COHb](t)}{\partial x_i} \qquad [A2]$$

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## Table A1

## LINEARIZED CFK EQUATION SENSITIVITIES

$$[COHb](t) = [COHb](0)\epsilon^{k_1 t} + k_2(1-\epsilon^{k_1 t})$$

$$k_1 = -P_cO_2/M[O_2Hb]BV_b$$

$$k_2 = (\dot{V}CO \cdot B + P_ICO)M[O_2Hb]/P_cO_2$$

$$B = 1/D_LCO + (P_B-PH_2O)/\dot{V}_A$$

$$Sx_i = \frac{\partial[COHb](t)}{\partial x_i}$$

$$Fx_i = \frac{\partial[COHb](t)}{\partial x_i} \cdot \frac{x_i}{[COHb](t)}$$

| $x_i$ | $Sx_i$ | $Fx_i$ |
|---|---|---|
| $[COHb](0)$ | $\epsilon^{k_1 t}$ | $[COHb](0)\epsilon^{k_1 t}/([COHb](0)\epsilon^{k_1 t}+k_2(1-\epsilon^{k_1 t}))$ |
| $[O_2Hb]$ | $\dfrac{k_2(1-\epsilon^{k_1 t}) - k_1 t\epsilon^{k_1 t}([COHb](0)-k_2)}{[O_2Hb]}$ | $\dfrac{k_2(1-\epsilon^{k_1 t})-k_1 t\epsilon^{k_1 t}([COHb](0)-k_2)}{[COHb](0)\epsilon^{k_1 t}+k_2(1-\epsilon^{k_1 t})}$ |
| $M$ | $\dfrac{k_2(1-\epsilon^{k_1 t})-k_1 t\epsilon^{k_1 t}([COHb](0)-k_2)}{M}$ | $\dfrac{k_2(1-\epsilon^{k_1 t})-k_1 t\epsilon^{k_1 t}([COHb](0)-k_2)}{[COHb](0)\epsilon^{k_1 t}+k_2(1-\epsilon^{k_1 t})}$ |

Table A1 (Continued)

| $x_i$ | $Sx_i$ | $Fx_i$ |
|---|---|---|
| $P_cO_2$ | $$\dfrac{k_1 t\epsilon^{k_1 t}([COHb](0)-k_2)-k_2(1-\epsilon^{k_1 t})}{P_cO_2}$$ | $$\dfrac{k_1 t\epsilon^{k_1 t}([COHb](0)-k_2)-k_2(1-\epsilon^{k_1 t})}{[COHb](0)\epsilon^{k_1 t}+k_2(1-\epsilon^{k_1 t})}$$ |
| $P_ICO$ | $$\dfrac{M[O_2Hb](1-\epsilon^{k_1 t})}{P_cO_2}$$ | $$\dfrac{M[O_2Hb](1-\epsilon^{k_1 t})P_ICO}{P_cO_2([COHb](0)\epsilon^{k_1 t}+k_2(1-\epsilon^{k_1 t}))}$$ |
| $V_b$ | $$\dfrac{-k_1 t\epsilon^{k_1 t}([COHb](0)-k_2)}{V_b}$$ | $$\dfrac{-k_1 t\epsilon^{k_1 t}([COHb](0)-k_2)}{[COHb](0)\epsilon^{k_1 t}+k_2(1-\epsilon^{k_1 t})}$$ |
| $\dot{V}_A$ | $$\dfrac{k_1 t(P_B-PH_2O)\epsilon^{k_1 t}([COHb](0)-k_2)}{B\dot{V}_A}-\dfrac{\dot{V}CO\cdot M[O_2Hb](P_B-PH_2O)(1-\epsilon^{k_1 t})}{P_cO_2\cdot\dot{V}_A^2}$$ | $$\dfrac{\dfrac{k_1 t(P_B-PH_2O)\epsilon^{k_1 t}([COHb](0)-k_2)}{B}-\dfrac{\dot{V}CO\cdot M[O_2Hb](P_B-PH_2O)(1-\epsilon^{k_1 t})}{P_cO_2\cdot\dot{V}_A}}{[COHb](0)\epsilon^{k_1 t}+k_2(1-\epsilon^{k_1 t})}$$ |
| $D_LCO$ | $$\dfrac{k_1 t\epsilon^{k_1 t}([COHb](0)-k_2)}{B\cdot D_LCO^2}-\dfrac{M[O_2Hb]\dot{V}CO(1-\epsilon^{k_1 t})}{P_cO_2\cdot D_LCO^2}$$ | $$\dfrac{\dfrac{k_1 t\epsilon^{k_1 t}([COHb](0)-k_2)}{B}-\dfrac{M[O_2Hb]\dot{V}CO(1-\epsilon^{k_1 t})}{P_cO_2}}{D_LCO([COHb](0)\epsilon^{k_1 t}+k_2(1-\epsilon^{k_1 t}))}$$ |

Table A1 (Continued)

| $x_i$ | $Sx_i$ | $Fx_i$ |
|---|---|---|
| $P_B$ | $$\dfrac{-k_1 t e^{k_1 t}([COHb](0)-k_2)}{B \cdot \dot{V}_A} + \dfrac{\dot{V}CO \cdot M[O_2Hb](1-e^{k_1 t})}{P_c O_2 \cdot \dot{V}_A}$$ | $$P_B \left(\dfrac{-k_1 t e^{k_1 t}([COHb](0)-k_2)}{B} + \dfrac{\dot{V}CO \cdot M[O_2Hb](1-e^{k_1 t})}{P_c O_2}\right) \Big/ \dot{V}_A([COHb](0)e^{k_1 t}+k_2(1-e^{k_1 t}))$$ |
| $PH_2O$ | $$\dfrac{k_1 t e^{k_1 t}([COHb](0)-k_2)}{B \cdot \dot{V}_A} - \dfrac{\dot{V}CO \cdot M[O_2Hb](1-e^{k_1 t})}{P_c O_2 \cdot \dot{V}_A}$$ | $$PH_2O \left(\dfrac{k_1 t e^{k_1 t}([COHb](0)-k_2)}{B} - \dfrac{\dot{V}CO \cdot M[O_2Hb](1-e^{k_1 t})}{P_c O_2}\right) \Big/ \dot{V}_A([COHb](0)e^{k_1 t}+k_2(1-e^{k_1 t}))$$ |
| $\dot{V}CO$ | $$\dfrac{B \cdot M[O_2Hb]}{P_c O_2}$$ | $$\dfrac{\dot{V}CO \cdot B \cdot M[O_2Hb]}{P_c O_2([COHb](0)+k_2)}$$ |

with respect to each independent variable, $x_i$, to determine (as a first approximation) which variables have the greatest effect on calculated [COHb]. These derivatives define the sensitivities of [COHb](t) to each variable in conventional instrumentation and measurement terms. This information is also a guide to the care with which each variable must be measured in an experimental verification of the equation as an accurate model.

A second expression,

$$Fx_i = \lim_{\substack{\Delta[COHb](t) \to 0 \\ \Delta x_i \to 0}} \frac{\Delta[COHb](t)}{[COHb](t)} \div \frac{\Delta x_i(t)}{x_i(t)} = \frac{\partial[COHb](t)}{\partial x_i(t)} \cdot \frac{x_i(t)}{[COHb](t)}$$

[A3]

was also obtained. This is a statement of the fractional change in calculated [COHb](t) for a fractional change in each independent variable, $x_i$. As an index of sensitivity, this latter expression is slightly more useful than Eq. [A2] for the perturbations in $x_i$ in which we have interest are usually fractional, expressed as a percent uncertainty or percent error.

RESULTS

The sensitivities and fractional sensitivity expressions for the linearized CFK equation are listed in Table A1. Substituting values for the variables as shown in Table A2 [drawn from Steinberg & Nielson (1977)], for three work loads, substituting into the fractional sensitivity equations, and plotting the results as functions of time (Figures A2 through A13), we find two distinct forms of behavior: 1) monotonic rises and declines from an intiial values and, 2) sensitivities which reach maxima or minima and return to their zero-time or near-zero-time values.

## TABLE A2.    CFK EQUATION DEFINITIONS AND TYPICAL VALUES

### Definitions

[COHb](0)      =   Initial blood concentration of COHb, mg/ml blood

$P_ICO$          =   Average partial pressure of CO in inspired air, mmHg

$P_cO_2$          =   Average partial pressure of $O_2$ in pulmonary capillaries, mmHg

M                  =   Haldane constant — relative affinity of hemoglobin for  CO
                         compared with $O_2$, dimensionless

$[O_2Hb]$        =   Average pulmonary capillary concentration of $O_2Hb$, mg/ml blood

[THb]            =   Total hemoglobin concentration, mg/ml blood

$V_b$             =   Total blood volume, ml

$\dot{V}_A$       =   Alveolar ventilation, ml/min

$P_B$             =   Barometric pressure, mmHg

$D_LCO$          =   Pulmonary diffusing capacity for CO, ml gas(min·mmHg)

$PH_2O$          =   Partial pressure of water vapor at body temperature, mmHg

$\dot{V}_{CO}$    =   Endogenous rate of CO production, ml/min

### Typical Values

| | | | Work Level | | |
|---|---|---|---|---|---|
| [COHb](0) | = | $1.54 \times 10^{-1}$ | 1 | 3 | 4 |
| $P_ICO$ | = | $7.6 \times 10^{-2}$ | $\dot{V}_A$   6,000 | 18,000 | 24,000 |
| $P_cO_2$ | = | 100 | $D_LCO$   30 | 40 | 50 |
| M | = | 215 | | | |
| $[O_2Hb]$ | = | 15.4 | | | |
| [THb] | = | 15.4 | | | |
| $V_b$ | = | 5,500 | | | |
| $P_B$ | = | 760 | | | |
| $PH_2O$ | = | 47 | | | |
| $\dot{V}_{CO}$ | = | 0.007 | | | |

73

## LINEARIZED CFK EQUATION SENSITIVITIES TO EXPLICIT VARIABLES

8.4-2     Fractional Sensitivity to [COHb](0)

8.4-3     Fractional Sensitivity to $P_I CO$

8.4-4     Fractional Sensitivity to $P_c O_2$

8.4-5     Fractional Sensitivity to M

8.4-6     Fractional Sensitivity to $[O_2 Hb]$

8.4-7     Fractional Sensitivity to [THb]

8.4-8     Fractional Sensitivity to $V_b$

8.4-9     Fractional Sensitivity to $\dot{V}_A$

8.4-10    Fractional Sensitivity to $P_B$

8.4-11    Fractional Sensitivity to $D_L CO$

8.4-12    Fractional Sensitivity to $PH_2 O$

8.4-13    Fractional Sensitivity to $\dot{V}_{CO}$

Note:    Saturation values for $[O_2 Hb]$, [COHb] are assumed to be [THb] $\times 1.39 \times 10^{-2}$ ml gas/ml blood.

SENSITIVITY TO COHb(0)

LINEAR

Figure A2

WORK LEVEL 1    WORK LEVEL 3    WORK LEVEL 4

LOG TIME IN MINUTES

FCOHb(0)

75
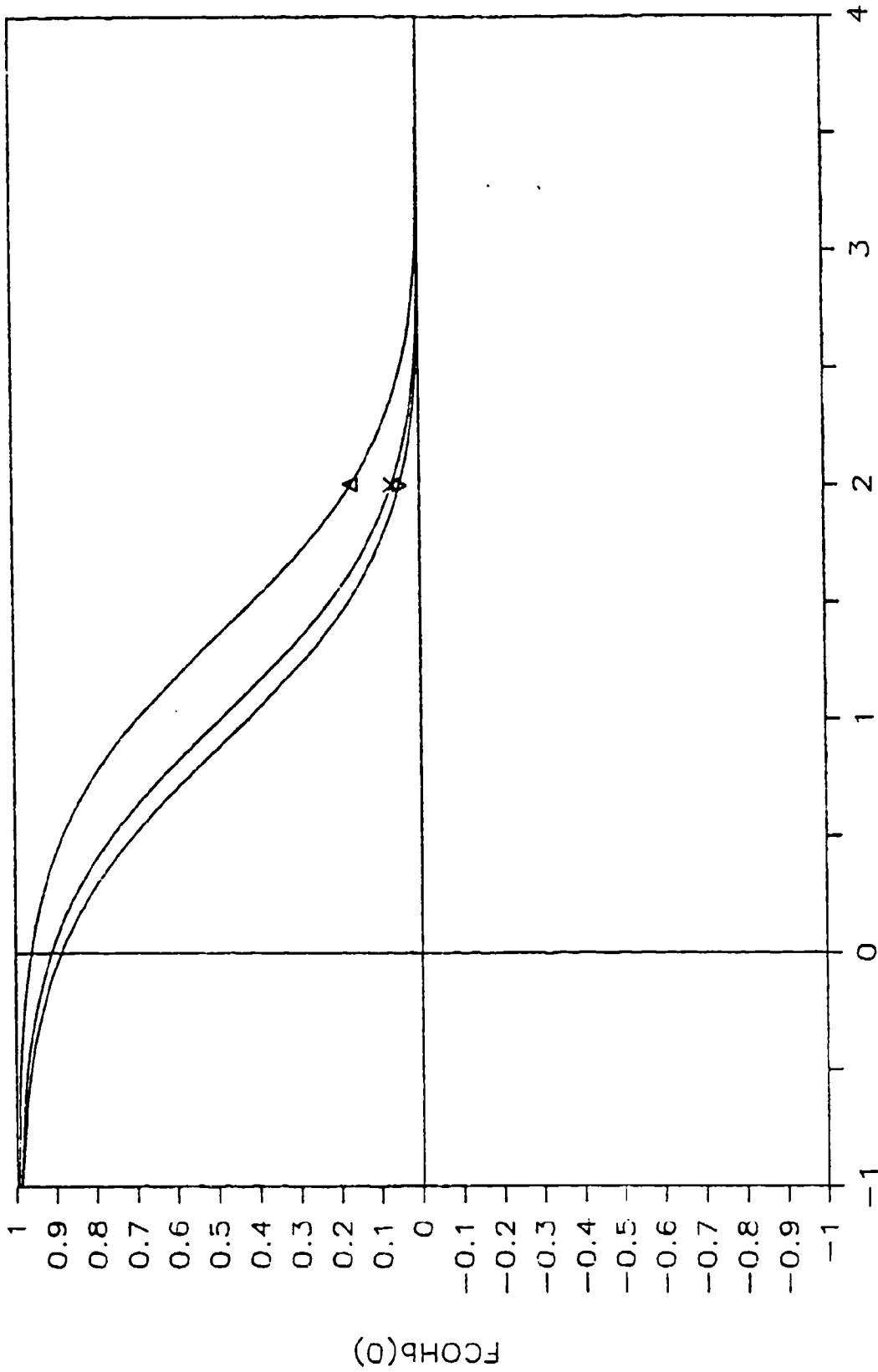
# SENSITIVITY TO PICO
## LINEAR
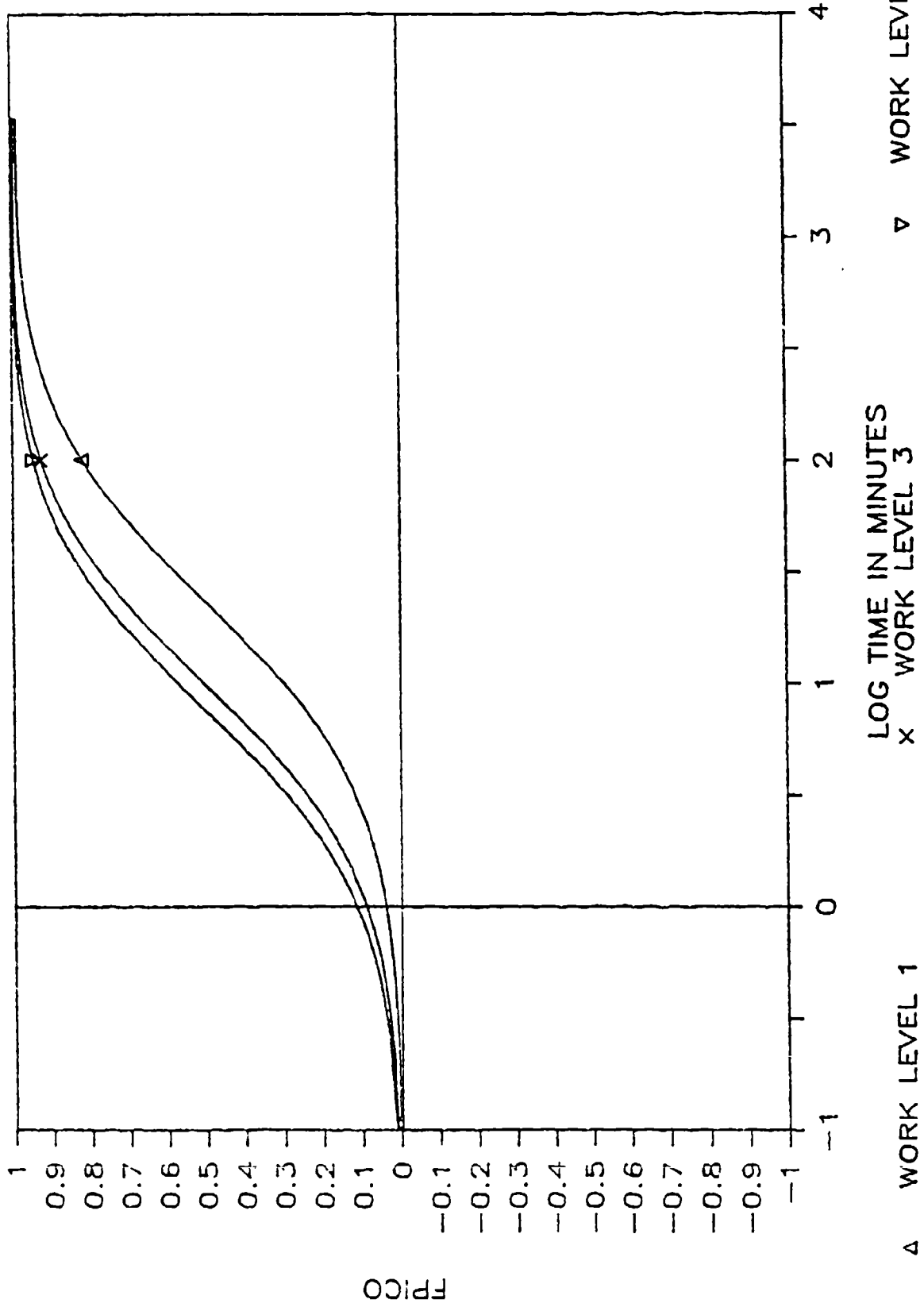


