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DEMONSTRATION OF A TECHNIQUE FOR ESTIMATING DETECTION LIMITS WITH SPECIFIED ASSURANCE PROBABILITIES

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1. INTRODUCTION

Procedures for determining critical concentrations below which chemical instrumentation or chemical methods are considered "unreliable" -- in terms of their capability to indicate the presence or absence of a given substance in some environmental medium -- have received a great deal of attention within the United States' Environmental Protection Agency (EPA). This concern has been manifested in the Agency's requirement that concentrations below the "detection limit" be clearly identified in data bases or in reported data. Unfortunately, different authors and researchers have used different terminology to refer to such limiting concentrations and have adopted different definitions of "detection limits." In many cases, procedures for determining such limits have been established and implemented with little conceptual or statistical justification.

The purposes of this report are (1) to describe an approach for defining and estimating detection limits, and (2) to demonstrate the use of this approach through an actual example relevant to EPA activities.

Errors inherent to chemical measurement of analyte responses -- a result of instrumental noise, chemical interferences, operator inconsistencies, etc. -- require that some type of "detection rule" be formulated for asserting that a given analyte either is or is not present in a given source matrix. For example, the rule might take the form: "declare analyte A to be present if the magnitude of a single response measurement exceeds some prescribed value". Such a "detection rule" is employed in EPA's current procedure for defining/estimating the "Method Detection Limit", for example. Based upon the use of any such rule, we may claim that the analyte of interest is present in the given matrix when in fact it is not (a Type I error, or false positive statement). On the other hand, we may conclude that the analyte is absent when it is in fact present (a Type II error, or false negative statement).

As shown in Chapter 3, EPA's current approach for defining/estimating the "Method Detection Limit" tends to emphasize achieving protection against false positives and tends to ignore achieving any specific level

of protection against false negatives. A preferred approach would permit protection against both types of errors at prescribed levels that are consistent with the objectives and needs of the specific EPA study or program. Such an approach is described in Chapter 3.

The approach advocated herein is based upon the notion that all of necessary parameters can be estimated using standard calibration procedures. (The calibration is carried out, however, over a range of "low" concentrations that is much narrower than the range that would normally apply to most calibration situations.) Chapter 2 therefore describes the calibration process. One major function of Chapter 2 is to introduce some of the terminology and notation that is used subsequently in Chapters 3 and 4. A second major function is to describe some of the important features and properties of calibration designs in relation to analyte detection.

Chapter 3 then defines the detection limit, describes procedures for its estimation, and compares the approach with traditional methods.

Chapter 4 describes a two-phased experiment designed to illustrate application of the recommended approach (Phase I) and to validate its performance empirically (Phase II).

Conclusions and recommendations are given in Chapter 5.

2. CALIBRATION

Consider a specific environmental medium, exposure source, or exposed population that may contain a specific analyte of interest. For a given medium (water, air, soil, sediment, human blood, etc.), assume that a well defined protocol exists for preparing and processing samples and taking observations. Let X denote the true, but unknown, concentration of the analyte and assume that the method produces an indirect measure, Y, that is related to X in some known (or assumed but testable) manner. The relationship between Y and X (i.e., the calibration curve) is assumed to be a monotonic function of concentration that depends on unknown parameters. These parameters are estimated during a calibration phase by fortifying "uncontaminated" media with known amounts of the analyte(s), processing these samples according to the established protocol, measuring the responses, and then fitting the calibration curve that relates Y to X. Estimated concentrations in subsequent samples are then determined by inverse interpolation of the estimated calibration curve (standard curve).

When some of the concentrations that may be estimated in this manner are "low", there is an immediate interest in whether the analyte is present ($X > 0$) or absent ($X = 0$). In such a case, it is desirable to be able to assert, with high probability of being correct, that the analyte either is or is not present. Therefore there is need for some rule, based on the magnitude of one or more observations, that provides the mechanism for making such assertions. Regardless of the actual rule adopted, such assertions may be incorrect in either of two ways:

Type I Error (false positive): assert that $X > 0$ when in fact $X = 0$

Type II Error (false negative): assert that $X = 0$ when in fact $X > 0$.

2.1 Calibration Models

Assume, for further specificity, that the underlying monotonic relationship between Y and X (i.e., ignoring unobservable, uncontrollable errors in observations) is such that Y increases as X increases, and that the expected background value of Y is an unknown constant, α. The calibration model therefore has the form

$$Y_x = \alpha + f(x,\beta) + \varepsilon \tag{2-1}$$

where

Y_x is an observed response when $X = x$,

β is an unknown parameter or vector of parameters,

$f(x,\beta)$ is a monotonic increasing function of x that satisfies $f(0,\beta) = 0$, and

ε is the observational error for the particular determination.

In order to provide a consistent context for the various approaches considered herein, we will assume that the observational errors, ε, from different determinations (at the same or different x values):

(i) are independent,

(ii) are normally distributed,

(iii) have zero mean, and

(iv) have constant variance, σ^2, which does not depend on concentration.

Clearly, there are cases where the distributional assumptions (ii) and/or (iv) may not be satisfied; in many such cases, a transformation of observations may be applied so that the assumptions will be tenable on the transformed scale.

The most straightforward example of model (2-1) is the straight-line calibration curve:

$$Y_x = \alpha + \beta x + \varepsilon \tag{2-2}$$

In this case, β represents the slope of the line. For (2-2) to be useful, the slope must not be zero. When dealing with model (2-2), we will assume (without loss of generality) that $\beta > 0$.

It should be noted that the model parameters of either (2-1) or (2-2) must remain constant over all sources (matrices) to which the analytical protocol is applied. Moreover, the distributional properties of the errors must not be dependent upon the particular matrices for which the calibration results are to be extrapolated. (Such assumptions are essentially required in any calibration situation involving extrapolation to other matrices.)

2.2 Estimation of the Calibration Model

In most situations, the form of the function f is unknown and the values of the parameters α, β, and σ^2 are unknown. The validity of distributional assumptions concerning ε may also be unknown. Generally, estimation of parameters and testing for adequacy of the chosen functional form and for validity of the variance homogeneity assumption are carried out as follows:

Step 1. A set of n "uncontaminated" sample aliquots are fortified at m different standard concentrations $x_1, x_2, \ldots, x_m$ and n_j determinations are made at x_j, where

$$n = \sum_{j=1}^{m} n_j .$$

Step 2. The resulting responses, y_i (i=1,2,...,n), are examined (e.g., via plots against the x_j) and sample means $\bar{y}_j$ and variances s_j^2 within each standard concentration level are computed.

Step 3. The sample variances s_j^2 are used to examine the reasonableness of the variance homogeneity assumption. Tests such as those of Bartlett or Levene can be used (see Snedecor and Cochran, 1980). Transformations are considered for stabilizing the variances, if necessary, and steps 2 and 3 are repeated.

Step 4. Based on steps 2 and 3, various functional forms (f) are considered and estimates of the model parameters are obtained using a least squares criterion (equivalent to maximum likelihood estimation by virtue of assumptions (i) - (iv)). If the functional form is a linear combination of the unknown parameters, then ordinary linear least squares procedures are used; otherwise, nonlinear least squares procedures would be needed to estimate the parameters.

Step 5. Adequacy and lack of fit of candidate functional forms are evaluated, and a satisfactory model is selected. When using a completely random design (see Section 2.3), for instance, the standard lack-of-fit test is conducted by computing

$$F = [(n-p)\,\hat{\sigma}^2 - (n-m)\,\tilde{\sigma}^2] \,/\, [(m-p)\,\tilde{\sigma}^2] \;,\; m > p \qquad (2\text{-}3)$$

where n and m are as defined previously; p is the number of model parameters (e.g., p = 2 for model [2-2]);

$$\tilde{\sigma}^2 = \sum_{j=1}^{m} (n_j - 1)s_j^2 \;/\; (n-m) = \text{pooled within-standards variance;} \tag{2-4}$$

and

$\hat{\sigma}^2$ is the residual mean square for the particular model

$$= \sum_{i=1}^{n} (y_i - \hat{y}_i)^2 \;/\; (n-p), \text{ where } \hat{y}_i \text{ is the fitted value for the } i^{th} \text{ observation.} \tag{2-5}$$

The statistic F is compared with the tabulated critical values for the F distribution with (m-p) and (n-m) degrees of freedom. Lack of fit of the particular model form is indicated if the F statistic exceeds the tabulated value. Since the alternative hypothesis for the test in (2-3) is not explicit, this test is broadly sensitive to any type of lack of fit, though not highly sensitive to any particular type. Rather than using (2-3), it is possible (even preferable) to examine model adequacy with respect to more specific alternatives (e.g., comparing fits of linear versus quadratic models).

As an example of step 4 above, assume that model (2-2), along with assumptions (i) through (iv), has been judged to be adequate. In this case, estimates of the model parameters (denoted by ^) are given by

$$\hat{\alpha} = \bar{y} - \hat{\beta}\bar{x} \tag{2-6}$$

and

$$\hat{\beta} = Q_{xy} \;/\; Q_{xx} \tag{2-7}$$

where $\bar{y}$ and $\bar{x}$ are the means (over all n observations) of the observed responses and of the standard concentrations, respectively, and

$$Q_{xy} = \sum_{j=1}^{m} n_j \,(x_j - \bar{x})\,(\bar{y}_j - \bar{y}), \text{ and}$$

$$Q_{xx} = \sum_{j=1}^{m} n_j \,(x_j - \bar{x})^2 \,.$$

Standard errors of the parameter estimates are given by

$$\text{s.e.}[\hat{\alpha}] = \hat{\sigma}\,\left[\frac{1}{n} + \frac{\bar{x}^2}{Q_{xx}}\right]^{\frac{1}{2}} \tag{2-8}$$

and

$$\text{s.e.}[\hat{\beta}] = \hat{\sigma}\, Q_{xx}^{-\frac{1}{2}} \,. \tag{2-9}$$

Under this model and related assumptions, the residual variance (with (n-2) degrees of freedom) provides the estimate

$$\hat{\sigma}^2 = [Q_{yy} - Q_{xy}^2 \ / \ Q_{xx}] \ / \ (n-2) \tag{2-10}$$

where

$$Q_{yy} = \sum_{i=1}^{n} (y_i - \bar{y})^2 \ .$$

Assume that an observation, Y, is obtained for some subsequent sample having unknown concentration; this concentration would generally be estimated by solving the calibration function for x. For model (2-2), for instance, this concentration would be estimated as

$$\hat{X} = (Y - \hat{\alpha}) \ / \ \hat{\beta} \ . \tag{2-11}$$

This estimate is not unbiased, and has infinite variance. It is, nevertheless, based upon the statistics y, $\bar{y}$, and $\hat{\beta}$, which are sufficient statistics for the involved parameters. (If σ^2 is known and $\beta>0$, then an unbiased estimator for the unknown concentration that is also based on sufficient statistics can be obtained [see Williams, 1969]).

Assume now that r subsequent determinations are made from the same source matrix with unknown concentration X = x. Let $\bar{Y}$ denote the mean of the r observed responses. Then $(\bar{Y} - \hat{\alpha} - \hat{\beta}x)$ is distributed normally with mean zero and variance $\sigma^2 w_x^2$, where

$$w_x^2 = \frac{1}{r} + \frac{1}{n} + \frac{(x-\bar{x})^2}{Q_{xx}} \ . \tag{2-12}$$

Similarly, $(\bar{Y} - \hat{\alpha})$ is distributed normally with mean βx and variance $\sigma^2 w_o^2$.

2.3 Calibration Designs

It is important to distinguish between analyte detection and analyte concentration estimation when considering calibration designs. Several considerations are common to both contexts, but others are specific to analyte detection and result in different criteria for optimal design. Complete elaboration of these matters is beyond the scope of this report which concentrates on detectability and detection limits; but the effects of variations in calibration design on detectability and its estimation cannot be ignored and we discuss them briefly in this section.

An initial consideration is a direct consequence of natural or imposed groupings of sample materials and their processing. This leads to the classical experimental design features of "blocking" and randomization (Cochran and Cox, 1957). It is common for groups or sets of samples to be collected, prepared, processed and analyzed in an organized fashion. Especially common is an imposed time structure, such as groups of samples that are prepared and/or processed together on the same day. Organization with spatial structure is another possibility. In this circumstance, it is often beneficial to arrange that such structure is incorporated in the calibration design. And when the magnitude of extraneous variations (attributable to levels of such structures) is important, it is essential to arrange that the calibration samples (known standards) and the unknown samples (subjects for detection inferences) are processed in an overall designed manner. As an example of such considerations, we cite Burrows et al. (1984) who employ lattice square designs to achieve two-way control over extraneous variations when calibrating antigen concentrations by enzyme-linked immunosorbent assay.

Choice of experimental design, and its consequences, are specific to context. We make no attempt to generalize here; indeed, we consider only the simplest choice (completely randomized designs (CRDs)) in subsequent chapters. Nevertheless, with appropriate modifications for parameter estimation and for degrees of freedom attached to the estimate $\hat{\sigma}$, the formulations and methods in Chapter 3 continue to apply.

A major consideration is choice of the actual standard analyte concentrations x_j, and their replication counts n_j, to include in the calibration design. The remainder of this section attempts to summarize the consequences of three interrelated and, unfortunately, antagonistic criteria for this choice:

(i) designs useful for exploring the calibration model,

(ii) designs yielding sensitive detection (minimum detection limits),

(iii) designs yielding precise estimates of detectability and detection limits.