Figure A.3

# SENSITIVITY TO PcO2

## LINEAR



Figure A4

LOG TIME IN MINUTES

△ WORK LEVEL 1    X WORK LEVEL 3    ▽ WORK LEVEL 4

FPcO2
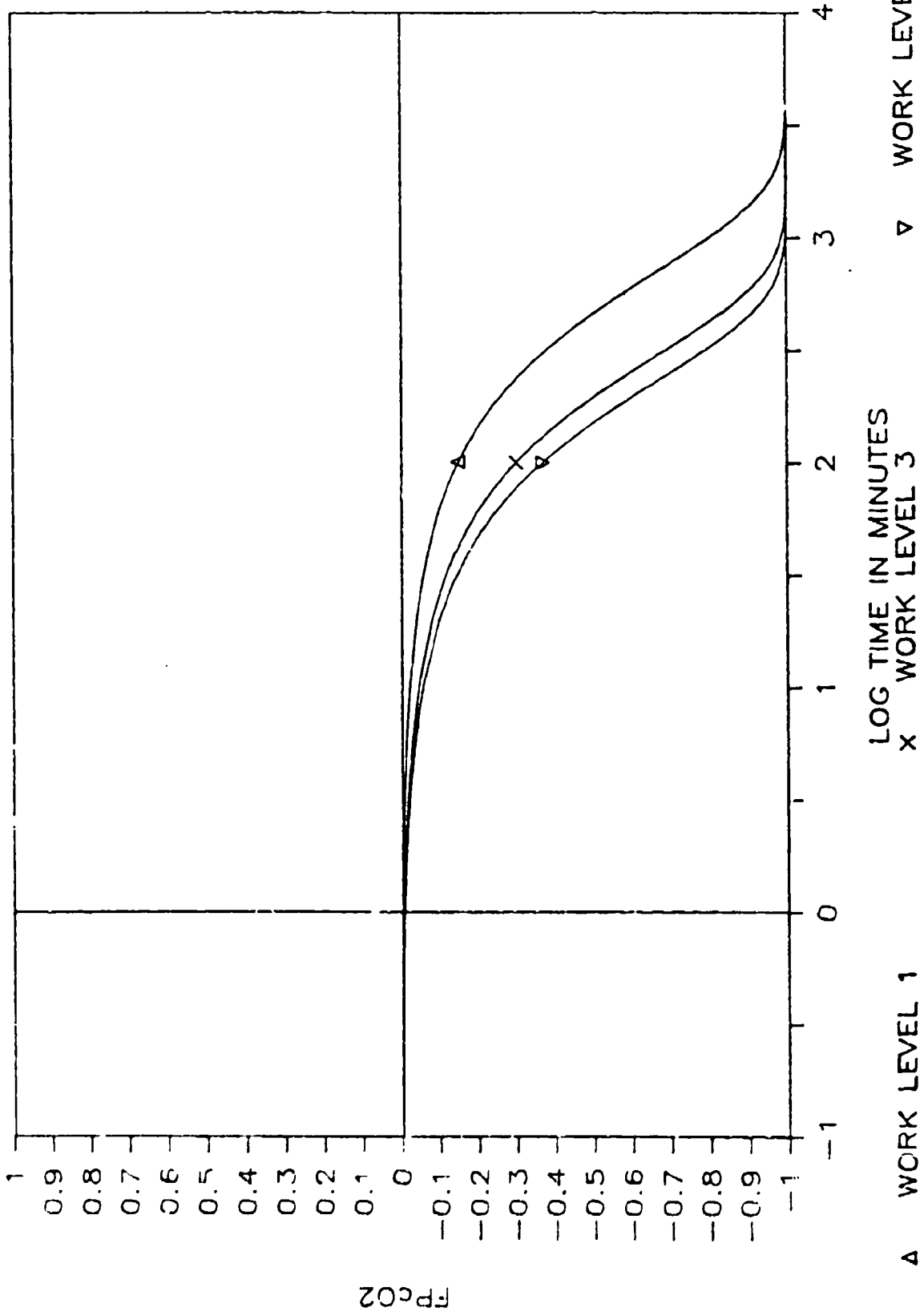
# SENSITIVITY TO M
## LINEAR



FM
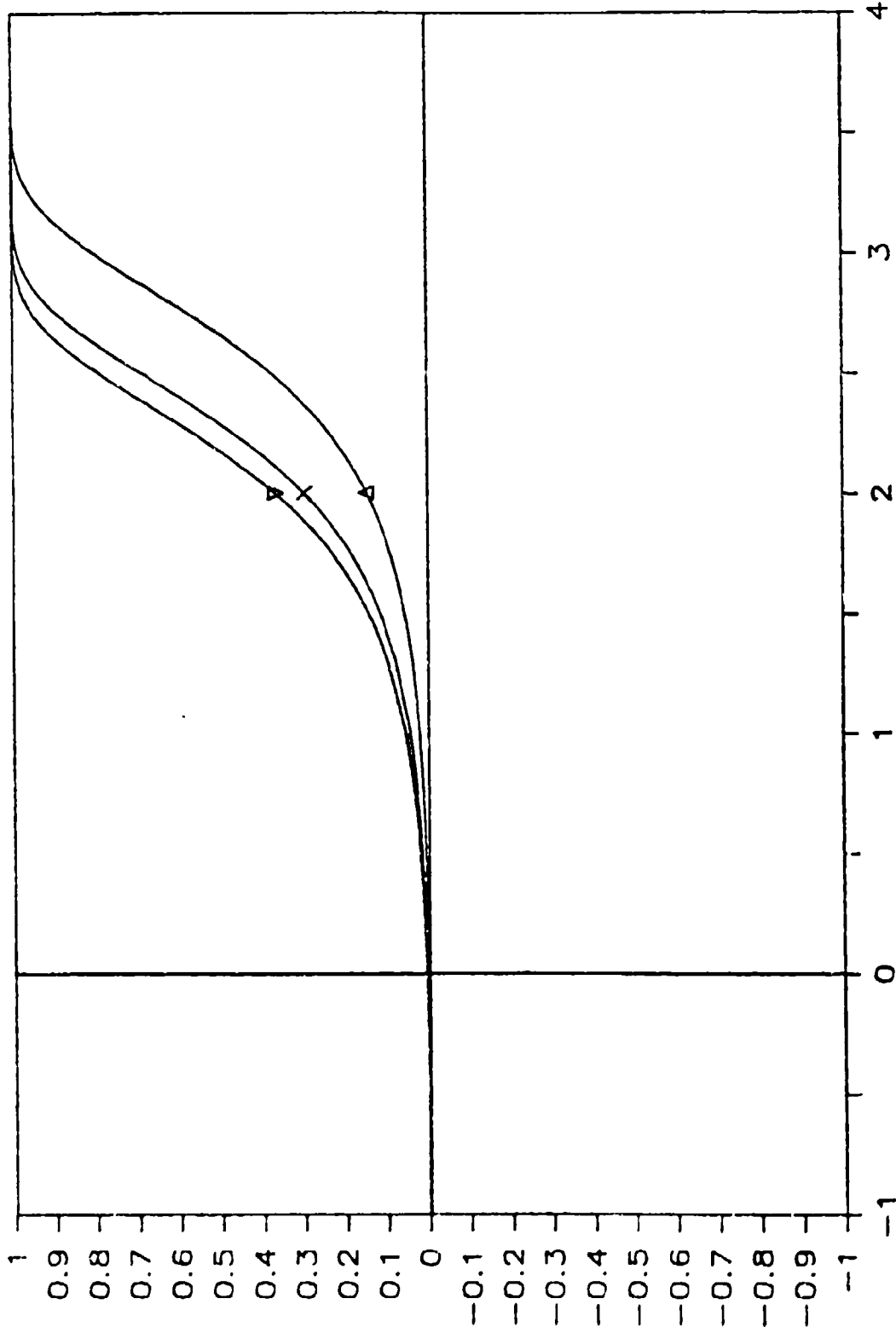
LOG TIME IN MINUTES

△ WORK LEVEL 1    × WORK LEVEL 3    ▽ WORK LEVEL 4

Figure A5

## SENSITIVITY TO O2HB
### LINEAR

FO2HB

LOG TIME IN MINUTES

△ WORK LEVEL 1  x WORK LEVEL 3  ▽ WORK LEVEL 4
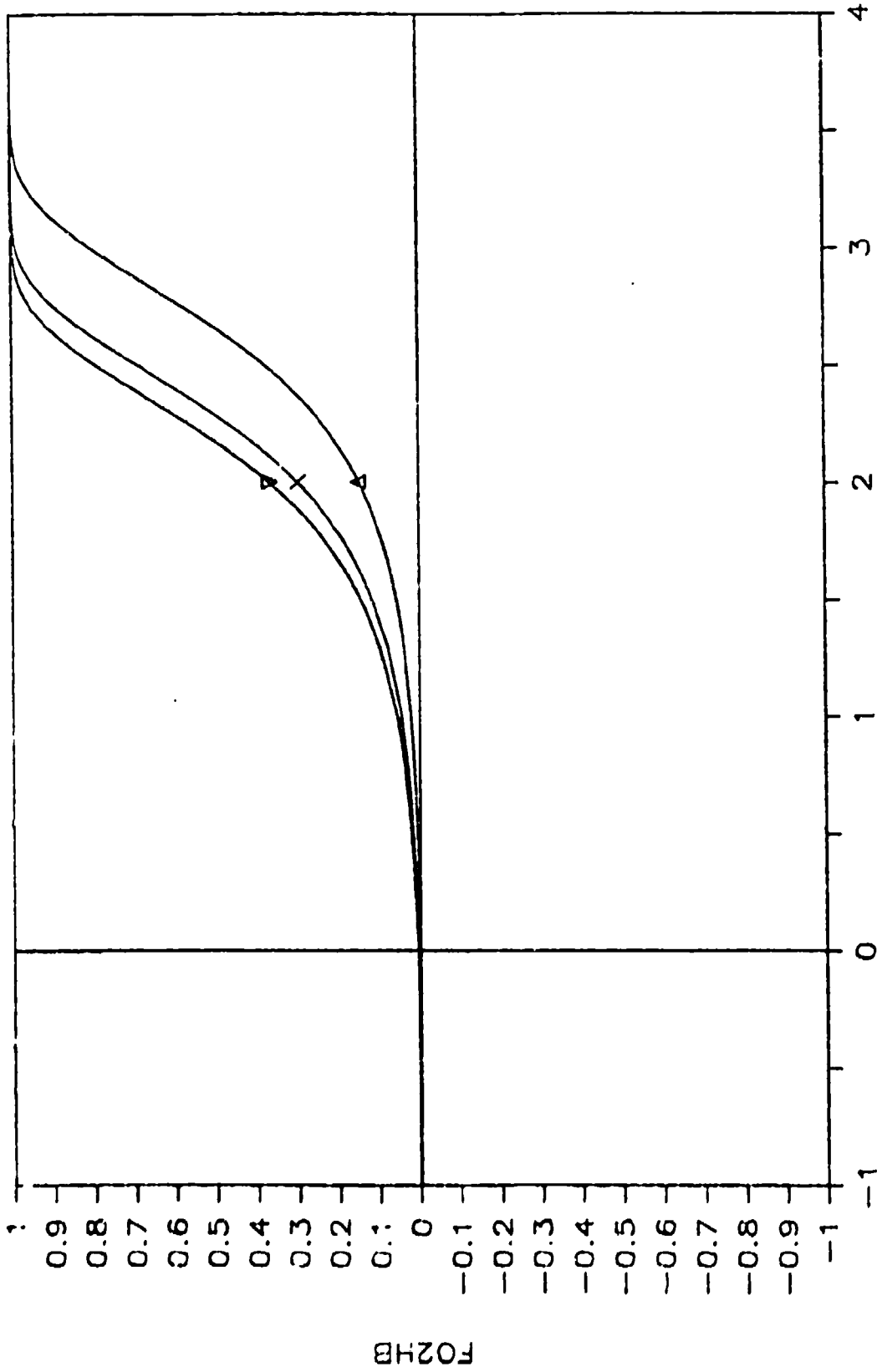
Figure A6