Prior information, such as might be available from preliminary evaluations of the chemical method and instrumentation, is invaluable. For

example, if the form of $f(x,\beta)$ is well-known or strongly suspected a priori, the choice of standards can be tailored to that specific form using criteria (ii) and (iii); otherwise, it is necessary to deploy the standards in a fashion (such as evenly spaced and equally replicated points) that conflicts with criteria (ii) and (iii) but enables a determination of that form for $f(x,\beta)$ considered adequate, as discussed in Section 2.2. Similarly, if it is known or suspected that the variance homogeneity assumption is tenable a priori, then fewer replications at each standard are necessary (implying that a larger number of distinct standards might be included). Finally, prior information, often quite informal, at low concentrations x (as opposed to calibrations against concentrations far beyond the region where detection is uncertain) enables choice of a range of standards in the neighborhood of the probable or speculated detection limit.

Henceforth it is assumed that the form of $f(x,\beta)$, with homogeneous error variance, has been determined and, for simplicity, that it yields the straight line model of (2-2). Hubaux and Vos (1970) discuss several variations in choices of x_j and n_j in this context and their general conclusions apply here also. But one of their recommendations is not applicable: They suggest arranging that $\bar{x}$ coincide with a prior guess for the detection limit because of an erroneous formulation of the latter which does not conform to our definition in Chapter 3.

It is convenient to characterize the calibration design (standards $x_1, x_2, \ldots, x_m$ and their replication counts $n_1, n_2, n_3, \ldots, n_m$) by four summary features that are open to choice:

(a) overall sample size: $n = \sum_j^m n_j$,

(b) location: $L = \min_j(x_j)$,

(c) span: $S = \max_j(x_j)-L$,

(d) pattern of relative spacings (free of location and span) and proportionate replication (free of overall size n): $u_j = (x_j-L)/S$ and $r_j = n_j/n$, so that the u_j's are free to be deployed over the interval [0,1] inclusive, and $\sum_j r_j = 1$.

Thus the important characteristics $\bar{x}$, Q_{xx} and w_o of Section 2.2 are given by

$$\bar{x} = L + S\bar{u}, \quad \bar{u} = \sum_j r_j u_j$$

$$Q_{xx} = nQS^2, \; Q = \Sigma\, r_j(u_j-\bar{u})^2, \; \mathrm{var}(\hat{\beta}/\sigma) = 1/nQS^2 \;, \quad (2\text{-}13)$$

$$w_o^2 = \frac{1}{r} + \frac{1}{n}\,[1+(\bar{u}+L/S)^2/Q], \quad \mathrm{var}(\hat{\alpha}/\sigma) = w_o^2 - 1/r. \quad (2\text{-}14)$$

Recall that r is the number of independent observations providing $\bar{Y}$ for a sample source of unknown concentration, and that degrees of freedom v are fixed at (n-2).

Criterion (ii), designs yielding sensitive detection, is met when w_o is minimized (this maximizes detection rates at all concentrations x>0 and so minimizes detection limits as described in Chapter 3). Observe first that w_o is minimized with respect to location, regardless of span or pattern, by choosing L=zero (that is, always include zero or blank as one of the standards), and that this choice does not affect Q or var $(\hat{\beta})$. Then w_o^2 reduces to

$$w_o^2 = \frac{1}{r} + \frac{1}{n}\,[1+\bar{u}^2/Q] \;. \quad (2\text{-}15)$$

Observe that w_o is minimized with respect to pattern by choosing $\bar{u}$=zero, that is, by placing all n observations at x=zero. This choice conflicts immediately with criterion (iii), because now S=zero and β is not estimable, so that detection rates and detection limits (as defined in Chapter 3), although optimized, are not estimable (since they depend upon β). Precision of estimates of detection rates and of detection limits, criterion (iii), improves as n increases and as the precision of the estimate $\hat{\beta}$ improves (which, with n fixed, occurs as Q and S increase). But this conflicts with criterion (ii), because Q is maximized when n/2 observations are placed at u=0 and n/2 at u=1 fixing $\bar{u}$=0.5 (instead of close to zero); fortunately, S can be increased without effect on $\bar{u}$. Thus, taking criteria (ii) and (iii) together, it is necessary to compromise between the desirability of small $\bar{u}$ and large Q. This is achieved by concentrating more of the u_j's closer to zero than to 1.0, or by increasing the proportionate replications, r_j, of those u_j's close to zero at the expense of those closer to 1.0.

Some examples of these considerations are given in Table 2-1 which contains nine design patterns with four concentrations each. The consequences of these manipulations of u_1, u_2, u_3 and u_4, and r_1, r_2, r_3, and r_4 are shown in Table 2-2 which contains n, and

$$SA = \text{standard deviation of } (\hat{\alpha}/\sigma), \tag{2-16}$$

$$SB = \text{standard deviation of } (S\hat{\beta}/\sigma), \tag{2-17}$$

$$WO_r = w_o \text{ at (2-12) for } r = 1, 2, \text{ and } 3.$$

Further discussion of design strategies is postponed until Section 3.2 where the concept of manipulating calibration design to achieve specified detectability is introduced.

TABLE 2-1. NINE DESIGN PATTERNS WITH FOUR STANDARD CONCENTRATIONS.

DESIGN NO.	CONCENTRATION UI (PROPORTIONATE REPLICATION RI) U1	(R1)	U2	(R2)	U3	(R3)	U4	(R4)
1	0.000	(.25)	0.333	(.25)	0.667	(.25)	1.000	(.25)
2	0.000	(.40)	0.333	(.20)	0.667	(.20)	1.000	(.20)
3	0.000	(.40)	0.333	(.30)	0.667	(.20)	1.000	(.10)
4	0.000	(.25)	0.200	(.25)	0.800	(.25)	1.000	(.25)
5	0.000	(.40)	0.200	(.20)	0.800	(.20)	1.000	(.20)
6	0.000	(.40)	0.200	(.30)	0.800	(.20)	1.000	(.10)
7	0.000	(.25)	0.250	(.25)	0.500	(.25)	1.000	(.25)
8	0.000	(.40)	0.250	(.20)	0.500	(.20)	1.000	(.20)
9	0.000	(.40)	0.250	(.30)	0.500	(.20)	1.000	(.10)