SENSITIVITY TO THB
LINEAR
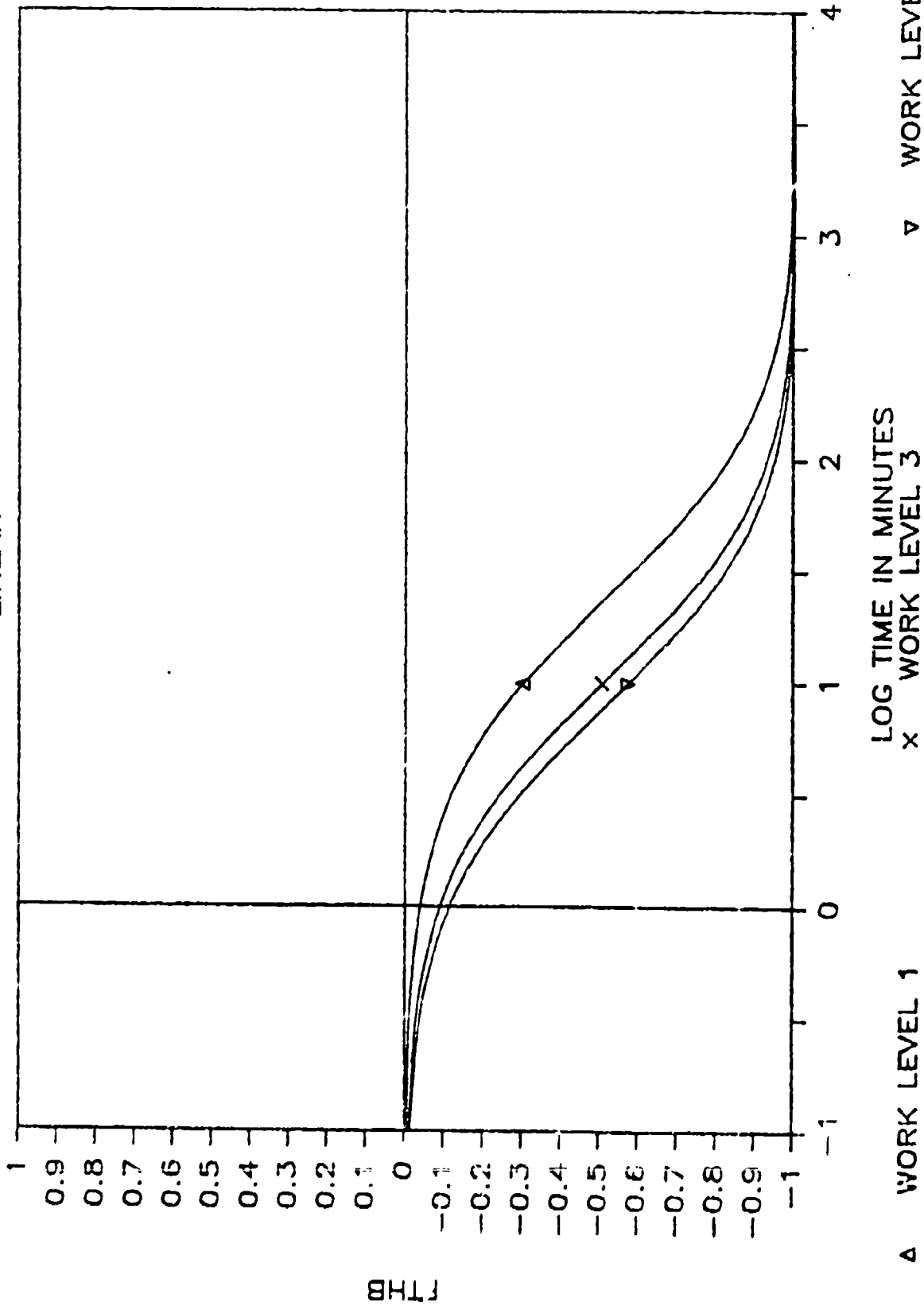
Figure A7

SENSITIVITY TO VB
LINEAR

FVB
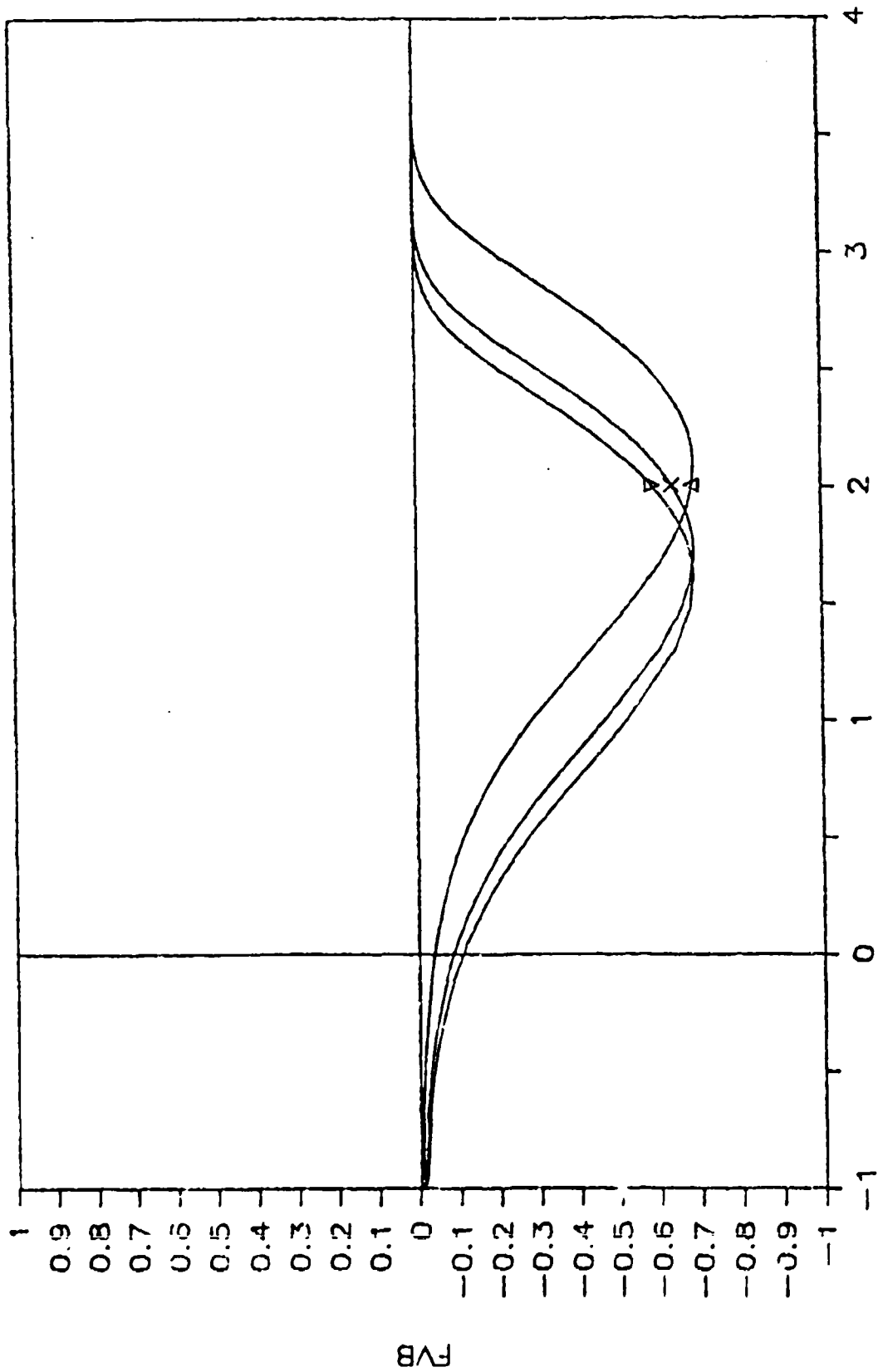
LOG TIME IN MINUTES
△ WORK LEVEL 1    × WORK LEVEL 3    ▽ WORK LEVEL 4

Figure A8

# SENSITIVITY TO V̇A

## LINEAR
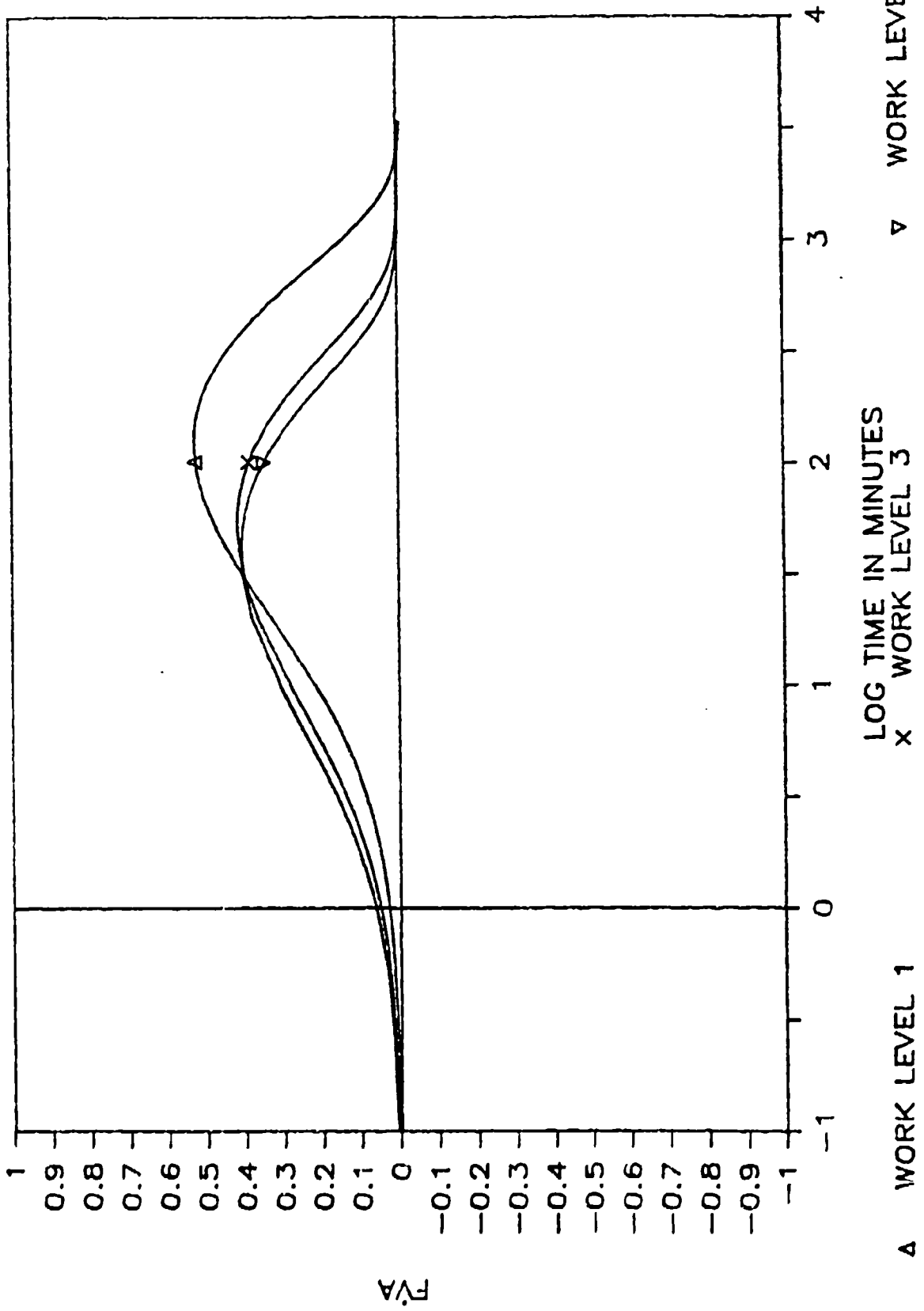


Figure A9

# SENSITIVITY TO PB
## LINEAR
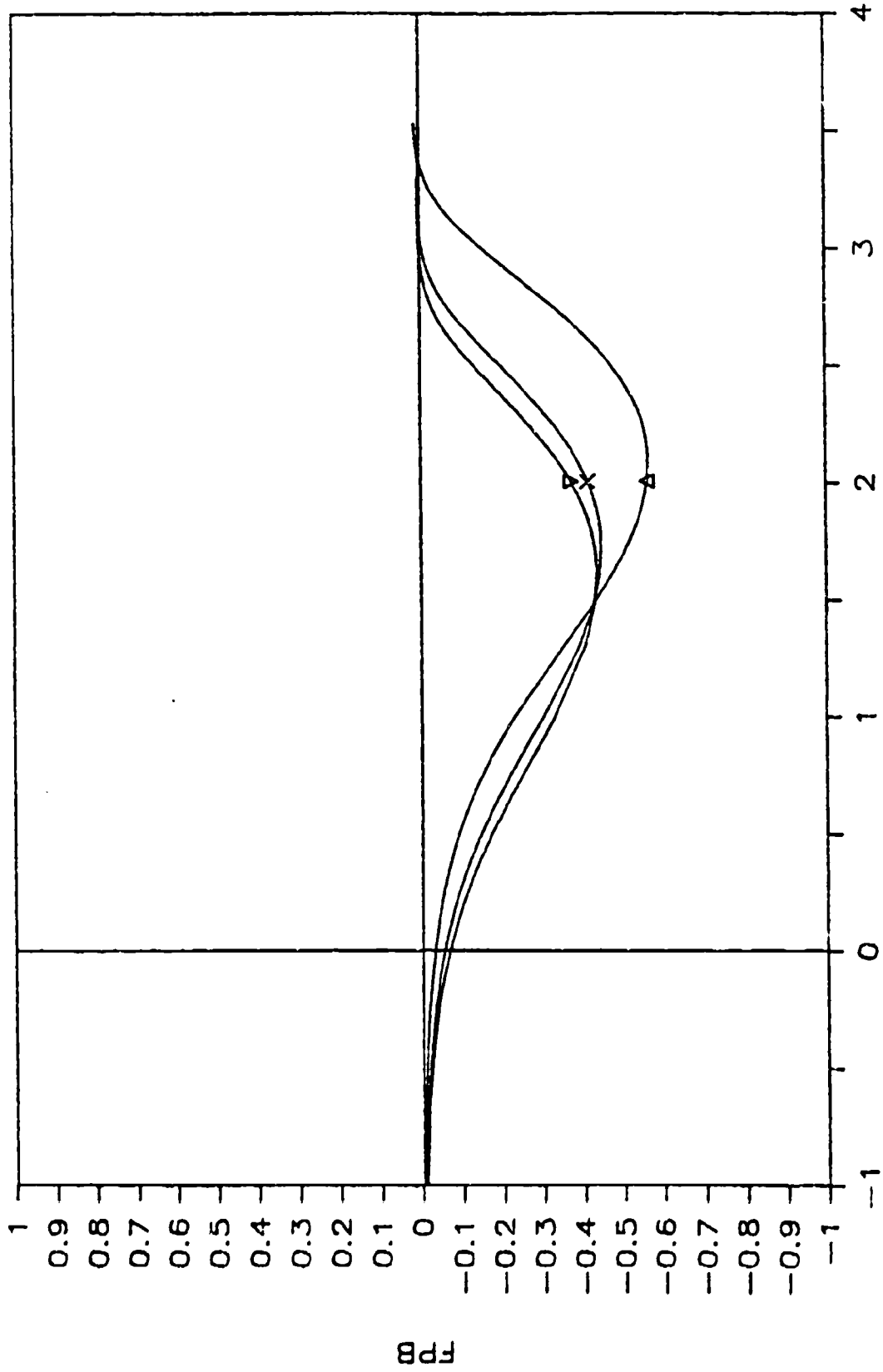


Figure A10

# SENSITIVITY TO DLCO
## LINEAR



LOG TIME IN MINUTES

x  WORK LEVEL 3

△  WORK LEVEL 1          ▽  WORK LEVEL 4
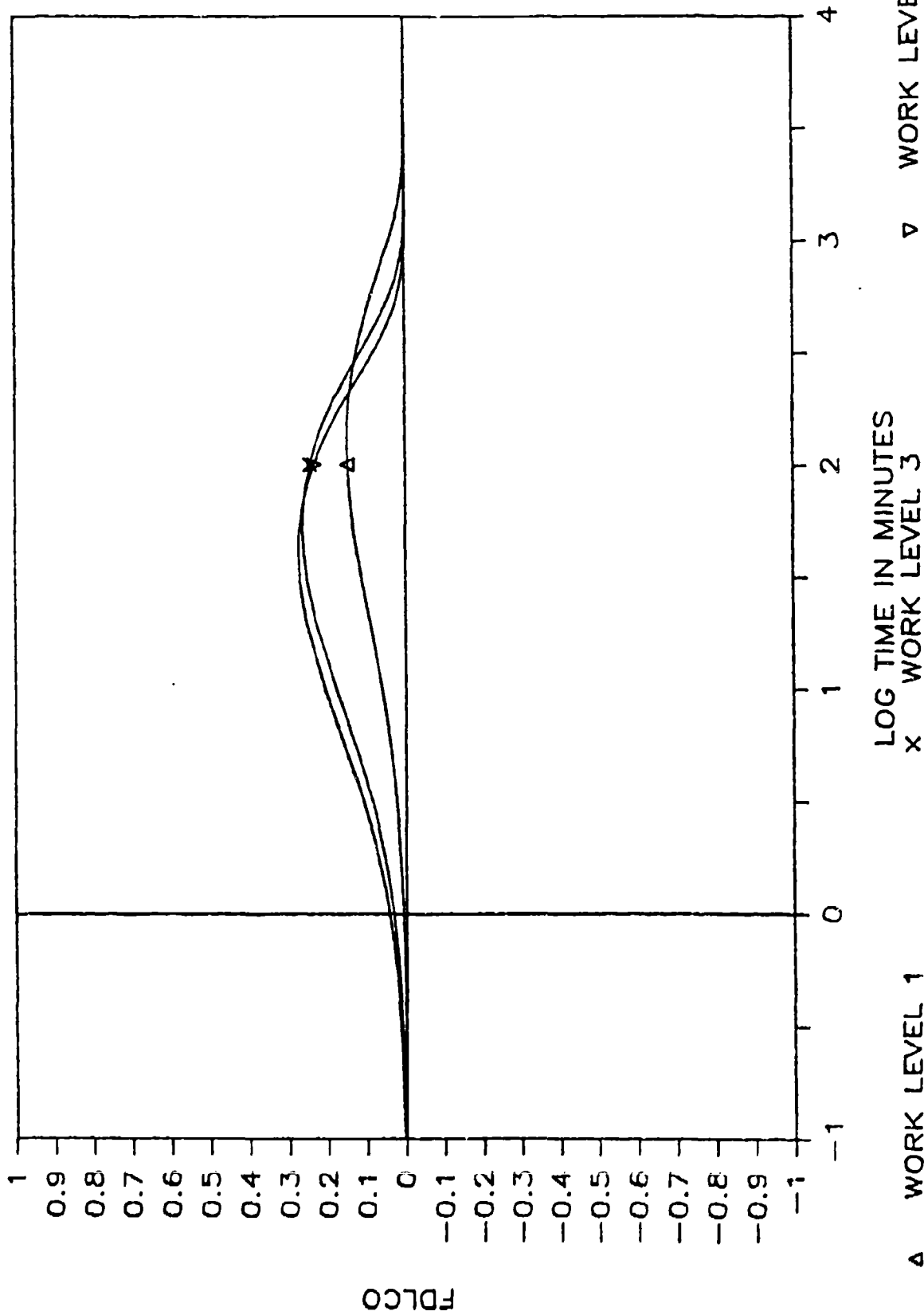
Figure A11

# SENSITIVITY TO PH2O
## LINEAR
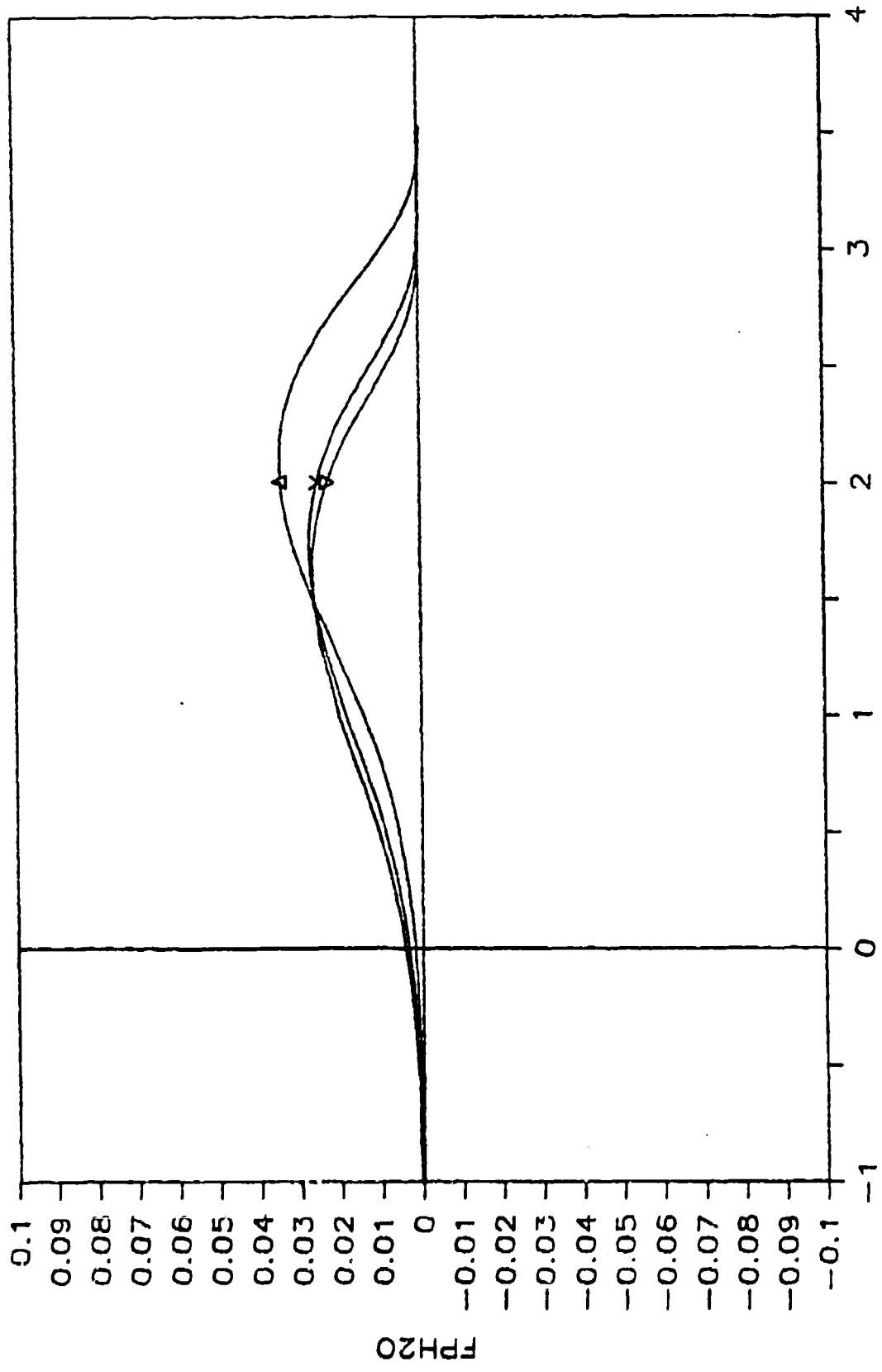


Figure A12

SENSITIVITY TO V̇CO

LINEAR
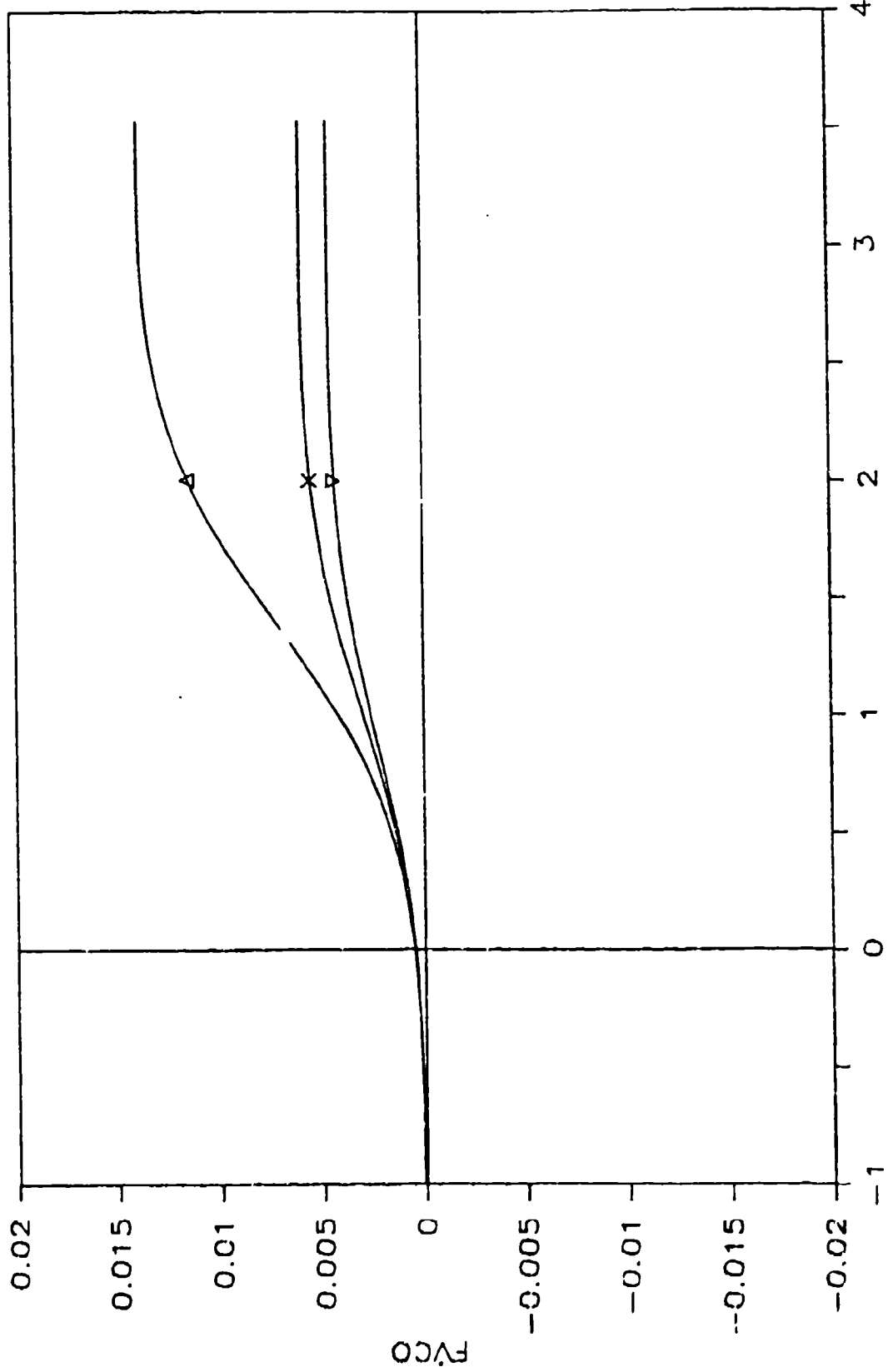
Figure A13

WORK LEVEL 1    x WORK LEVEL 3    ▽ WORK LEVEL 4

LOG TIME IN MINUTES

△ WORK LEVEL 1

33

Before any interpretation of these sensitivities is attempted, it should be emphasized that the calculations are valid for perturbations of only a single variable at a time, and that those perturbations are infinitesimal. A further caveat is that the independent variables are considered to be truly independent and error-free. This means that the range of [COHb] is sufficiently small that the value of [$O_2$Hb] does not change and that $P_ICO$ is a vanishingly small fraction of $P_cO_2$. The infringement of COHb on $O_2$Hb can be accounted for (e.g., Marcus, 1980a and 1980b), but it must be done before the CFK equation in differential form is integrated. Because the resulting differential equation is nonlinear and does not have a closed form integral, it is not treatable with the analytic methods used here.

As a second level of study, a nonlinear form of the CFK Equation was subjected to a numerical sensitivity analysis. In this analysis, [$O_2$Hb] was replaced by 0.97([THb] − [COHb]), where [THb] is total hemoglobin concentration and the blood is assumed to always contain 3% reduced hemoglobin. No serious error is anticipated by these assumptions (Marcus, 1980a, has discussed several means of dealing with less than total saturation of arterial blood).

In the numerical analysis, each variable was allowed to vary a small amount (1%) about the "typical" values of Table A2 and [COHb](t) was calculated for the high, typical, and low values for the selected variable as time ranged from zero to $10^4$ minutes or until the derivative became less than $10^{-9}$. Fractional sensitivities were calculated as before and the results are plotted in Figures A14 through A25. All of the caveats stated for the analytical model calculations apply here also.