TABLE 2-2. PROPERTIES OF DESIGNS IN TABLE 2-1 UNDER LINEAR CALIBRATION

DESIGN	N	SA	SB	WO_1	WO_2	WO_3
1	8	0.591532	0.948493	1.16186	0.921906	0.826585
	12	0.482984	0.774442	1.11053	0.856314	0.752733
	16	0.418276	0.670686	1.08395	0.821556	0.712943
	20	0.374118	0.599880	1.06769	0.799977	0.687966
	24	0.341521	0.547613	1.05671	0.785262	0.670798
	28	0.316187	0.506991	1.04880	0.774580	0.658261
	32	0.295766	0.474247	1.04282	0.766471	0.648699
	36	0.278851	0.447124	1.03815	0.760104	0.641164
	40	0.264541	0.424179	1.03440	0.754972	0.635071
2	10	0.453708	0.813369	1.09811	0.840150	0.734292
	15	0.370451	0.664113	1.06641	0.798269	0.685979
	20	0.320820	0.575139	1.05020	0.776483	0.660499
	25	0.286950	0.514420	1.04036	0.763112	0.644728
	30	0.261949	0.469599	1.03374	0.754067	0.633996
	35	0.242517	0.434764	1.02899	0.747539	0.626217
	40	0.226854	0.406685	1.02541	0.742605	0.620319
3	10	0.447146	0.948493	1.09542	0.836624	0.730256
	20	0.316180	0.670686	1.04879	0.774577	0.658258
	30	0.258160	0.547613	1.03279	0.752759	0.632440
	40	0.223573	0.474247	1.02469	0.741610	0.619127
4	8	0.555719	0.857493	1.14404	0.899346	0.801347
	12	0.453743	0.700140	1.09813	0.840168	0.734313
	16	0.392953	0.606339	1.07444	0.808957	0.698387
	20	0.351468	0.542326	1.05997	0.789639	0.675916
	24	0.320844	0.495074	1.05021	0.776493	0.660511
	28	0.297044	0.458349	1.04319	0.766965	0.649283
	32	0.277859	0.428746	1.03789	0.759741	0.640733
	36	0.261968	0.404226	1.03374	0.754074	0.634004
	40	0.248525	0.383482	1.03042	0.749510	0.628568
5	10	0.436931	0.753778	1.09129	0.831209	0.724046
	15	0.356753	0.615457	1.06173	0.792006	0.678680
	20	0.308957	0.533002	1.04664	0.771657	0.654819
	25	0.276340	0.476731	1.03748	0.759186	0.640076
	30	0.252262	0.435194	1.03133	0.750757	0.630055
	35	0.233550	0.402911	1.02691	0.744678	0.622799
	40	0.218466	0.376889	1.02359	0.740086	0.617301
6	10	0.417635	0.852493	1.08371	0.821230	0.712567
	20	0.295312	0.602804	1.04269	0.766296	0.648493
	30	0.241121	0.492187	1.02866	0.747087	0.625678
	40	0.208817	0.426246	1.02157	0.737295	0.613953
7	8	0.547723	0.956183	1.14018	0.894427	0.795822
	12	0.447214	0.780720	1.09545	0.836660	0.730297
	16	0.387298	0.676123	1.07238	0.806226	0.695222
	20	0.346410	0.604743	1.05830	0.787401	0.673300
	24	0.316228	0.552052	1.04881	0.774597	0.658281
	28	0.292770	0.511101	1.04198	0.765320	0.647339
	32	0.273861	0.478091	1.03682	0.758288	0.639010
	36	0.258199	0.450749	1.03280	0.752773	0.632456
	40	0.244949	0.427618	1.02956	0.748331	0.627163
8	10	0.433013	0.845154	1.08972	0.829156	0.721688
	15	0.353553	0.690066	1.06066	0.790569	0.677003
	20	0.306186	0.597614	1.04583	0.770552	0.653516
	25	0.273861	0.534522	1.03682	0.758288	0.639010
	30	0.250000	0.487950	1.03078	0.750000	0.629153
	35	0.231455	0.451754	1.02644	0.744024	0.622017
	40	0.216506	0.422577	1.02317	0.739510	0.616610
9	10	0.425685	1.03626	1.08683	0.825353	0.717315
	20	0.301005	0.73274	1.04432	0.768508	0.651105
	30	0.245770	0.59828	1.02976	0.748600	0.627484
	40	0.212843	0.51813	1.02240	0.738446	0.615334

3. DETECTION LIMITS WITH SPECIFIED ASSURANCE PROBABILITIES

Within EPA, calibrations of analyte responses versus standard concentrations x_j ($j = 1, 2, \ldots, m$) are usually performed for the purpose of estimating analyte concentrations, as opposed to the purpose of detecting analyte presence/absence. The former purpose implies that the range covered by the x_j should encompass all of the concentrations occurring in matrices for which the analytical protocol and calibration results apply. The results of Section 2.3, on the other hand, indicate that calibration designs directed towards the latter purpose would cover a narrower range of concentrations -- namely, that range over which detectability was considered uncertain. The definitions and methods of this chapter presume that a calibration of this latter type will be/has been conducted. (This does not imply that an overall calibration design aimed at both purposes should not be considered; on the contrary, efficient use of resources dictates that such a composite calibration design be utilized whenever both of the aforementioned purposes are deemed relevant.)

Section 3.1 below provides the basic concepts associated with developing detection limits that achieve specified assurance probabilities (i.e., specified rates of protection against both Type I and Type II errors). In that section, the simple straight-line calibration model is assumed and its parameters are assumed to be known. The latter assumption, though obviously impractical, is temporary: it allows many of the technical details relating to estimation to be avoided. For example, the methods described in Section 2.2 are unnecessary.

In Section 3.2, the straight-line calibration model is still assumed but the definitions and methods are developed for the case in which this relationship between Y and X must be estimated.

Detection limits, as formulated in Section 3.2, are functions of unknown parameters. Hence, Section 3.3 deals with the estimation of detection limits and associated parameters for the linear calibration case. A more general calibration framework, along with additional details concerning estimation of the calibration model, detection

limits, etc., can be found in Burrows (1985) which is included as Appendix A.

The chapter concludes with Section 3.4, which furnishes a brief review of EPA's current approach for defining/estimating the "Method Detection Limit."

3.1 Linear Calibration With Known Parameters

We assume temporarily that the calibration model (2-1) relating Y and X is known (i.e., both the model form and the parameters α, β, and σ^2 are known) and, for simplicity, we assume that the model is of the form (2-2). As in Chapter 2, we presume the observed responses Y at any given concentration x are distributed normally, and assume that r independent determinations will be made from each source matrix. Hence the mean of the r responses, $\bar{Y}$, has mean $(\alpha + \beta x)$ and variance σ^2/r.

With $\beta > 0$, it is reasonable to declare that the analyte of interest is present if $\bar{Y}$ is "sufficiently large". We formalize this notion by adopting the following detection rule:

'Assert $X > 0$ whenever $\bar{Y} > y_p$', (3-1)

where y_p is a threshold response value chosen so that the rate of false positives, in repeated applications of the rule to (blank) samples, is fixed at some desired (low) level p.

Let Z denote a random variable having a standard normal distribution (mean = 0, variance = 1), and let z_p denote the upper 100p percentage point of this distribution, i.e.,

$$\Pr[Z > z_p] = p.$$

With the assumptions given above, the rule (3-1) is specified if we choose y_p such that the probability of making a Type I error is

$$p = \Pr[\text{false positive}] = \Pr[\bar{Y} > y_p | X=0]$$

$$= \Pr\left[\frac{\bar{Y} - \alpha}{\sigma/\sqrt{r}} > \frac{y_p - \alpha}{\sigma/\sqrt{r}} \,|X=0\right] = \Pr\left[Z > \frac{y_p - \alpha}{\sigma/\sqrt{r}}\right].$$

Thus we can satisfy the condition of having a Type I error rate equal to p by choosing

$$y_p = \alpha + \sigma z_p / \sqrt{r}\,. \tag{3-2}$$

The value y_p shown in Figure 3-1a, for instance, illustrates such a response threshold value. Note that the shaded region under the normal

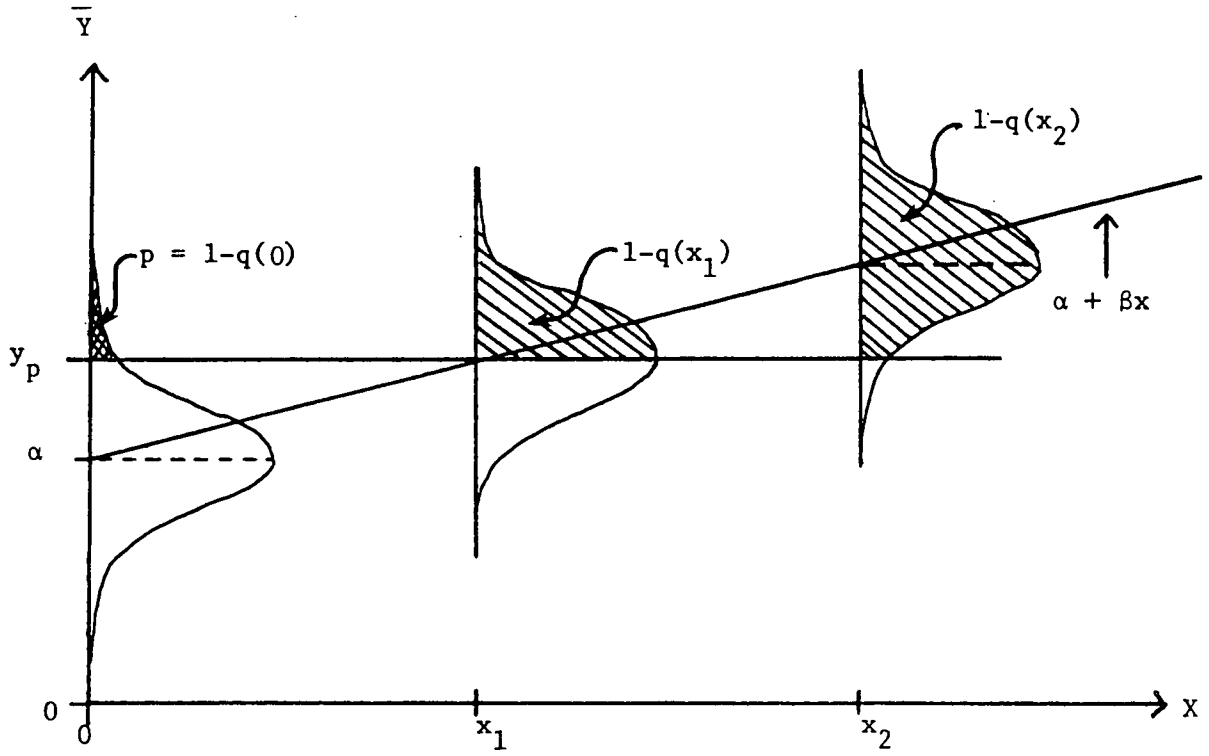

Figure 3-1a. Observed Response vs. Concentration, With $\bar{Y}$ Distributions.

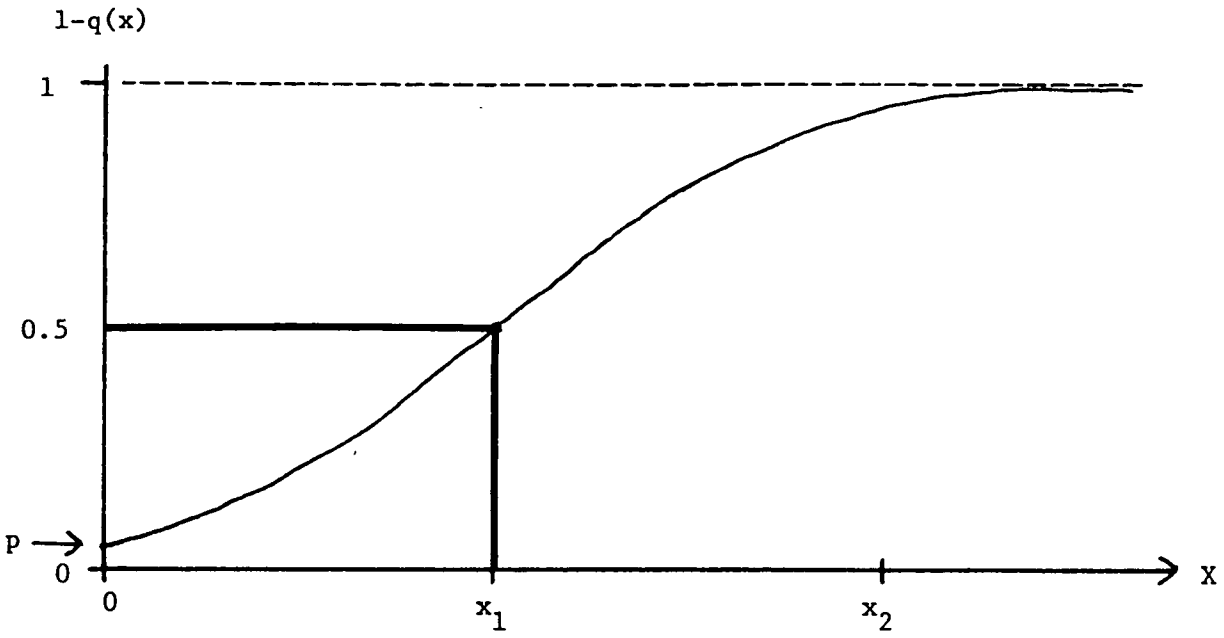

Figure 3-1b. Detection Probability vs. Concentration.

Figure 3-1. Illustration of How Detection Probability, 1-q(x), Varies With Concentration x When Using the Rule 'Assert X > 0 Whenever $\bar{Y} > y_p$'

distribution pictured at X=0 is equal to the specified value p. (This distribution represents the distribution of values that would be expected if a large number of $\bar{Y}$ values were independently determined for a source matrix having X=0.)

Now suppose that r determinations are made on a single source matrix having some unknown, but positive, concentration x. We then compute the probability that the mean of the r determinations, $\bar{Y}$, will exceed y_p, the threshold response value defined by (3-1) and (3-2). Since $\bar{Y}$ is normally distributed with mean $(\alpha + \beta x)$ and variance σ^2/r, the detection probability at x is given by

$$\Pr[\bar{Y} > y_p \mid X = x] = \Pr\left[\frac{\bar{Y} - (\alpha + \beta x)}{\sigma/\sqrt{r}} > \frac{\alpha + \sigma z_p/\sqrt{r} - (\alpha + \beta x)}{\sigma/\sqrt{r}}\right]$$

$$= \Pr\left[Z > z_p - \frac{\beta x}{\sigma/\sqrt{r}}\right]$$

$$= 1 - \Pr\left[Z \leq z_p - \frac{\beta x}{\sigma/\sqrt{r}}\right] \equiv 1 - q(x) \ . \qquad (3\text{-}3)$$

Note the following:

(i) The function q(x), defined by (3-3), depends not only on the true concentration x but also on the values p, r, and β/σ (as well as the distributional assumptions).