NONLINEAR CFK EQUATION SENSITIVITIES TO EXPLICIT VARIABLES

# SENSITIVITY TO COHb(0)
## NON-LINEAR

FCOHb(0)

LOG TIME IN MINUTES

△ WORK LEVEL 1

x WORK LEVEL 3

▽ WORK LEVEL 4

Figure A14

# SENSITIVITY TO PICO
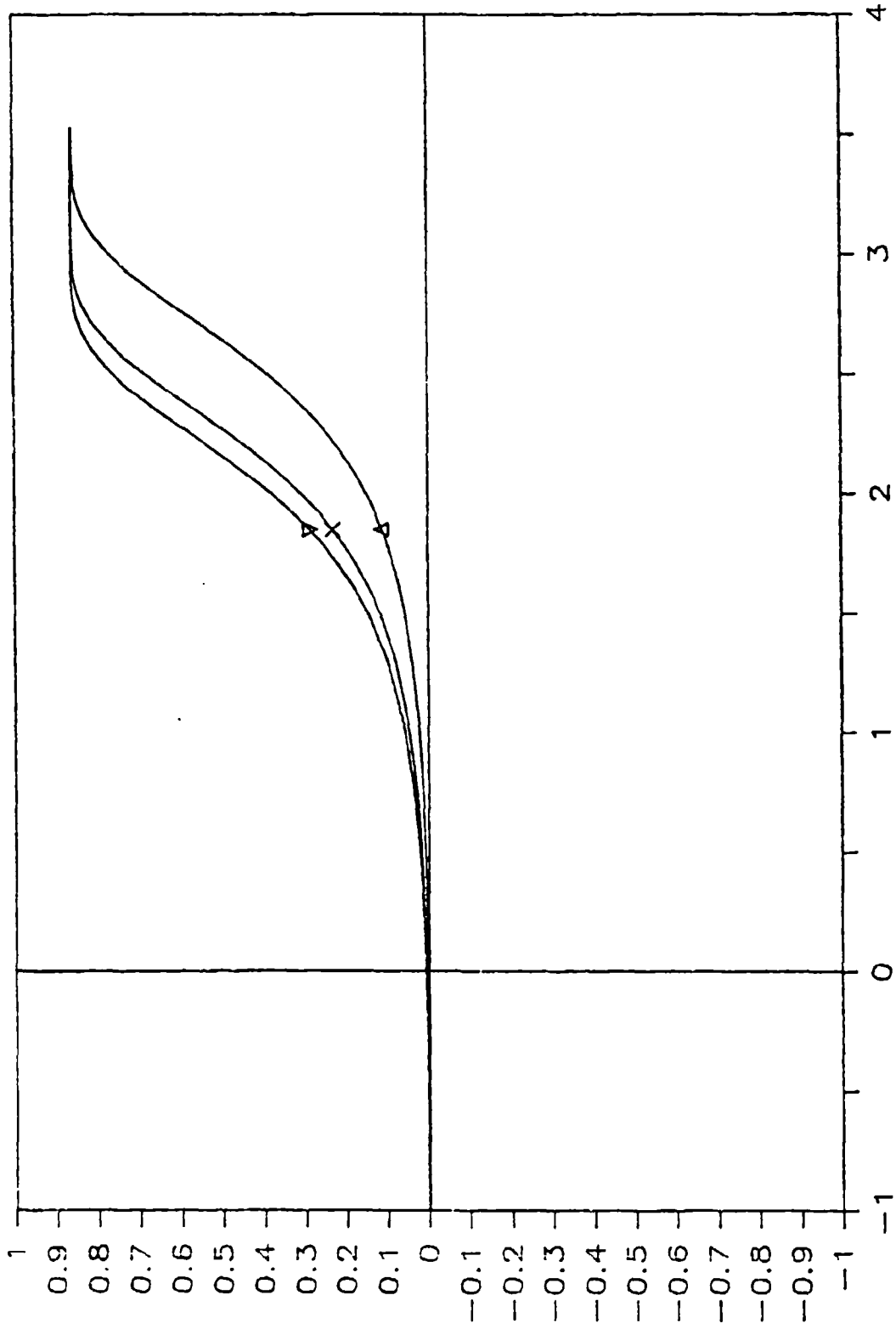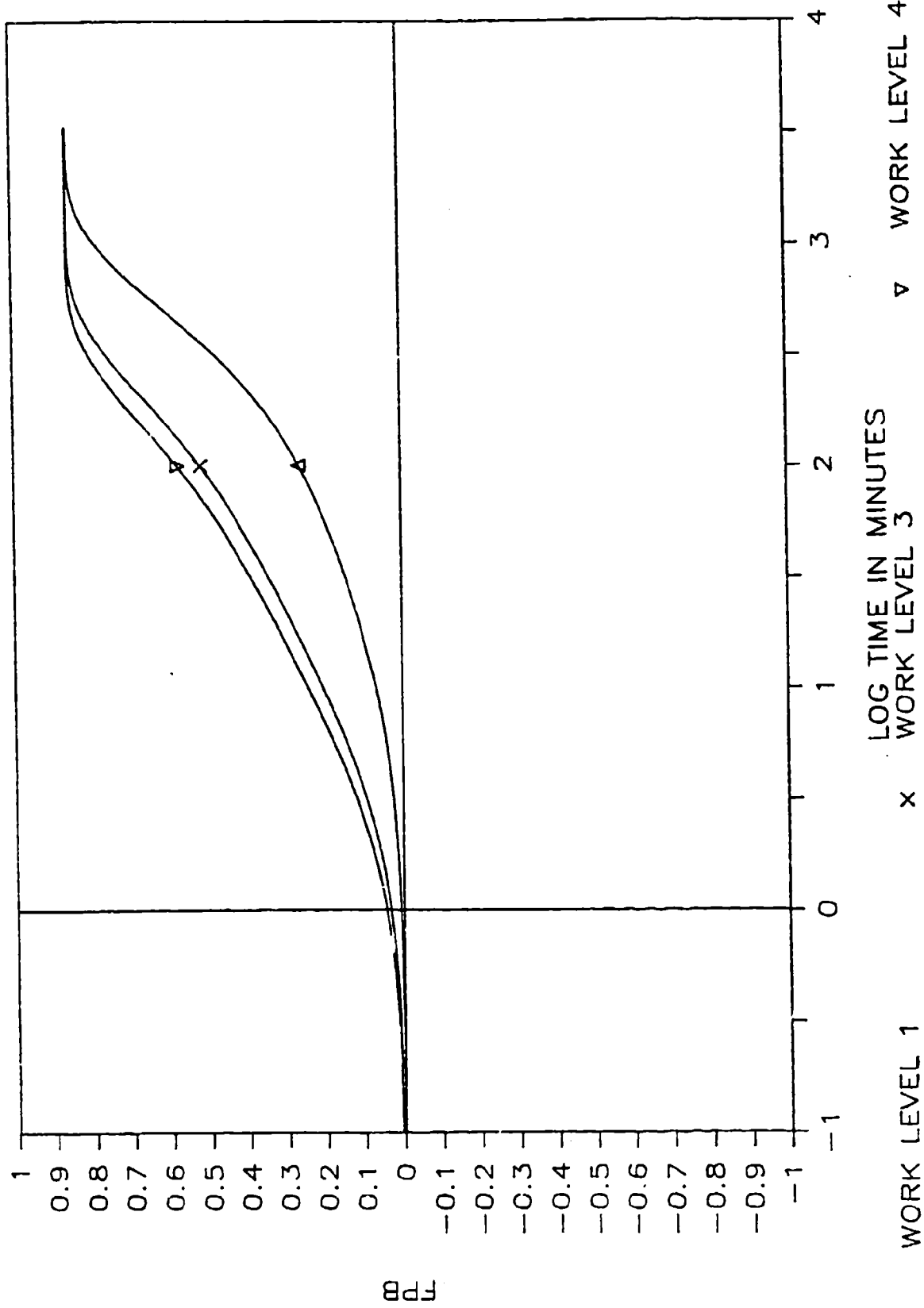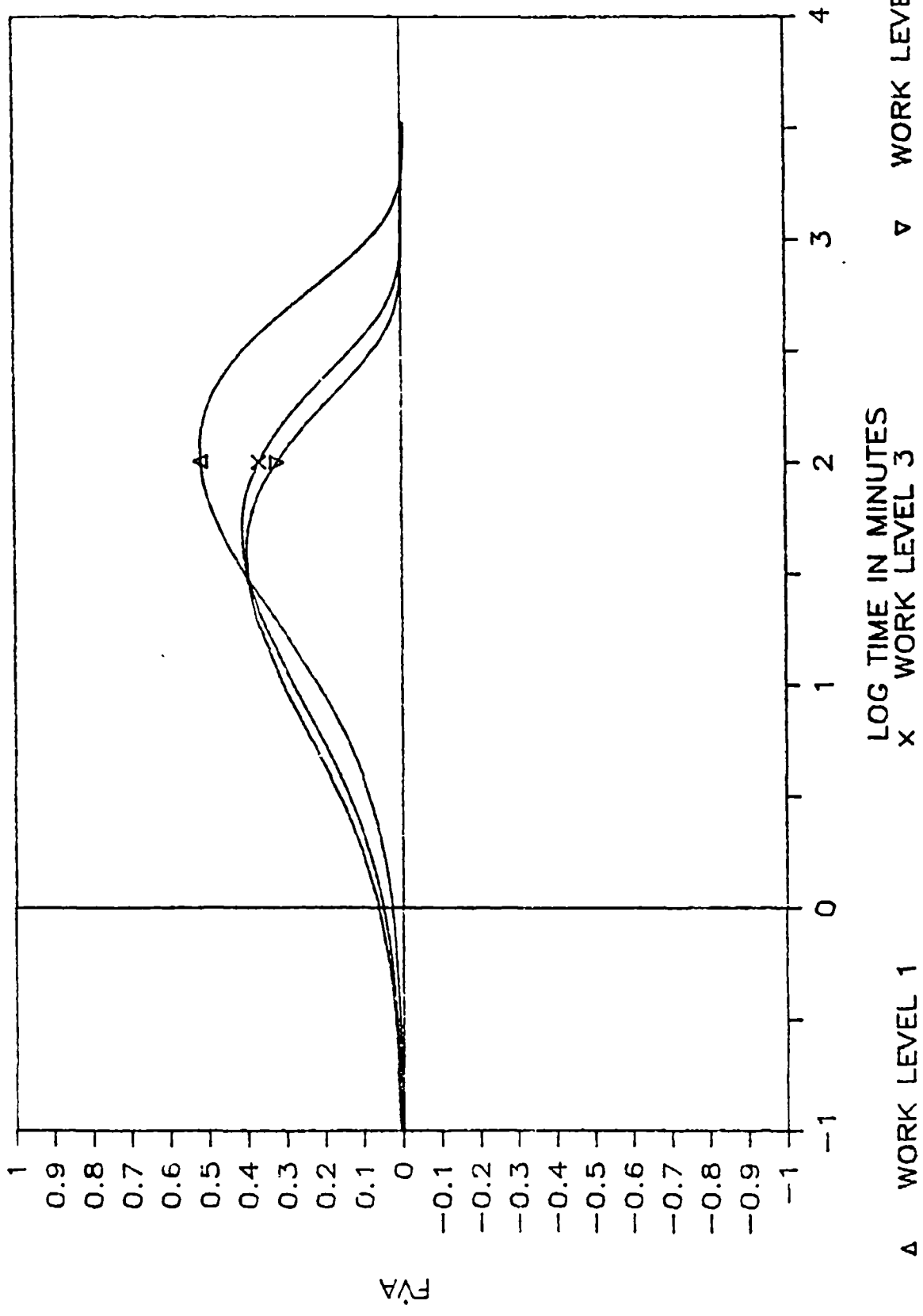## NON-LINEAR
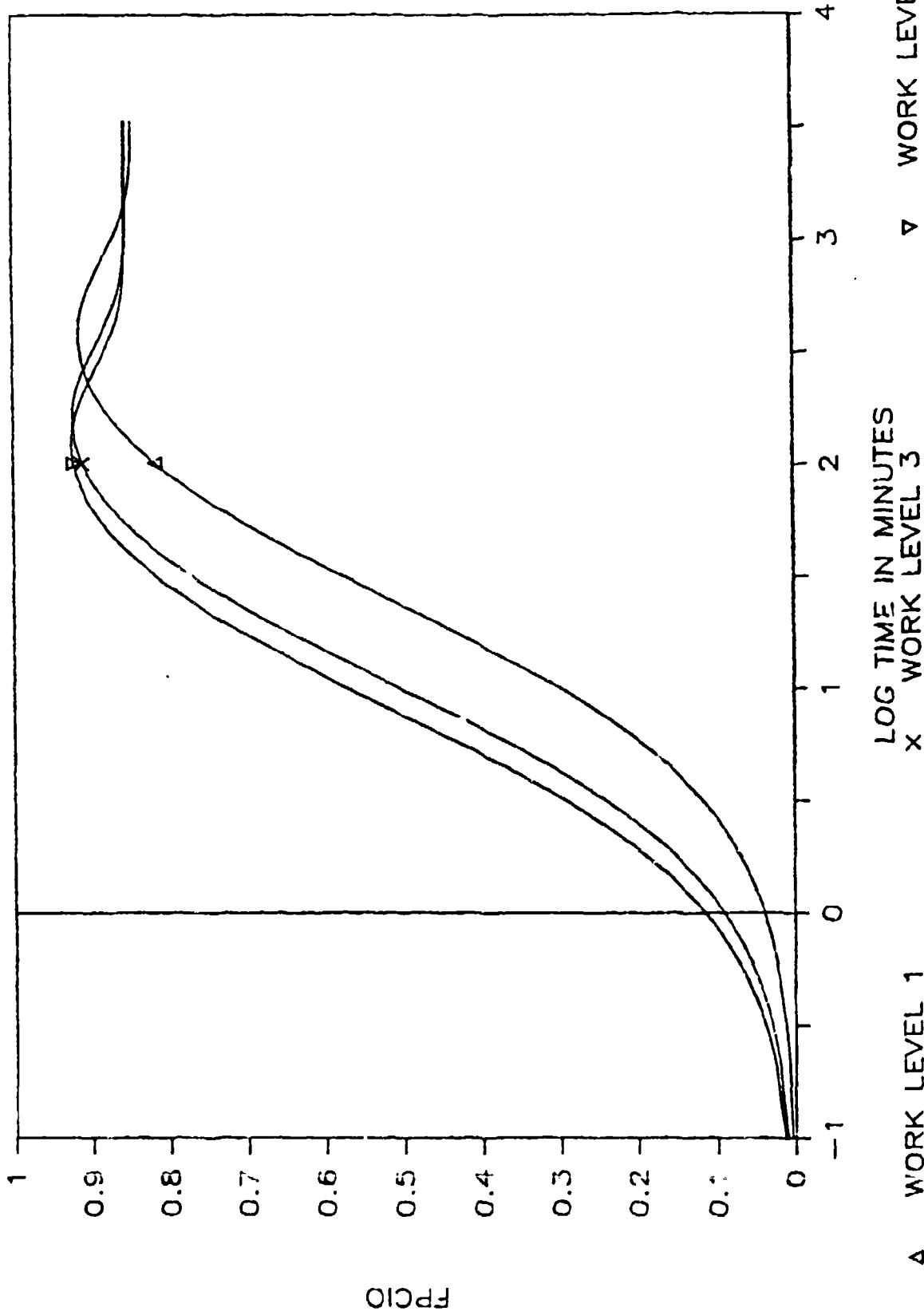


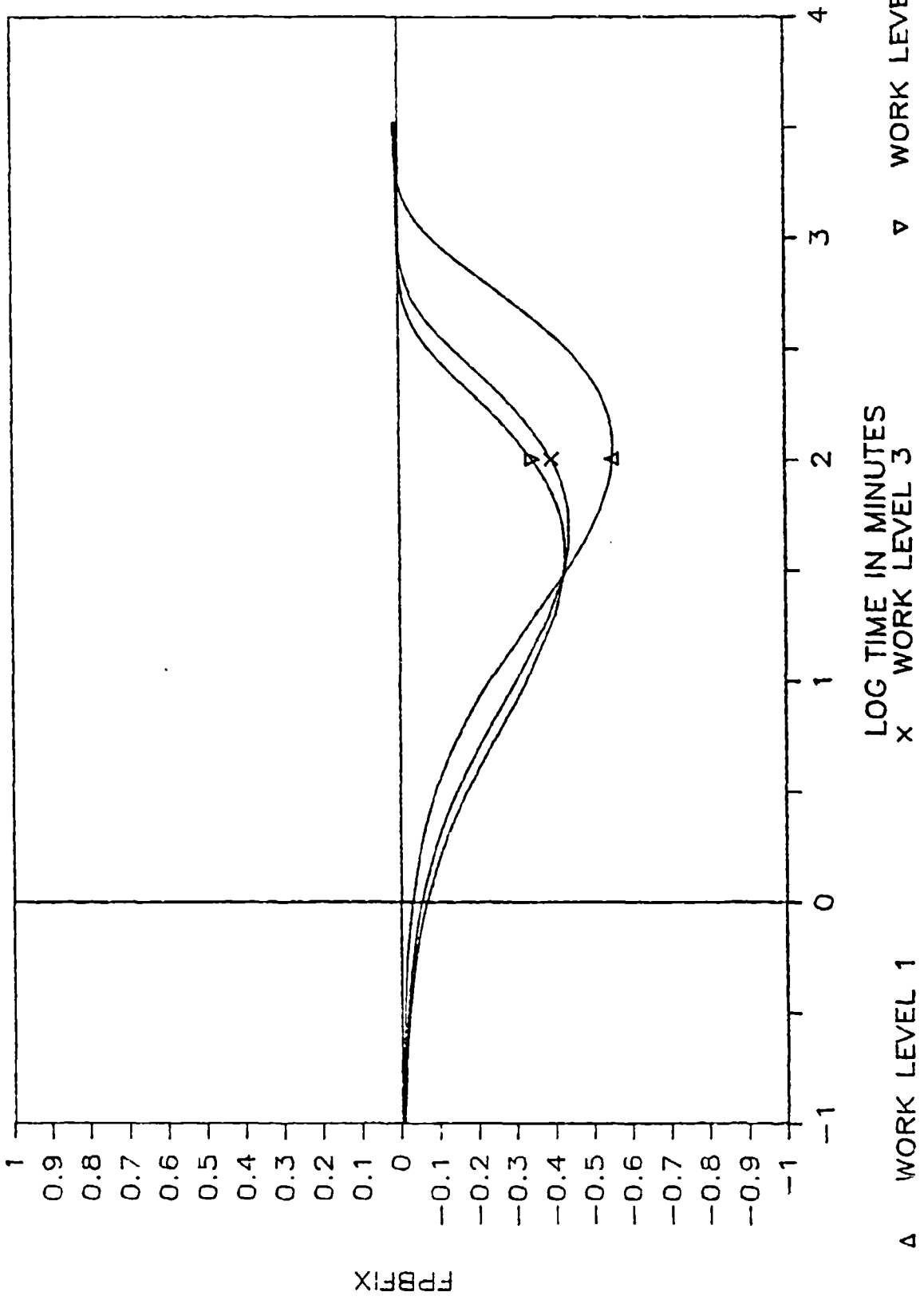Figure A15

SENSITIVITY TO PCO2
NON-LINEAR

FPCO2
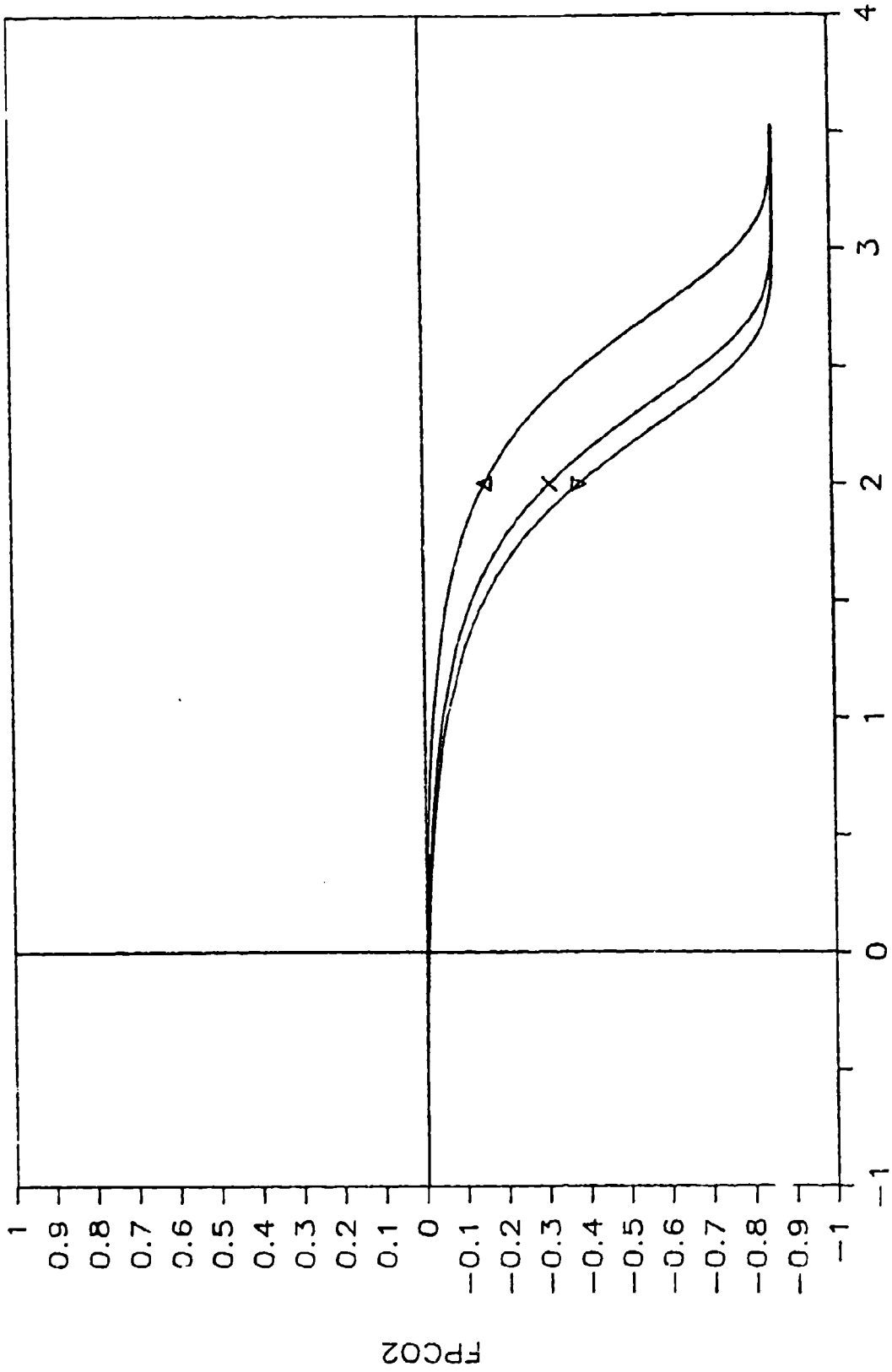
LOG TIME IN MINUTES

△ WORK LEVEL 1    x WORK LEVEL 3    ▽ WORK LEVEL 4

Figure A16

97

SENSITIVITY TO M
NON-LINEAR

FM

LOG TIME IN MINUTES

△ WORK LEVEL 1    x WORK LEVEL 3    ▽ WORK LEVEL 4

Figure A17

SENSITIVITY TO PB
NON-LINEAR

LOG TIME IN MINUTES

Figure A18

WORK LEVEL 1    × WORK LEVEL 3    ▽ WORK LEVEL 4

FPB

# SENSITIVITY TO VB
## NON-LINEAR
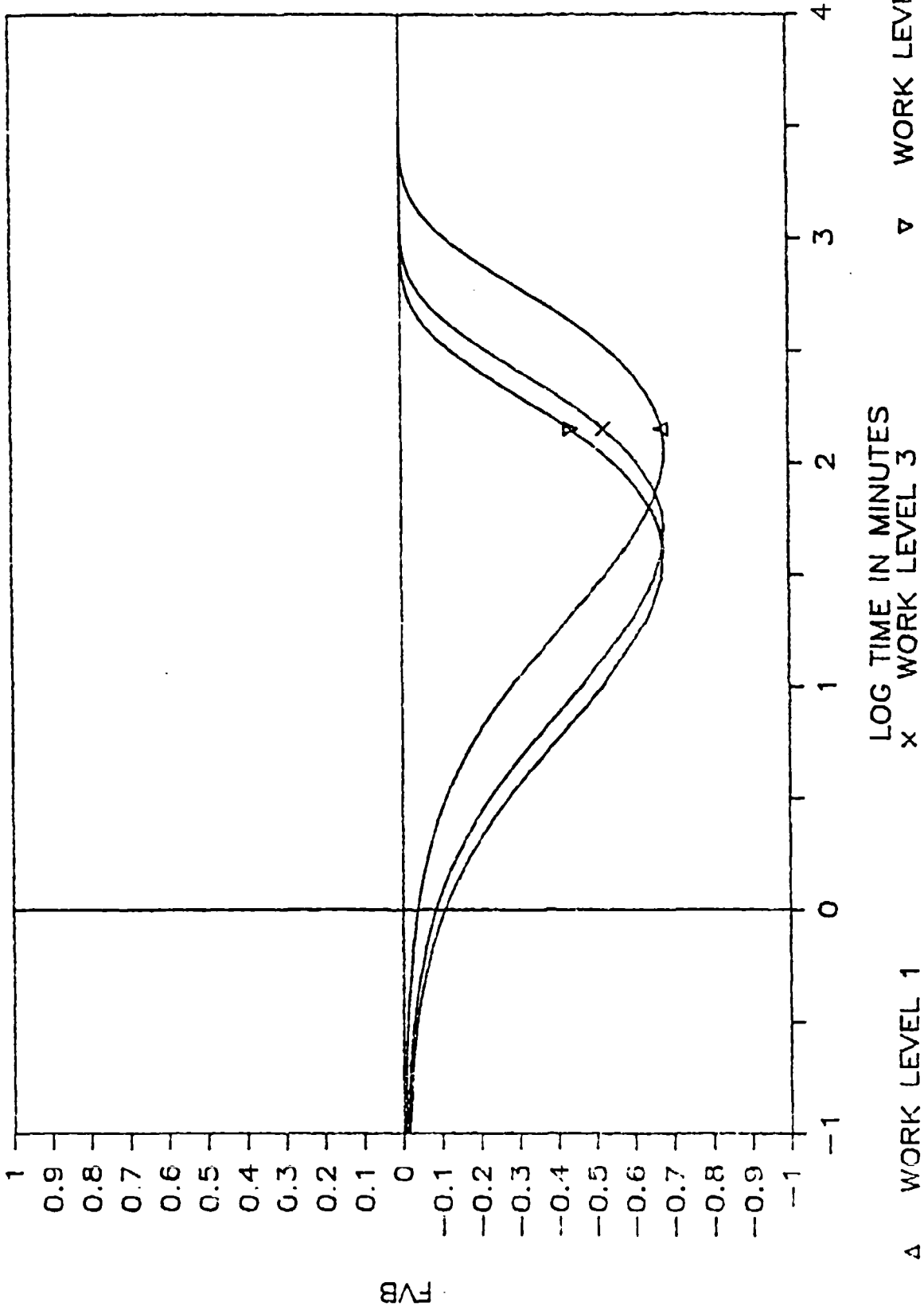


Figure A19

SENSITIVITY TO THB
NON-LINEAR

Figure A20

FTHB

LOG TIME IN MINUTES

WORK LEVEL 1
WORK LEVEL 3
WORK LEVEL 4

95
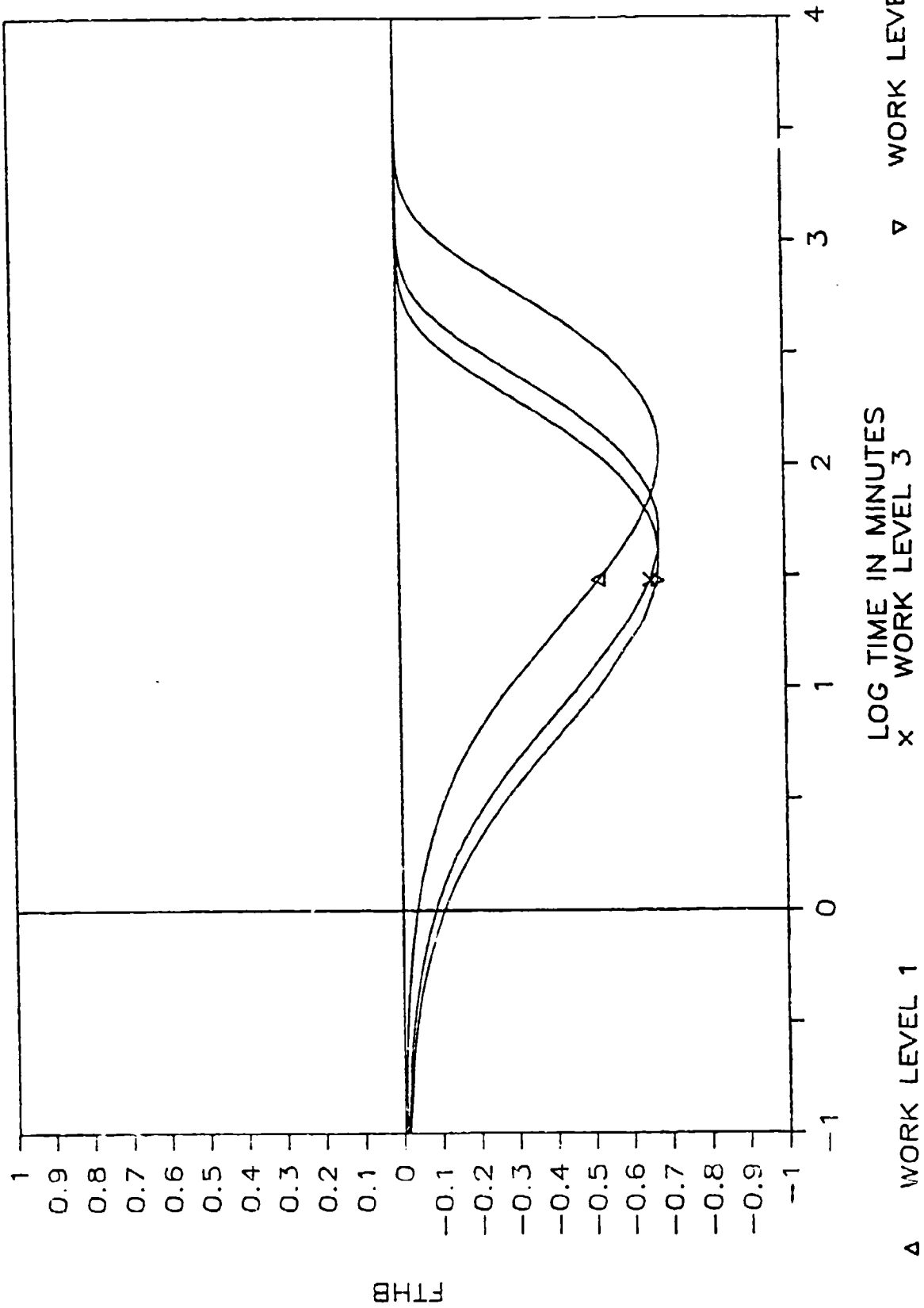
SENSITIVITY TO V̇A
NON-LINEAR

Figure A21

△ WORK LEVEL 1    x WORK LEVEL 3    ▽ WORK LEVEL 4
LOG TIME IN MINUTES
WORK LEVEL 3

F/V̇A

SENSITIVITY TO PBFIX

NON-LINEAR

Figure A22

SENSITIVITY TO DLCO

NON-LINEAR

Figure A23

WORK LEVEL 1

WORK LEVEL 3

WORK LEVEL 4

LOG TIME IN MINUTES

FDLCO

# SENSITIVITY TO PH2O
## NON-LINEAR



WORK LEVEL 1

FPH2O
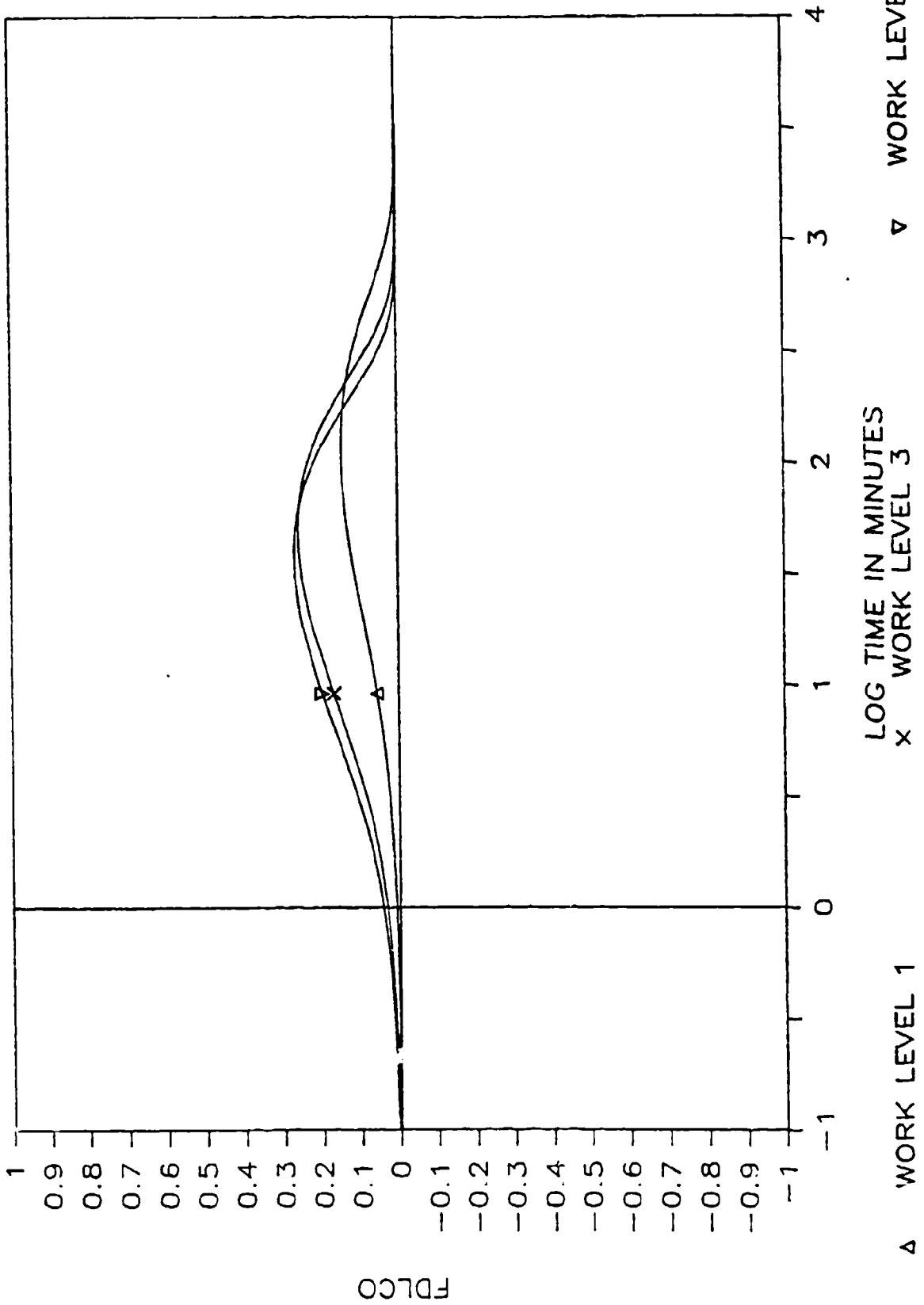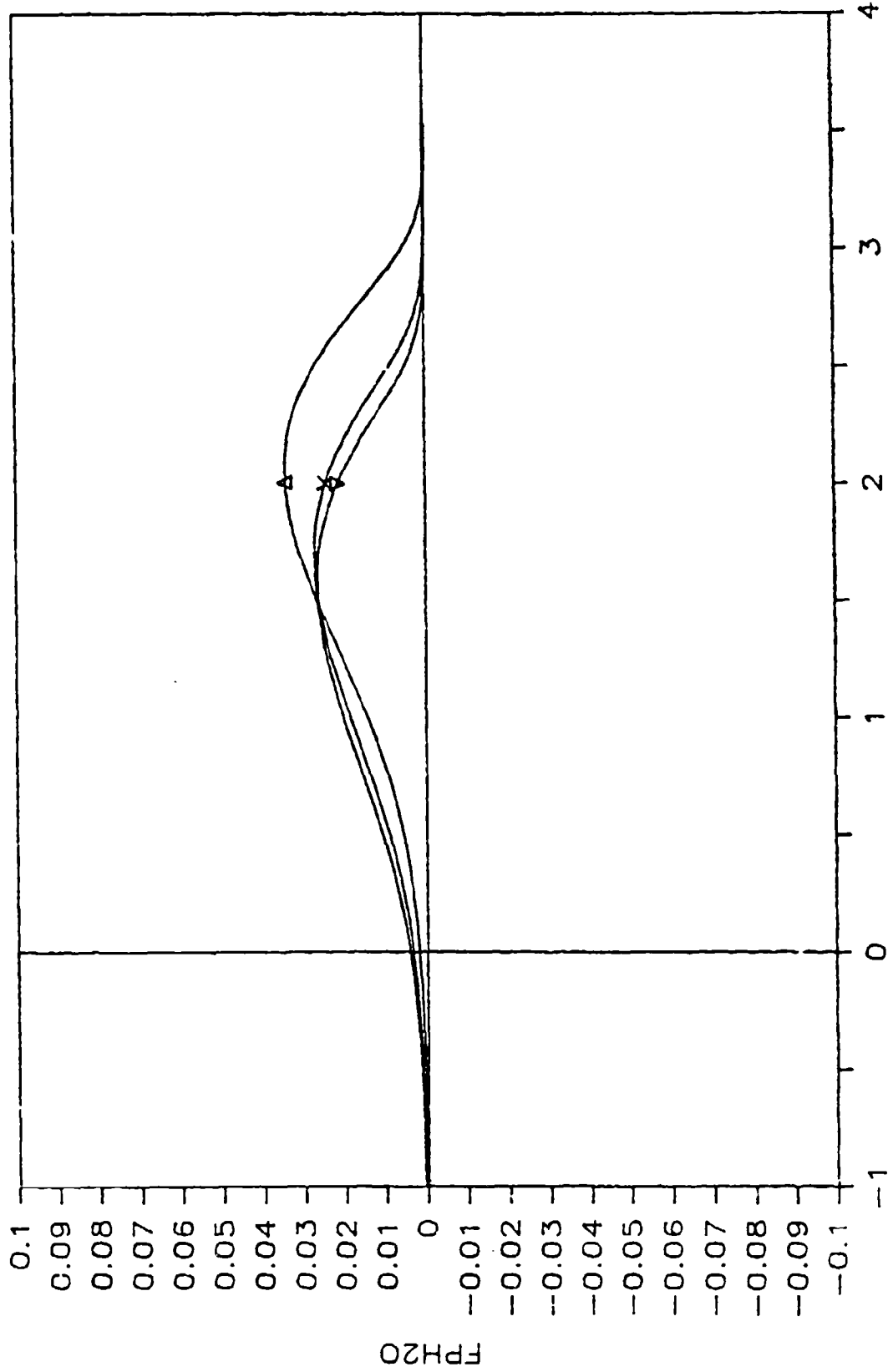
LOG TIME IN MINUTES
x  WORK LEVEL 3

△  WORK LEVEL 4

▽  WORK LEVEL 4

Figure A24

# SENSITIVITY TO V̇CO

## NON-LINEAR



FV̇CO

LOG TIME IN MINUTES
X WORK LEVEL 3
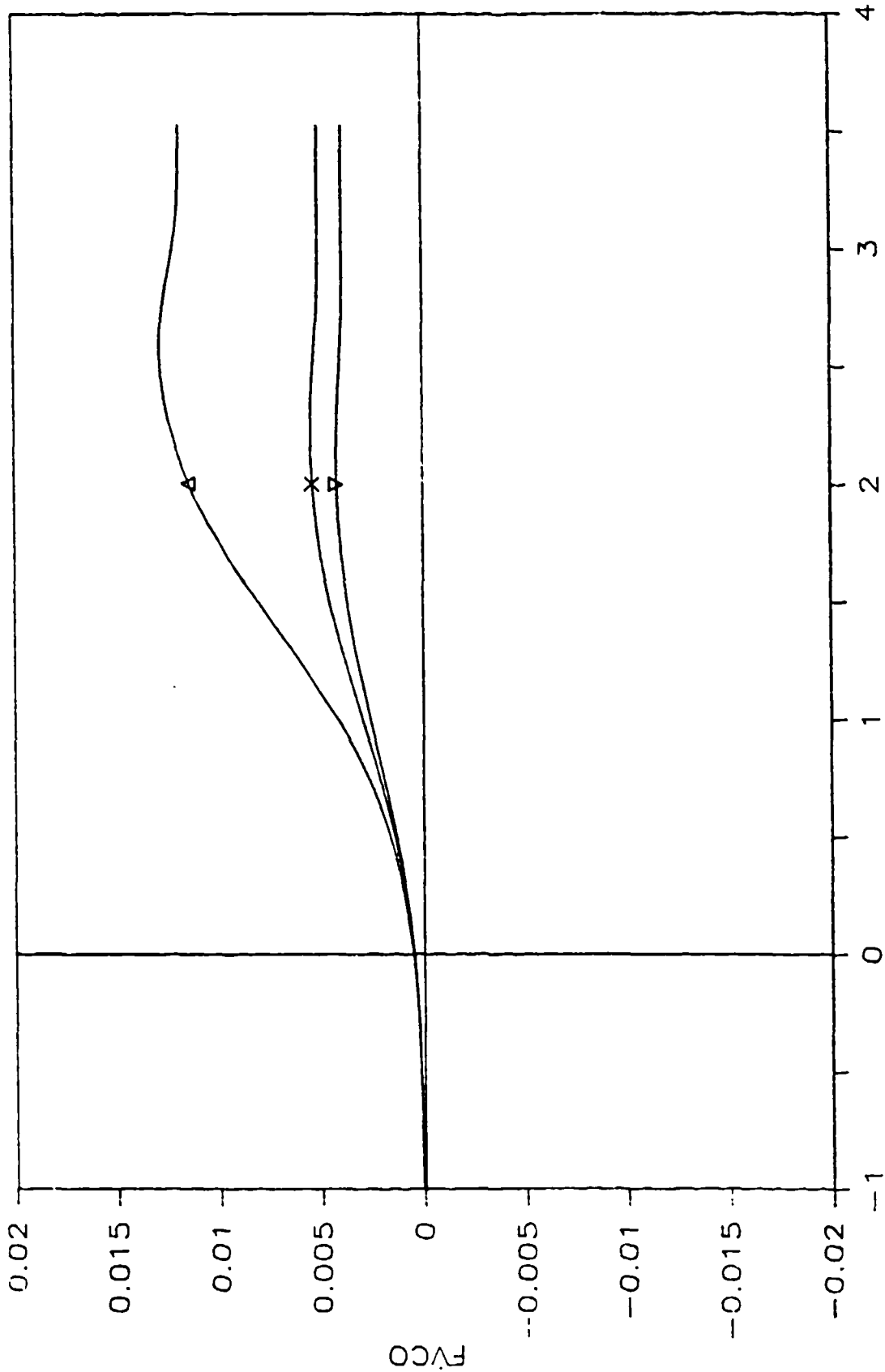
△ WORK LEVEL 1

▽ WORK LEVEL 4

Figure A25

100

Both sets of figures were generated by solving the difference equations using a fourth-order Runge-Kutta algorithm. In the linear cases, the results were compared with the analytic solutions. The numerical and analytical results agree within 1 part in $10^6$.