(ii) The function q(x) represents the rate of false negatives that would be expected in repeated applications of the rule (3-1) -- i.e., the probability of erroneously concluding that the analyte was absent (X = 0) when it was not (X > 0).

(iii) The detection probability, [1 - q(x)], is an increasing function of x bounded as follows:

$$1 - q(0) = p \quad \text{and} \quad 1 - q(\infty) = 1.$$

Figure 3-1b illustrates the function [1 - q(x)]. This curve is simply the power curve associated with the test of the null hypothesis that the analyte concentration is zero, and derives directly from the classical Neyman-Pearson theory. For a given x, the point on the curve in Figure 3-1b represents the area above the point y_p (in Figure 3-1a) under the normal distribution centered at $\alpha + \beta x$. The shaded regions in Figure 3-1a, for instance, illustrate these areas for three such x values:

$$X = 0,\ X = x_1,\ \text{and}\ X = x_2.$$

Of particular interest is the concentration level x_1 in Figure 3-1, because when $X = x_1$, we have

$$y_p = \alpha + \beta x_1$$

or

$$x_1 = (y_p - \alpha) / \beta .$$

Frequently, researchers have adopted x_1 as a definition of the "detection limit". It is clear from the above discussion that using $y_p = (\alpha + \beta x_1)$ as the detection threshold for $\bar{Y}$ does in fact achieve protection against Type I errors at the specified rate p. However, it is also clear from Figure 3-1 that if a particular source matrix had analyte concentration actually equal to x_1, then we would expect to assert $X = 0$ (i.e., a false negative) 50 percent of the time. Although a number of authors (for example, Currie, 1968) have pointed out this problem with such traditional "detection limits", their use has continued to be widespread.

Here, we prefer to adopt the notion that a detection limit should be an analyte concentration that is almost assured of detection. That is, in addition to fixing the Type I error rate p at some low value, we also want to specify the Type II error rate q at a realistic level. Given the relationship between [1-q] and x (for fixed p), as depicted in Figure 3-1b, it is clear that specification of q is equivalent to specifying a concentration level x_ℓ as a detection limit. For example, we might report the value x_2 in Figure 3-1 as a detection limit that achieves a satisfactory value for q given p. Clearly, such a formulation of detection limits is inseparable from the detection rule.

In the case of a known relationship between X and Y, as assumed in this section, the detection rule is given by (3-1); for this situation, we state the following definition:

> if $x_\ell = x_\ell(p,q)$ is the lowest analyte concentration for which the rate of detection is at least (1-q) when using the rule (3-1), then x_ℓ is <u>the detection limit with assurance probability (1-q)</u> for that rule; alternatively, the rate of false negatives is at most q for all analyte concentrations exceeding x_ℓ.

With appropriate modification of the detection rule, the above definition also applies more generally. Values of $x_\ell(p,q)$ for fixed p, q and r are determined from (3-3) as

$$x_\ell(p,q) = (z_p + z_q)\frac{\sigma}{\beta\sqrt{r}} . \tag{3-4}$$

3.2 Linear Calibration With Unknown Parameters

In this section, we again assume the straight-line calibration model (2-2) but in contrast with the previous section, we now assume that α, β, and σ^2 are unknown and must be estimated. The least squares methods of Section 2.2 are adopted.

In addition to the notation developed previously, we adopt the following:

S_v is a random variable that is independent of the random variable Z (see Section 3.1) and is such that vS_v^2 is distributed as chi-square with v degrees of freedom.

$T_v(\delta)$ is a random variable having a noncentral t-distribution with v degrees of freedom and noncentrality parameter δ. Note that $T_v(0)$ follows Student's t-distribution with v degrees of freedom, and that $T_v(\delta) = (Z + \delta)/S_v$.

$t_{v,p}$ represents the upper 100p percentage point of Student's t-distribution with v degrees of freedom, i.e., $\Pr[T_v(0) > t_{v,p}] = p$.

In most cases, we will suppress the v subscript appearing in the above to simplify the notation. We will assume that the residual variance $\hat{\sigma}^2$ (eq. (2-5), with p = 2) is used to provide an estimate of σ^2; hence $v = (n-2)$.

Proceeding in the manner of Section 3.1, we first specify a detection rule that will provide the desired level of protection against false positives. In the rule (3-1), the threshold concentration y_p was a function of σ and of α, both of which were assumed to be known. In this section, they must be estimated, so that the threshold level becomes a random variable, which we will denote as $\hat{y}_p$. We then adopt the <u>detection rule</u>:

$$\text{'Assert } X > 0 \text{ whenever } \bar{Y} > \hat{y}_p\text{'} \tag{3-5}$$

where $\hat{y}_p$ is chosen so that the rate of false positives [in repeated applications of (3-5)] will be fixed at the desired level p. The prescribed level p will be achieved if we choose

$$\hat{y}_p = \hat{\alpha} + w_o \hat{\sigma} t_p \ , \tag{3-6}$$

where w_o is defined by (2-12), since this choice of $\hat{y}_p$ gives

$$\Pr[\text{false positive}] = \Pr[\bar{Y} > \hat{y}_p | X=0]$$

$$= \Pr\left[\frac{\bar{Y} - \hat{\alpha}}{w_o \hat{\sigma}} > t_p | X=0 \right]$$

$$= \Pr\left[\frac{Z}{S} > t_p \right] \qquad (\text{since } \bar{Y} - \hat{\alpha} = \sigma w_o Z \text{ and } \hat{\sigma} = \sigma S)$$

$$= \Pr[T(0) > t_p] = p.$$

Use of (3-5) and (3-6) corresponds to using a t-statistic to test the hypothesis that the true analyte concentration is zero versus alternatives that it is greater than zero. The power of this test (or alternatively, the rate of false negatives) is obtained from the appropriate noncentral t-distribution (rather than the normal distribution, as was the case in Section 3.1).

As in the previous section, we now need to determine the detection probability for any given x. First, we define the following parameter:

$$\Delta = \frac{x\beta}{w_o \sigma} \ . \tag{3-7}$$

Detection rates when X=x, using the rule (3-5), are given by

$$\Pr[\text{detection} | X=x] = \Pr[\bar{Y} > \hat{y}_p | X=x]$$

$$= \Pr[\bar{Y} > \hat{\alpha} + w_o \hat{\sigma} t_p | X=x] \qquad [\text{from } (3\text{-}6)]$$

$$= \Pr\left[\frac{\bar{Y} - \hat{\alpha}}{w_o \hat{\sigma}} > t_p | X=x \right]$$

$$= \Pr\left[\frac{\beta x + \sigma w_o Z}{w_o \sigma S} > t_p \right] \qquad (\text{since } \bar{Y} - \hat{\alpha} = \beta x + \sigma w_o Z \text{ and } \hat{\sigma} = \sigma S)$$

$$= \Pr\left[\frac{\Delta + Z}{S} > t_p \right]$$

$$= \Pr[T(\Delta) > t_p]. \tag{3-8}$$

From (3-7) and (3-8), it is clear that (with β, σ, and w_o fixed) we can achieve a specified detection rate (1-q) at x by choosing a value of Δ appropriately; this value is a function of p and q (and v) and hence is

denoted as $\Delta(p,q)$. Using (3-7) and this choice of Δ yields an analyte concentration level

$$x_\ell(p,q) = w_o\Delta(p,q)(\sigma/\beta) \tag{3-9}$$

such that the detection probability will be (1-q) when we use the rule (3-5). Thus $x_\ell(p,q)$ is the <u>detection limit associated with (3-5) that achieves an assurance probability equal to (1-q)</u>.