The third analysis uses the following expressions:

$$\frac{d[COHb](t)}{dt} = \frac{1}{V_b} [\dot{V}_{CO} - \frac{[COHb](t)\ P_c O_2}{M[O_2Hb](t)} \cdot \frac{1}{1/D_LCO + P_D/\dot{V}_A}$$

$$+ \frac{P_I CO}{1/D_LCO + P_D/\dot{V}_A} ]$$

$$V_b = \frac{V_{rbc} - 3n(HCT/100)}{(0.91)(0.97)(HCT/100)}$$

$$[O_2Hb](t) = 0.97([THb](1-\%MHb/100) - [COHb](t)) \times 1.39 \times 10^{-2}$$

$$P_D = P_B - (31.3269 - 1.172975T_B + 0.0583063T_B^2)$$

$$\dot{V}_A = k\ \dot{V}_E(273/(273 + T_B))(P_B/760)$$

$$P_{ICO} = FICO*(10-6)(P_B - (31.3269 - 1.172975TB + 0.0583063TB^2)$$

$$[COHb](0) = \%COHb(0) \cdot [THb] \times 1.39 \times 10-2$$

The experimental variables (measured or assumed) with respect to which sensitivities are calculated are then:

HCT      -  Hematocrit, %    (= 45)

$V_{rbc}$    -  Red blood cell volume, ml    (= 2185)

$\dot{V}_{CO}$    -  Endogenous CO production rate, ml/min    (= 0.007)

$P_cO_2$ — Average pulmonary capillary oxygen partial pressure, mmHg    (=         M         — Haldane coefficient   (= 215)

[THb] — Total hemoglobin concentration, g/dl    (= 15.4)

%MHb — Methemoglobin saturation, %   (= 0.10)

$P_B$ — Barometric pressure, mmHg    (= 760)

$T_B$ — Body temperature, °C    (= 37)

$D_LCO$ — CO transfer factor for the lung, $ml \cdot min^{-1} \cdot mmHg^{-1}$
(= 30;40;50)

$\dot{V}_E$ — Minute expired ventilation rate, ml/min
(= 8791; 26374; 35165)

$F_ICO^*$ — Ambient CO concentration, ppm   (= 100)

%COHb(0) — initial (seed) COHb saturation, %   (= 1.0)


The  values  above were chosen to yield approximately the same values for  the explicit variables used in the previous analyses.

## NONLINEAR CFK EQUATION SENSITIVITIES TO MEASURED VARIABLES

SENSITIVITY TO %COHB(O)-MEASUREMENT
NON-LINEAR

Figure 8.26

WORK LEVEL 1    WORK LEVEL 3    WORK LEVEL 4

LOG TIME IN MINUTES

FCOHB

104
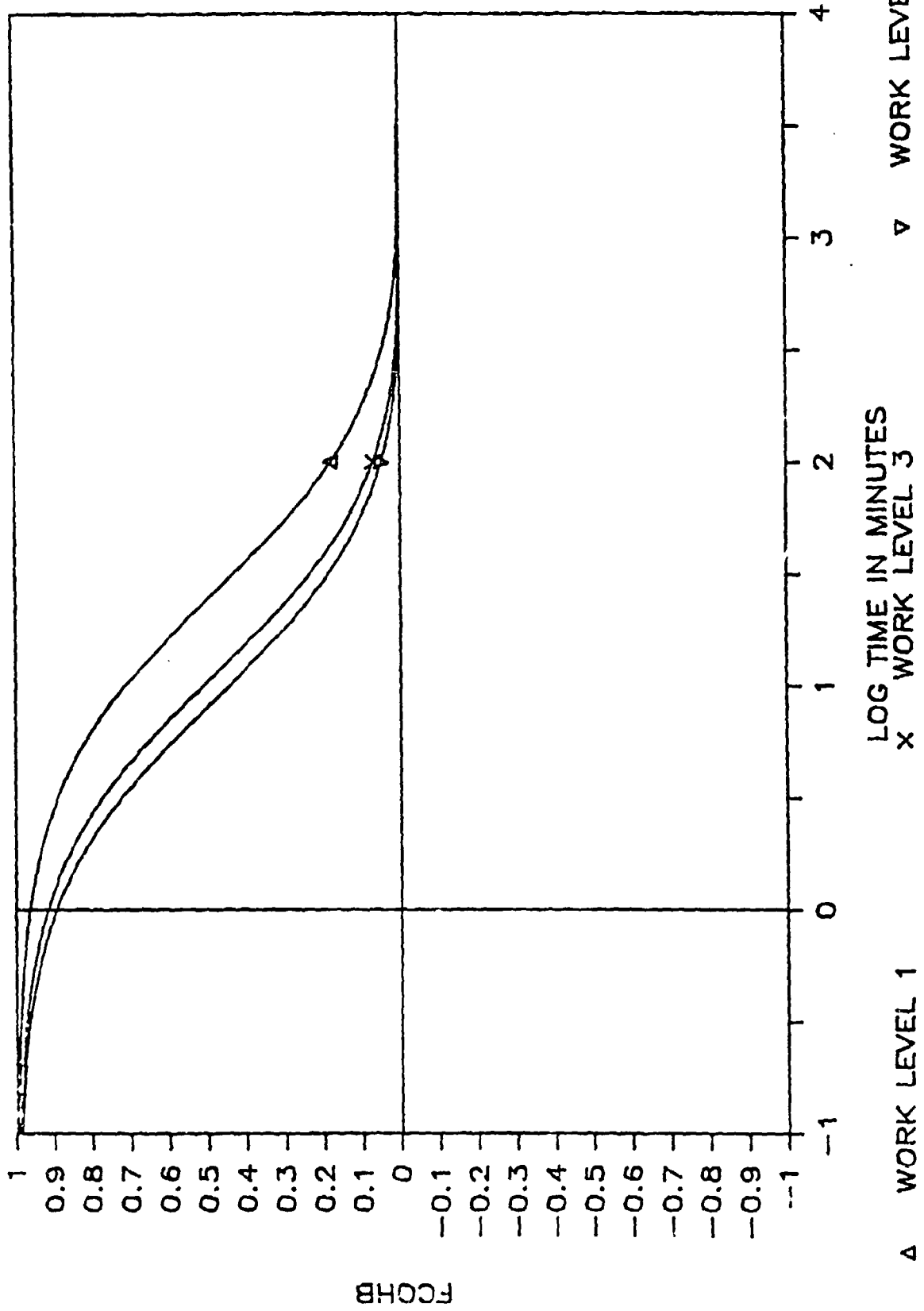
# SENSITIVITY TO PB — MEASUREMENT
## NON-LINEAR



LOG TIME IN MINUTES

FPB
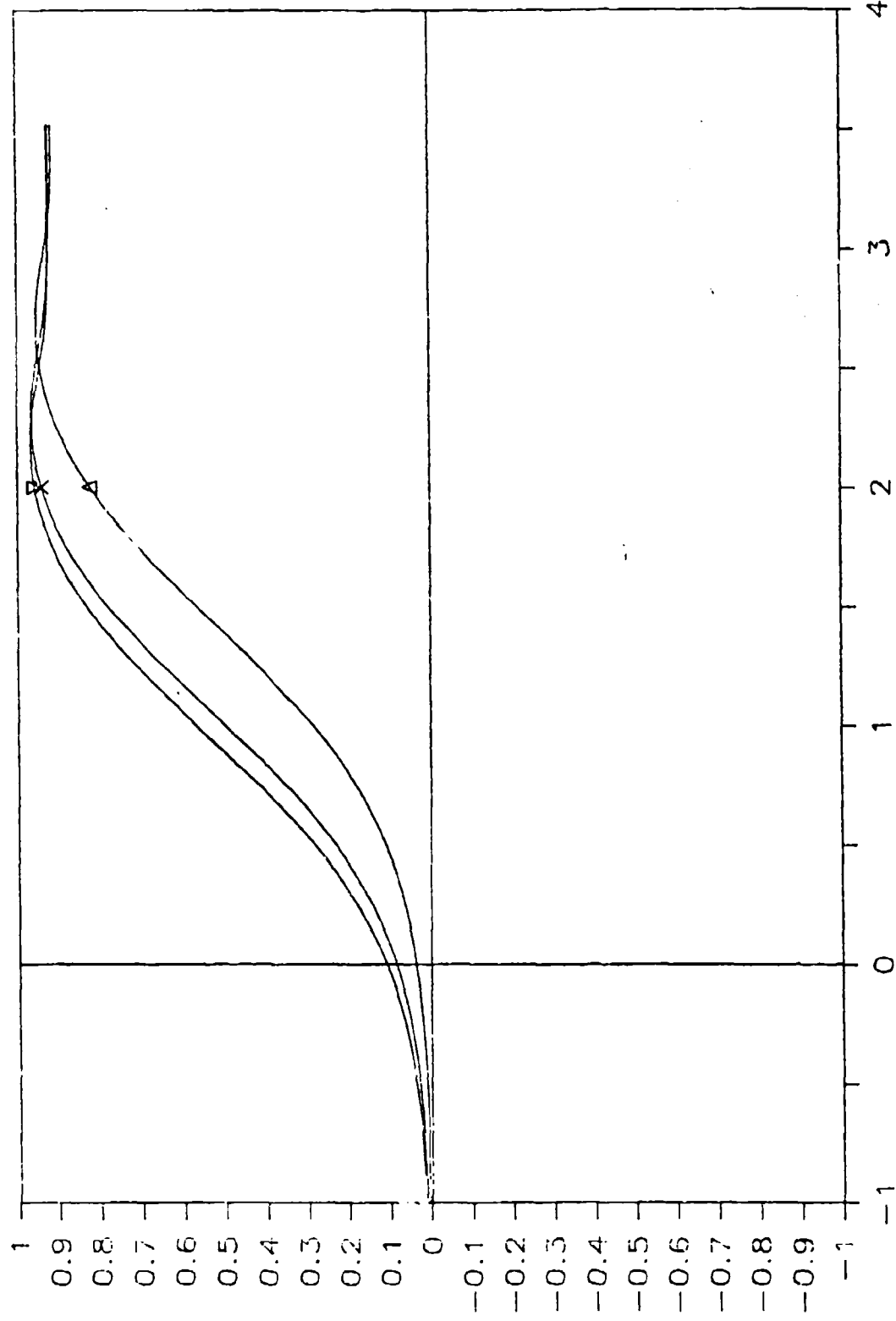
WORK LEVEL 1

x   WORK LEVEL 3

△   WORK LEVEL 1

▽   WORK LEVEL 4

Figure A27

# SENSITIVITY TO FICO — MEASUREMENT

## NON-LINEAR



Figure A.28

Δ WORK LEVEL 1    x WORK LEVEL 3

LOG TIME IN MINUTES

▽ WORK LEVEL 4

FFICO
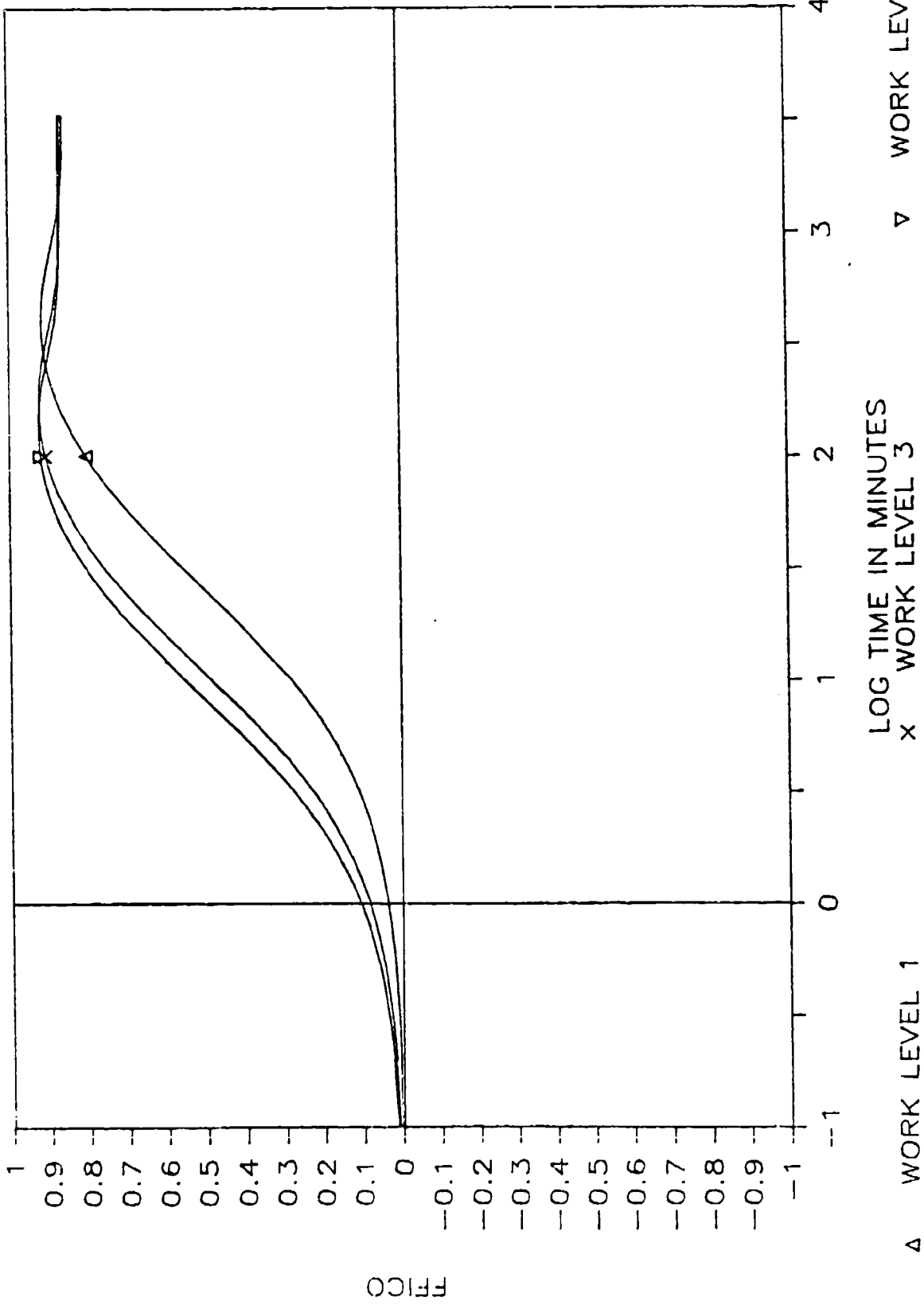
# SENSITIVITY TO M — MEASUREMENT
## NON-LINEAR



LOG TIME IN MINUTES

△ WORK LEVEL 1     X WORK LEVEL 3     ▽ WORK LEVEL 4

Figure A29

# SENSITIVITY TO PCO2 — MEASUREMENT
## NON-LINEAR



Figure A30

WORK LEVEL 1      $\triangle$   WORK LEVEL 4

$\times$   WORK LEVEL 3

LOG TIME IN MINUTES

FPCO2

# SENSITIVITY TO HCT — MEASUREMENT

## NON-LINEAR



Figure A31

WORK LEVEL 1

△ WORK LEVEL 4    ▷ WORK LEVEL 3    × WORK LEVEL 3

LOG TIME IN MINUTES

FHCT

# SENSITIVITY TO THB — MEASUREMENT

## NON-LINEAR



LOG TIME IN MINUTES
x WORK LEVEL 3

FTHB

△ WORK LEVEL 1

▽ WORK LEVEL 4

Figure A32

110
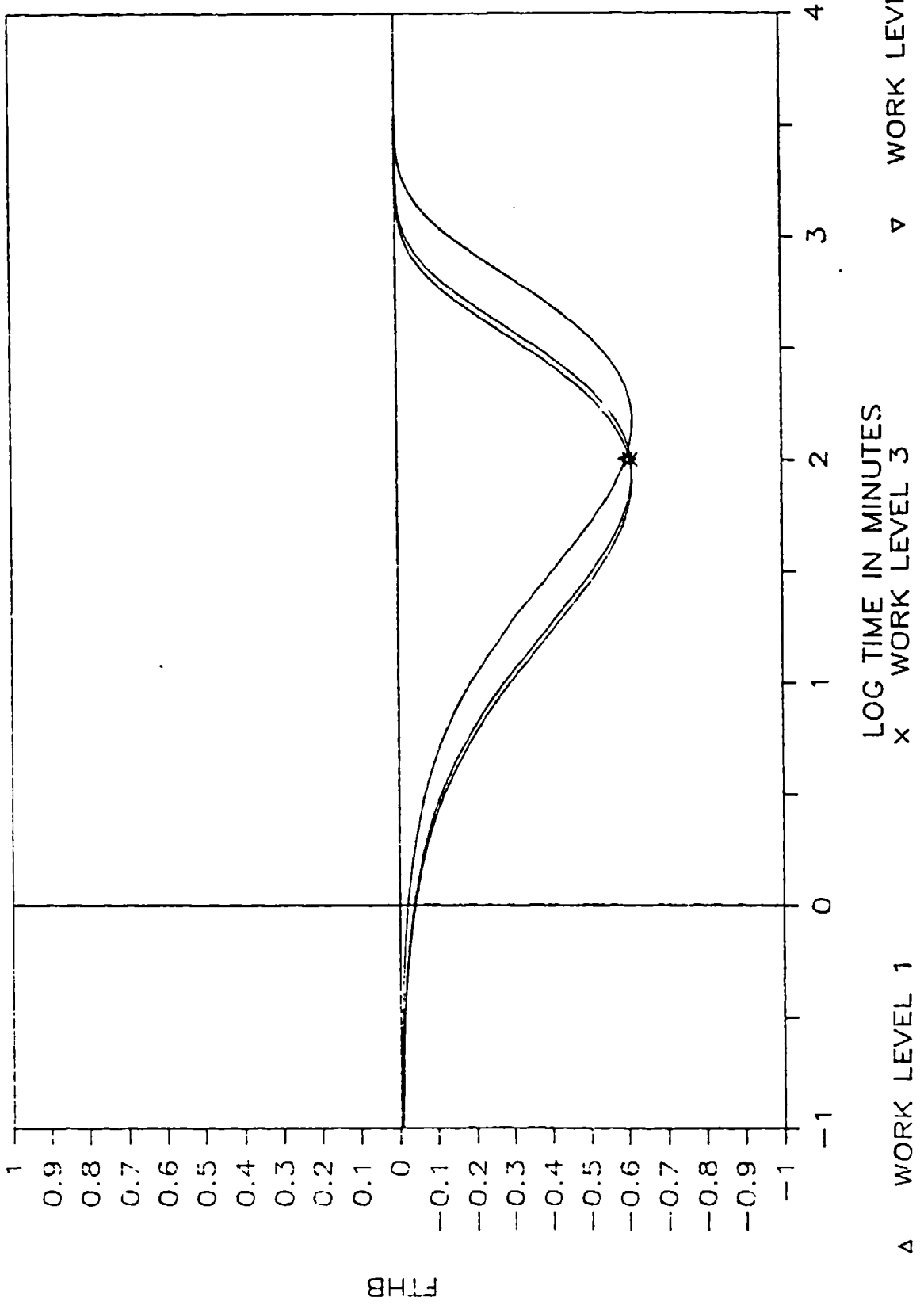
# SENSITIVITY TO VRBC — MEASUREMENT
## NON-LINEAR

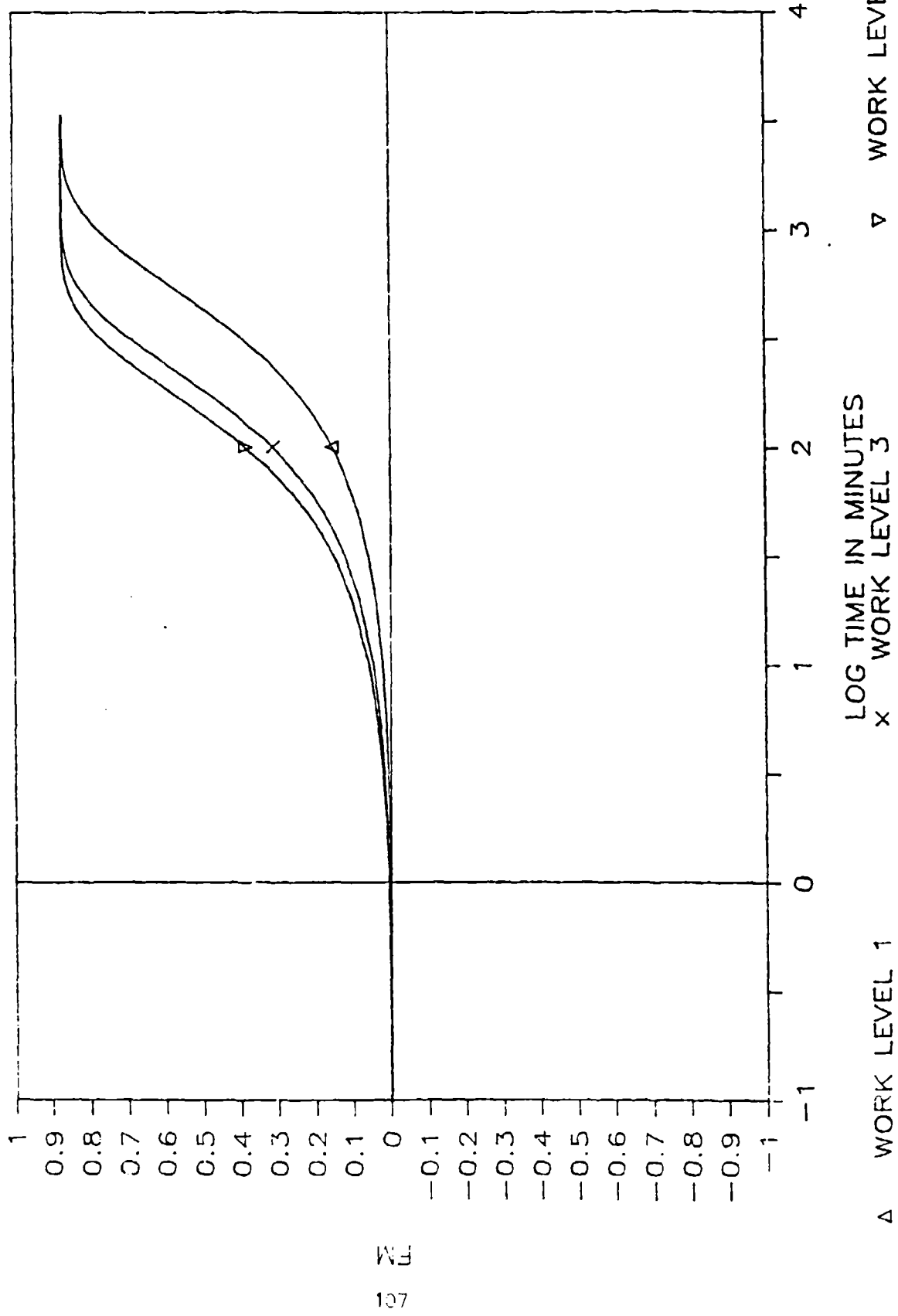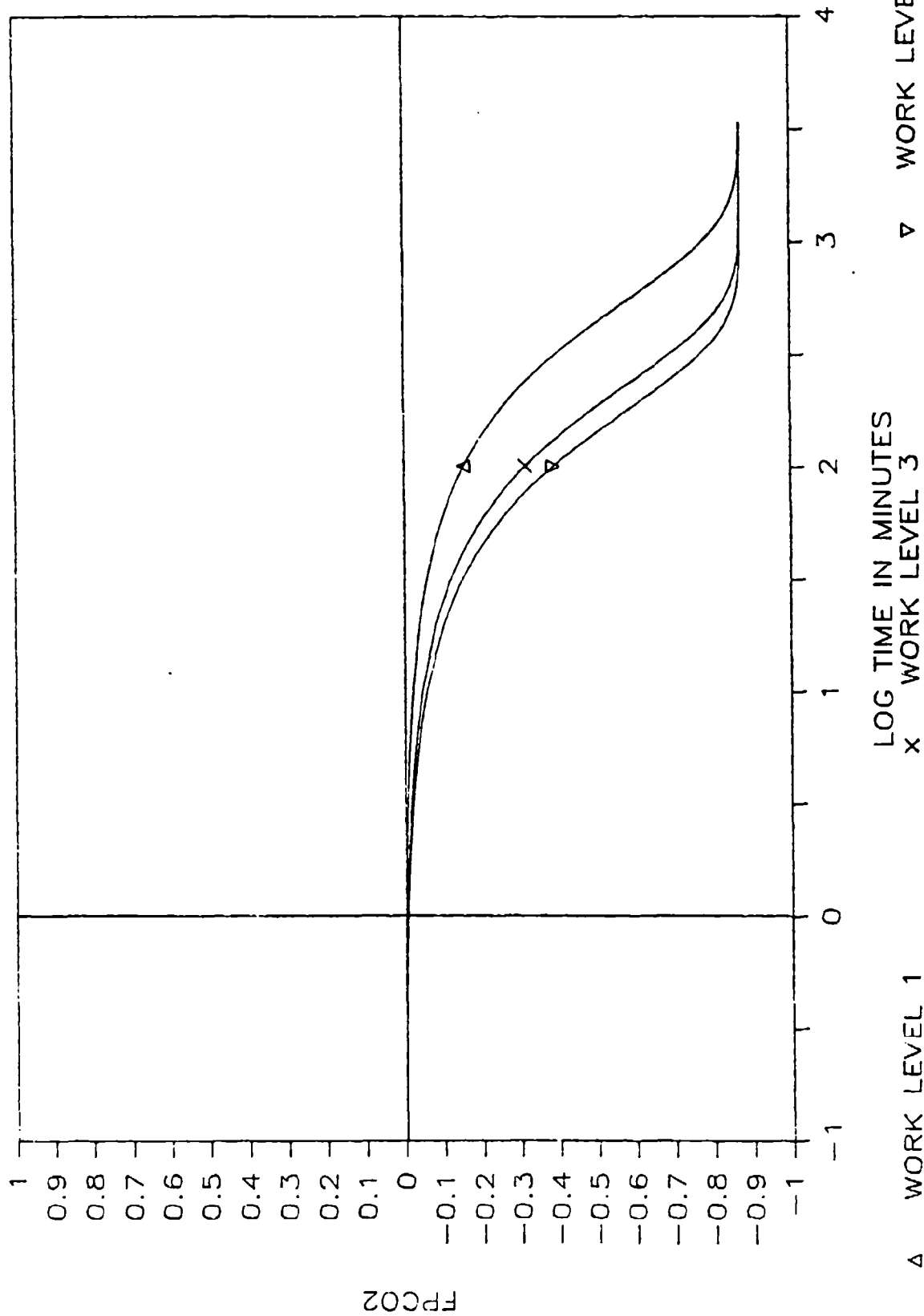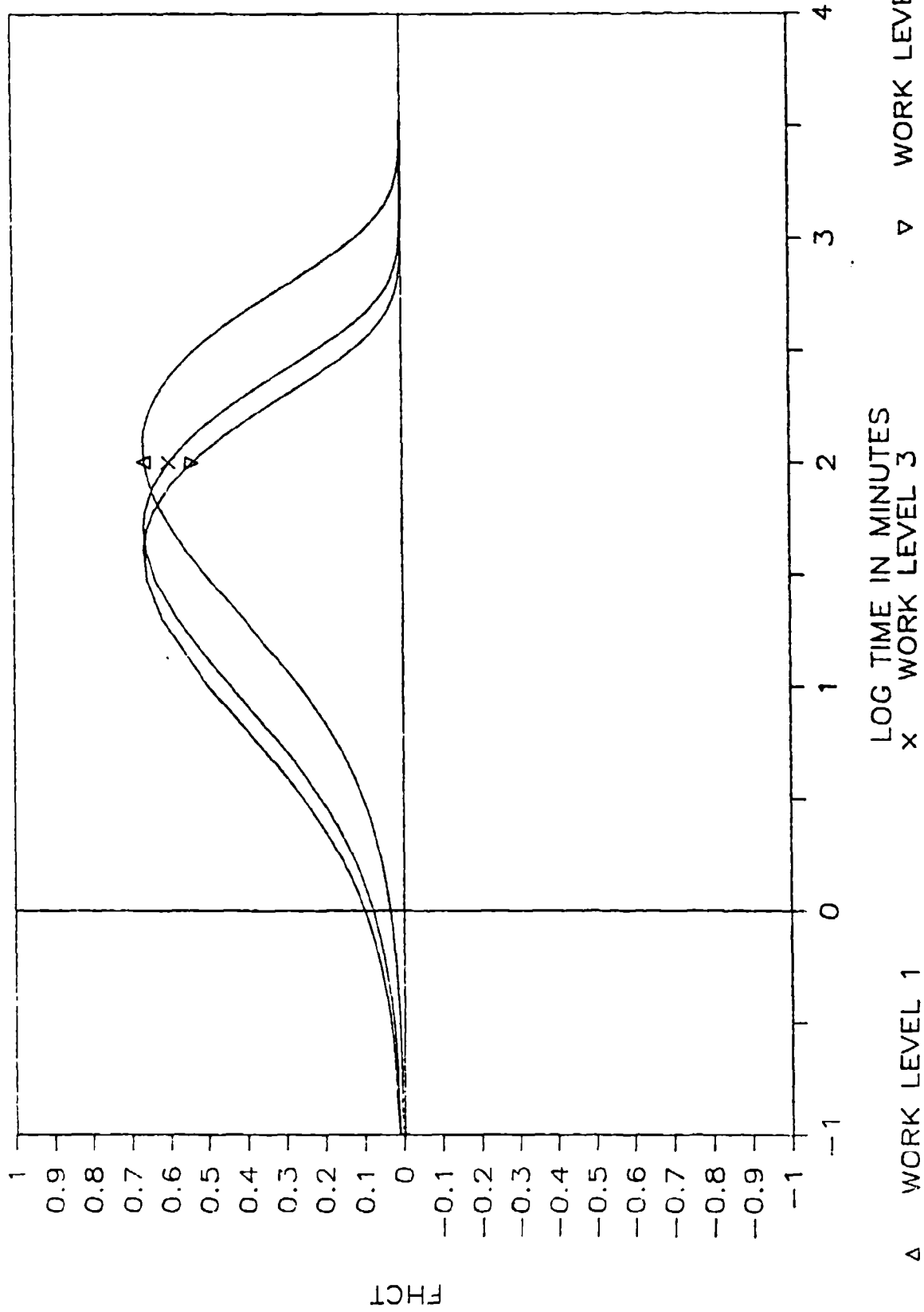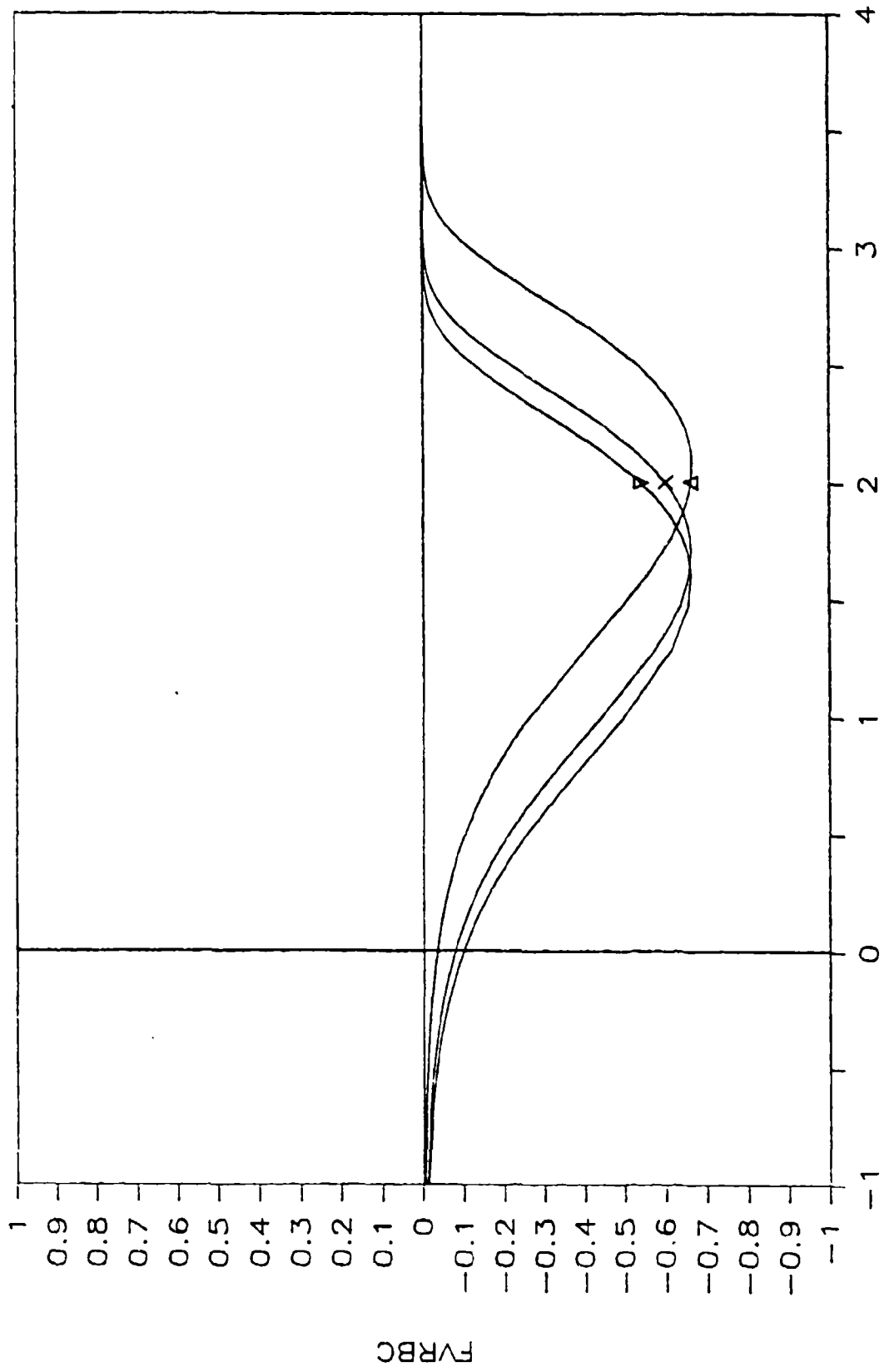

Figure A33

SENSITIVITY TO VMIN — MEASUREMENT

NON-LINEAR

LOG TIME IN MINUTES

FVMIN

△  WORK LEVEL 1

×  WORK LEVEL 3

▽  WORK LEVEL 4

Figure A34

112
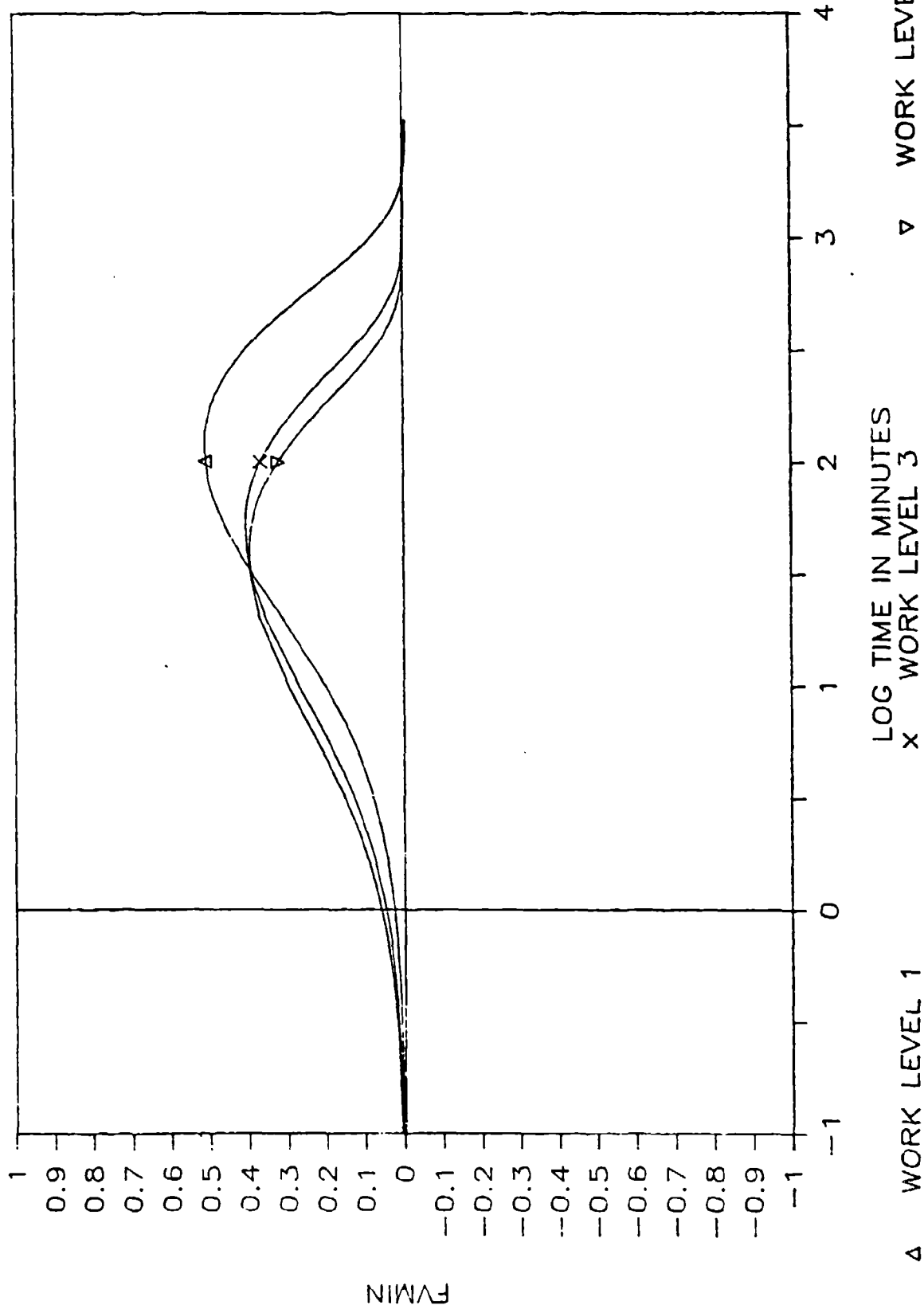
SENSITIVITY TO DLCO — MEASUREMENT

NON-LINEAR

LOG TIME IN MINUTES

WORK LEVEL 1    WORK LEVEL 3    WORK LEVEL 4

Figure A35

113
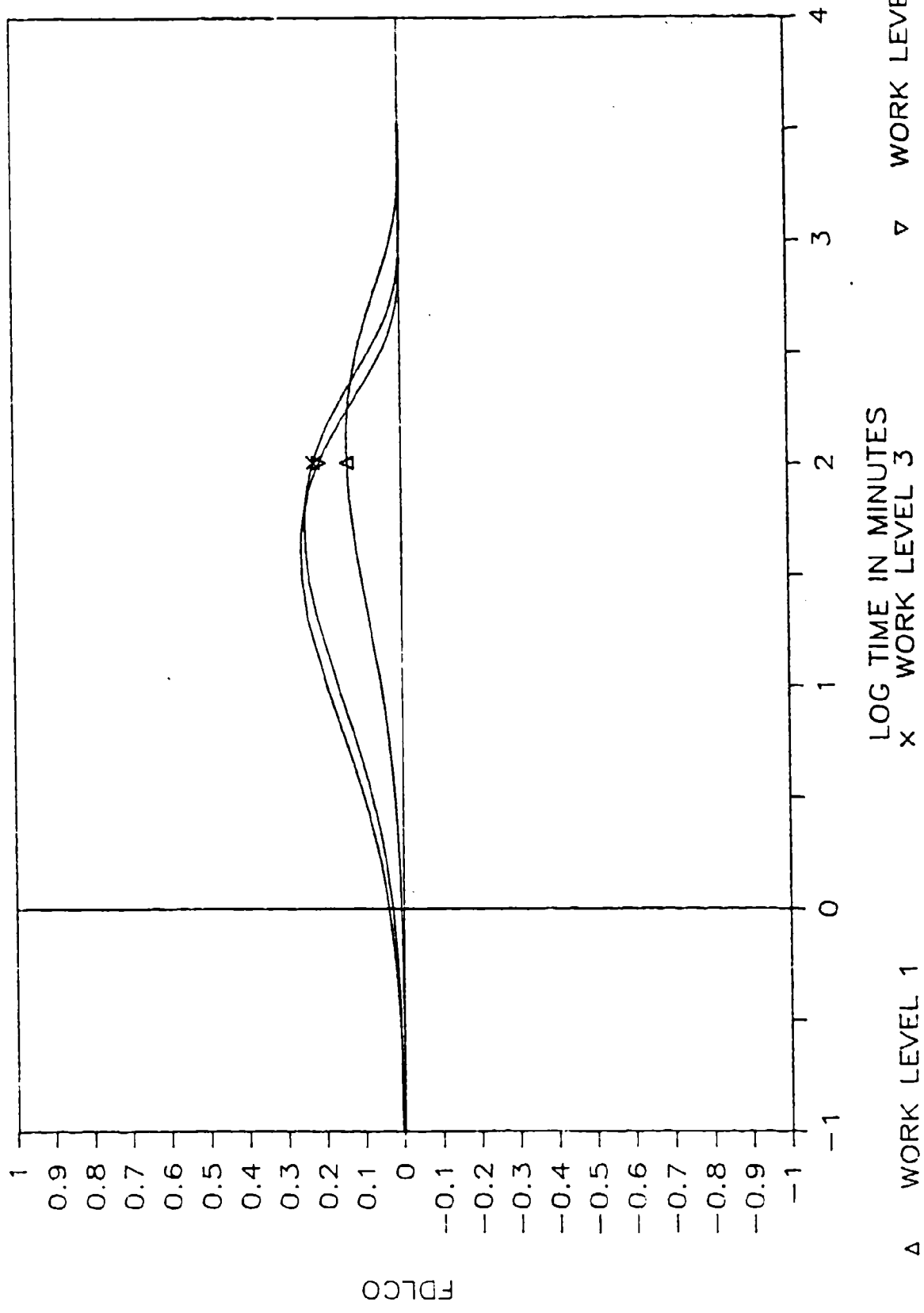
# SENSITIVITY TO TB — MEASUREMENT
## NON-LINEAR