Note the following:

i) The detection limit $x_\ell(p,q)$ is a <u>parameter</u> that characterizes the <u>methods</u> employed to produce the observed responses; it is not an estimate based on a particular set of observations.

ii) Those aspects of the method that influence detectability are evident in (3-9); they can be separated into three factors:

- (σ/β), determined by sensitivity of the protocol leading to observations Y;
- Δ, determined by v and specification of p and q, the rates of false positives and false negatives that can be tolerated; and
- w_o, determined by r and characteristics of the calibration design $(n, \bar{x}, Q_{xx})$.

iii) As $n\to\infty$, $w_o\to 1/\sqrt{r}$, $\Delta\to(z_p+z_q)$, and $x_\ell(p,q)\to(z_p+z_q)\sigma/\beta\sqrt{r}$, exactly as given at (3-4) in the previous section.

Apart from the unknown factor (σ/β), which is analyte- and measurement-specific and which must be estimated (see Section 3.3), all of the components of (3-9) can be determined by

a) specifying a calibration design,

b) specifying a value for p, and

c) specifying a value for q.

In practice, it would be desirable to specify several values of q, and determine the corresponding values of

$$x_\ell^* = x_\ell\frac{\beta}{\sigma} = w_o\Delta(p,q) \tag{3-10}$$

which are values of x_ℓ expressed as multiples of (σ/β) instead of in original concentration units.

As an example, suppose we choose design number 4 from Table 2-1 as the calibration design and assume that eight replicates will be utilized at each of the four standard concentrations (see Table 2-2). If we also fix r and p and let 1-q vary over the interval (p,1), then values of x_ℓ^*

can be determined for each such q. This produces curves such as those shown in Figure 3-2 and Figure 3-3. Figure 3-2 shows the case p = 0.05 while Figure 3-3 shows the case p = 0.01. The three curves in each figure correspond to r = 1, 2, and 3. Once an estimate of σ/β is obtained from the calibration, the horizontal axes of these figures can be scaled in original concentration units to provide appropriate estimated detection curves (henceforth referred to as power curves).

Values of $\Delta(p,q)$ for generating the x_ℓ^* values in these figures were determined using Gaussian quadrature algorithms developed by Peter M. Burrows; the SAS[1/] Macros are given in Appendix B. Values of $\Delta(p,q)$ for special cases p=q set at 0.05, 0.01 and 0.001 are given in Table 1 of Appendix A.

Figures 3-2 and 3-3 illustrate an important feature of the concepts and methods above: Since such curves can be developed prior to actual experimentation, we can assess the impact of using alternative calibration designs (as well as alternative p, q, or r values) as a part of the overall experimental plan. Moreover, after estimates of the calibration model parameters become available, we can determine threshold response value(s) and estimate detection limit(s) <u>for calibration designs and r values other than those actually used</u>. This permits the strategy of modifying designs for subsequent calibrations so as to produce a detection limit (with the same p and q) that, in expectation, differs from that for the initial calibration design. It also permits detection limits from two different calibrations to be compared in a realistic fashion (e.g., did laboratory A report lower limits of detection than laboratory B because of a more sensitive protocol, or because of a different calibration design, or because of different choices for p, q, or r?).

3.3 Estimation of Detection Limits for Linear Calibration

As noted in the previous section, if we fix p, q, and the calibration design, then all quantities needed for determining $x_\ell(p,q)$ according to (3-9) are known except for the factor σ/β. Given an estimate of σ/β, we can therefore provide an <u>estimate</u> of the detection limit having

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1/ SAS is the registered trademark of SAS Institute, Cary, N.C.