FTB

0.2  0.15  0.1  0.05  0  -0.05  -0.1  -0.15  -0.2

-1  0  1  2  3  4

LOG TIME IN MINUTES
x  WORK LEVEL 3

△  WORK LEVEL 1  ▽  WORK LEVEL 4

Figure A36

114
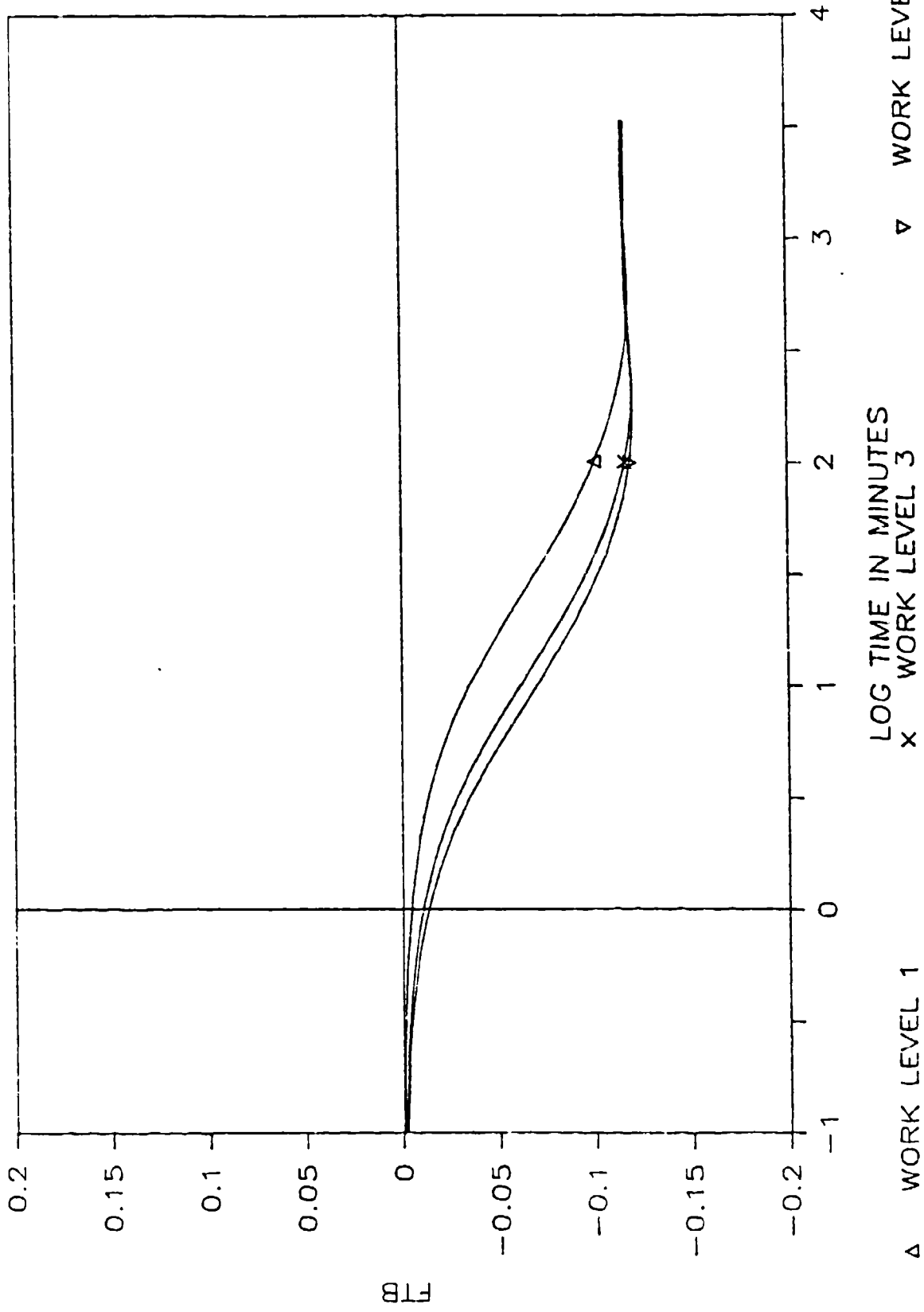
# SENSITIVITY TO V̇CO — MEASUREMENT
## NON-LINEAR



Figure A37

WORK LEVEL 1    Δ  WORK LEVEL 3    ∇  WORK LEVEL 4
LOG TIME IN MINUTES
×  WORK LEVEL 3

115
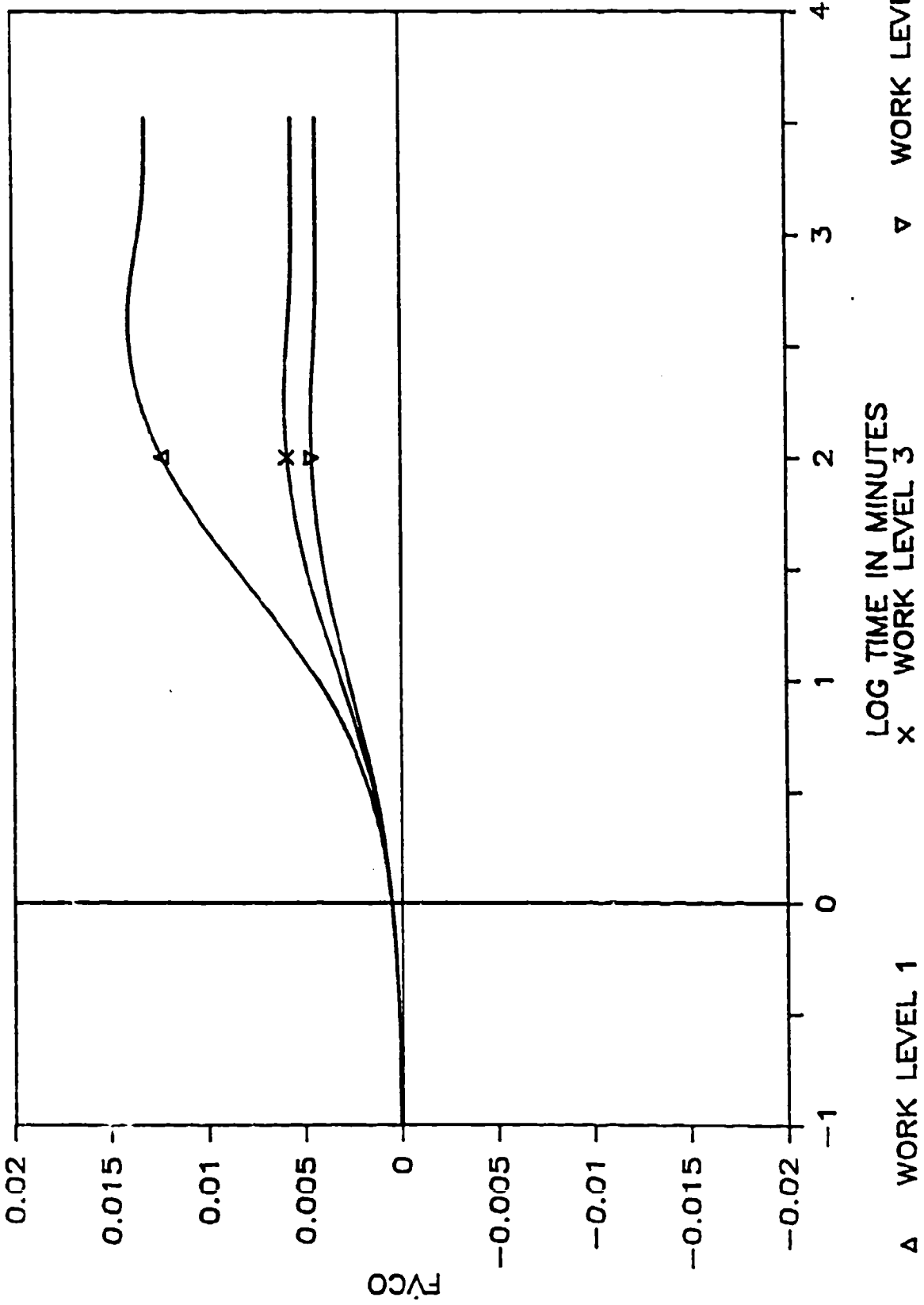
# SENSITIVITY TO MHB — MEASUREMENT
## NON-LINEAR



LOG TIME IN MINUTES

FMHB

Δ   WORK LEVEL 1      X   WORK LEVEL 3      ▽   WORK LEVEL 4
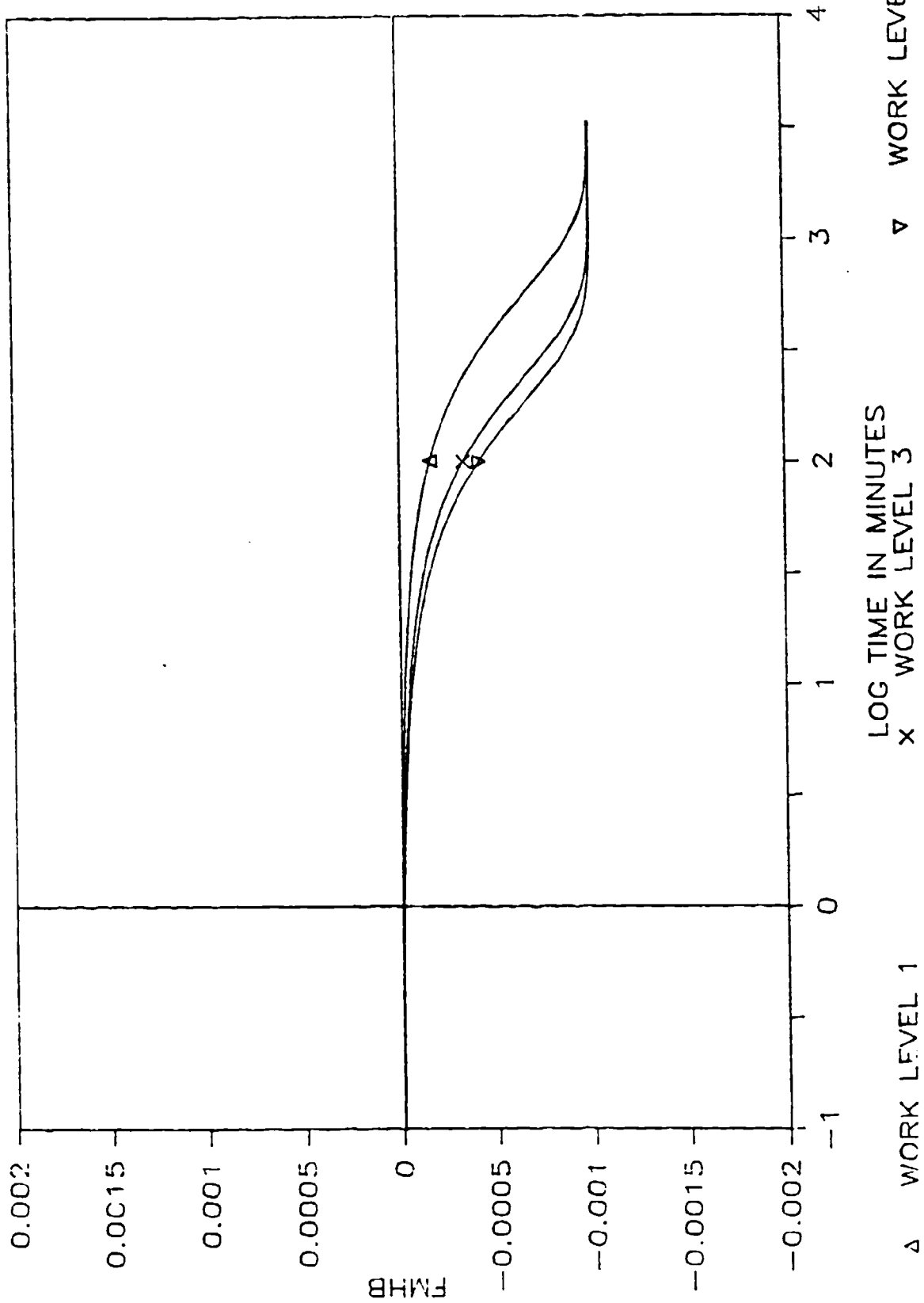
Figure  A38

The use of these sensitivity curves should be viewed as merely illustrative rather than as rigidly quantitative. Their interpretation is straightforward, as an example will show.

The contribution of error in (or deliberate perturbation of) a given variable to error in (or perturbation of) %COHb is obtained from

$$\Delta\%COHb(t) = Fx_i(t) \cdot \Delta\%x_i$$

where $Fx_i(t)$ is the ordinate value on a sensitivity curve for variable $x_i$ at some time of interest (t). The fractional perturbation in $x_i$ expressed as percent ($\Delta\%x_i$) multiplied by the fractional sensitivity then yields the fractional perturbation in calculated %COHb.

Consider figure A38, the fractional sensitivity of calculated %COHb to measured methemoglobin saturation. Under the exposure conditions assumed (i.e., a constant background of 100 ppm CO, and a constant exercise level), if %MHb is the only variable with error, then we observe that for a time of exposure less than about 10 minutes (log time <1.0), the effect of error in %MHb is essentially zero. If we allow an experiment to run for 1000 minutes (log time = 3.0), then a 1% error in %MHb will appear as approximately 0.001% error in calculated %COHb (the ordinate value for $Fx_i$ at 1000 min (~0.001) times the error in %MHb), and this limiting error is reached more rapidly at higher levels of exercise than at lower levels.

Because %MHb is typically in the range of 0.1% and because the error in %MHb can be 100% (primarily because 0.1% is the resolution with which it can be obtained), then we expect calculated %COHb to suffer an error of 0.1% (100% x 0.001) as a result of 100% %MHb error. Similar treatments of the other variables are easy to envision.

The use of exercise level as a parameter in these analyses illustrates the interaction among each of the variables with $\dot{V}_A$ and $D_LCO$, which are

primary determinants of CO dose.    In general,   higher exercise levels cause a more   rapid   achievement   of the equilibrium sensitivity   to   each   variable.

The   results   of these three sensitivity analyses are   substantially   the same   for those variables which appear as independent in all three   equations, with the notable exception of barometric pressure,   $P_B$.   The sensitivity to $P_B$ as a measured variable is much greater than as an explicit variable because it is a factor in so many of the explicit variables.   For this reason, it should be measured with care.    Fortunately,   however, it is the measurement in which we generally have the greatest confidence.

We note that the variables can be sorted according to whether their major effect   is   on   the initial or equilibrium values of [COHb], on the   rate   of equilibration, or on more than one of these.  Because the modeling studies are based on the measured variables,   those will be briefly discussed here and the remaining   two   sensitivity studies will not be further   commented   upon.    It should   be emphasized again that the curves and ensuing discussion apply only to the exposure conditions assumed in their generation.    The use of exposures which   result   in substantial reduction of $[O_2Hb]$ will be   expected   to   cause considerable deviation from the behaviors presented here.

The   variables which are expected to have a major effect on [COHb](t) for small values of t are,   of course, %COHb(0) and [THb], the product of which is [COHb](0).    This initial value constitutes an impulse input for the substan- tially linear first-order model.    As expected,   %COHb(0) has an exponentially decreasing effect   on   calculated   [COHb](t) as   time   increases, with   its influence on equilibrium decaying to zero.

The behavior of [THb] appears somewhat anomalous.    As shown,   the sensi- tivity   to   [THb] is zero at very small and very large values of   time,   while sensitivity   to %COHb(0) at small values of time is unity.    Because [COHb]   =

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[%COHb·[THb], one would expect that the fractional sensitivities near t=0 would be similar. The results differ, however, because the fractional sensitivity plots are created from calculated % saturation values of COHb, and these are derived from the perturbed values of [THb]. If the fractional sensitivity were plotted for [COHb] rather than %COHb, then near t=0, the plots would be essentially the same as those for %COHb(0). Per cent saturation plots were chosen because %COHb is the measurement obtained from the CO-Oximeter™ and [COHb] must be calculated from it using [THb] supplied by the instrument.

Variables which affect only the equilibrium value of [COHb](t) are $P_B$, $F_ICO^*$, M, $P_cO_2$, $T_B$, $\dot{V}_{CO}$ and %MHb. We have mentioned the behavior of $P_B$ above but note here that error in $P_B$ propagates essentially unattenuated to equivalent error in calculated [COHb](t) for t>100 minutes..

The atmospheric concentration of CO, $F_ICO^*$, would be expected to have a definite proportional effect on equilibrium [COHb](t) for values which correspond to less than 100% saturation. The error in $F_ICO^*$ does not appear totally unattenuated at equilibrium because $\dot{V}_{CO}$ also contributes to [COHb].

Because the blood capacity for CO depends on both the amount of blood present and its affinity for CO, the Haldane constant is expected to influence both equilibrium and the rate at which it is achieved. Its effect on [COHb] at equilibrium is attenuated only about 10% (i.e., a 1% error in the assumed value of M appears as approximately 0.9% error in calculated [COHb]).

Pulmonary capillary oxygen partial pressure, $P_cO_2$, is in direct competition with CO for sites on the hemoglobin, consequently errors in $P_cO_2$ estimation result in [COHb] calculation errors of similar magnitude but opposite sign.

Body temperature influences [COHb](t) through its use to calculate saturation pressure of water vapor in tracheal air. The exact form of this effect

will depend on the algorithm chosen to describe the relation between $T_B$ and $P_{H_2O}(T)$, but the effect in the normal range of body temperatures is small compared with most of the other measurements (e.g., a 1°C error in estimation of $T_B$ at 37 °C yields an error of <0.35% in [COHb](t) at equilibrium).

Because methemoglobin in also "in competition" with COHb, an error in its estimate results in an error of the opposite sign in calculated [COHb]. In all but serious pathologies, the contribution of %MHb can be ignored because both its fractional sensitivity and its absolute values are small.

The effect of endogenous CO production rate is quite small in all but severe hemolytic anemias or drug-induced heme destruction. Higher equilibrium values of [COHb] are anticipated for higher $\dot{V}_{CO}$ at a fixed exercise level and alternatively for a fixed $\dot{V}_{CO}$ and lower exercise level. For acute exposures to exogenous CO, however, the effect of errors in $\dot{V}_{CO}$ estimation is seen to be relatively small.

The remaining variables affect primarily the rate at which equilibrium is achieved. Hematocrit affects only the calculation of blood volume. An over-estimate of hematocrit results in an underestimate of blood volume which then causes an underestimate in calculated CO uptake time constant. As a result, calculated [COHb] rises more rapidly and equilibrium is obtained earlier. Because total blood volume does not affect the equilibrium value of [COHb], neither will either of the measurements from which it is calculated (HCT, $V_{rbc}$). This is seen in the sensitivity for $V_{rbc}$, in which an overestimate of $V_{rbc}$ results in an overestimate of blood volume, a consequent overestimate of time constant, and a transient negative undercalculation of [COHb].

Ventilation, in the absence of endogenously-produced CO, would affect only the equilibrium time constant, with positive error resulting in transient positive errors in calculated [COHb]. Because CO is also produced by the

body, the rate at which it is exchanged with the atmosphere is a function of ventilation, hence the equilibrium value of [COHb] will also be slightly affected (note the slight negative excursion at the highest exercise level).

The lung transfer factor also substantially affects only the rate at which equilibrium is achieved. An effect similar to that for ventilation, however, does occur, and at higher exercise levels, the equilibrium [COHb] will be affected because of the increased rate of elimination of endogenously-produced CO.

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