FIGURE 3-2

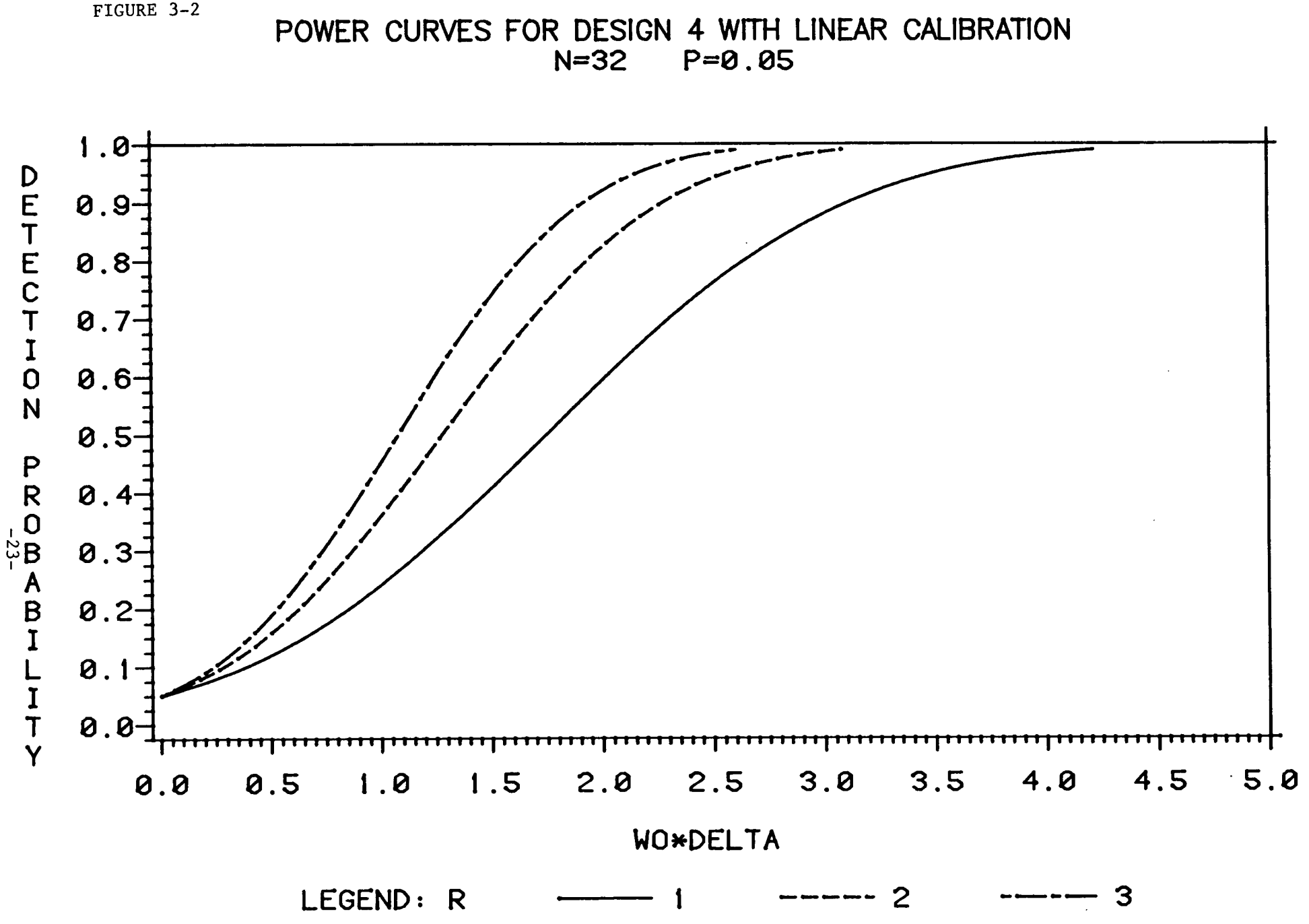
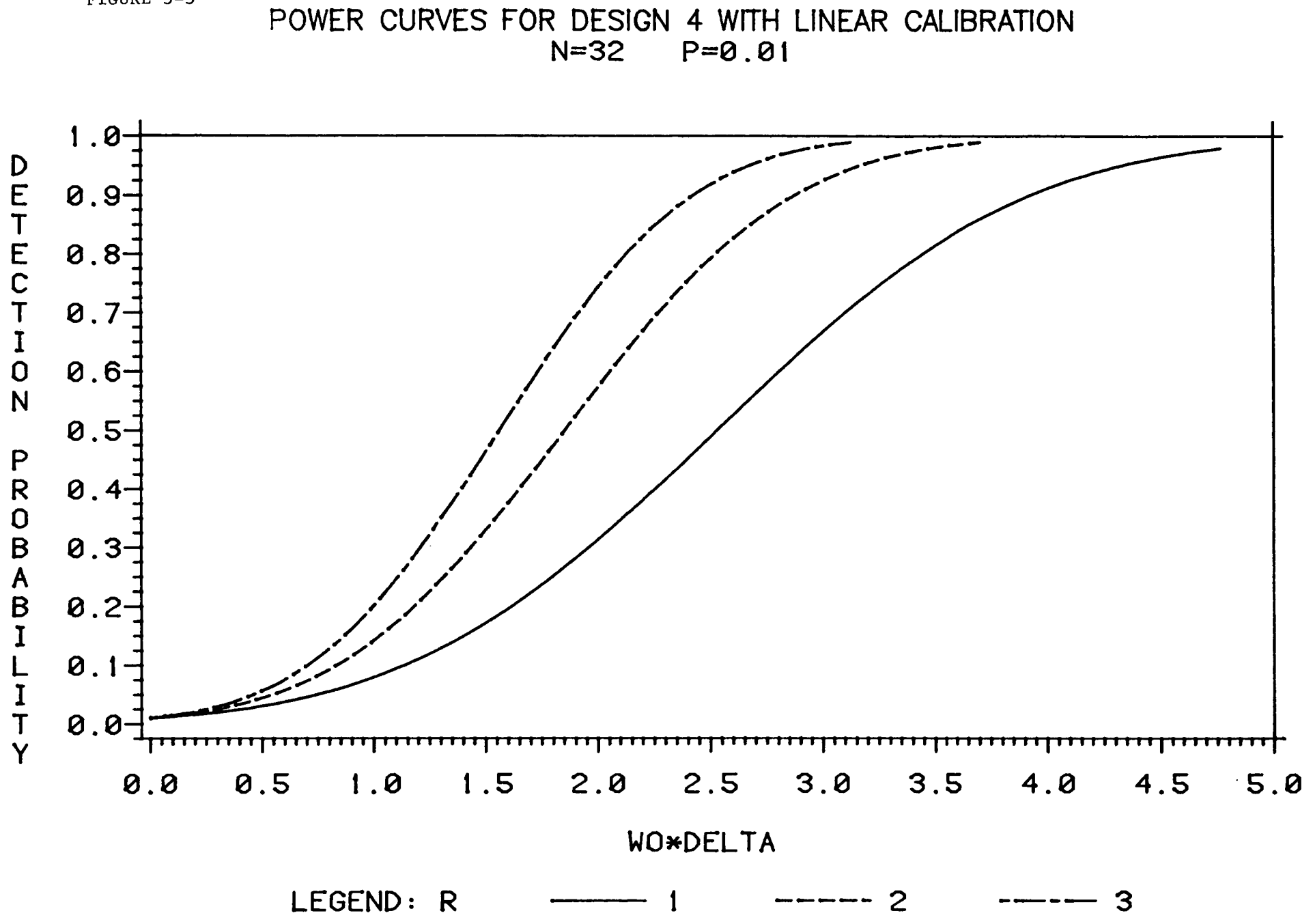

POWER CURVES FOR DESIGN 4 WITH LINEAR CALIBRATION
N=32 P=0.05
DETECTION PROBABILITY
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0
WO*DELTA
LEGEND: R 1 2 3

FIGURE 3-3


POWER CURVES FOR DESIGN 4 WITH LINEAR CALIBRATION
N=32 P=0.01
DETECTION PROBABILITY
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0
WO*DELTA
LEGEND: R 1 2 3

specified assurance probability for the detection rule (3-5). Alternatively, if an estimate of β/σ is used in (3-7) to provide an estimated noncentrality parameter $\hat{\Delta}$ (for fixed p, x, and calibration design), then an estimate of the detection rate for that x can be determined.

Estimation of β/σ. An intuitive point estimate of β/σ is $\hat{\beta}/\hat{\sigma}$, where $\hat{\beta}$ and $\hat{\sigma}$ are determined from the calibration results, as described in Chapter 2. This estimate is not unbiased, since the expectation of $\hat{\sigma}^{-1}$ is not σ^{-1}. Rather, the expectation of $\hat{\sigma}^{-1}$ is $M_v\sigma^{-1}$, where v is the degrees of freedom for $\hat{\sigma}$, and

$$M_v = \begin{cases} (v/2)^{\frac{1}{2}}2^{v-2}\{[(v-3)/2]!\}^2/\pi^{\frac{1}{2}}(v-2)! & \text{if } v \text{ is odd,} \\ (v/2)^{\frac{1}{2}}\pi^{\frac{1}{2}}(v-2)!/2^{v-2}\{[(v-2)/2]!\}^2 & \text{if } v \text{ is even.} \end{cases}$$

An approximation for M_v, suitable for $v > 8$, is given by Owen (1968):

$$M_v \approx 1 + \frac{3}{4(v-1.042)} .$$

Thus the (minimum variance) unbiased estimator for β/σ is

$$\widehat{(\beta/\sigma)} = \hat{\beta}/M_v\hat{\sigma} . \tag{3-11}$$

An interval estimate for β/σ with coverage probability $(1-\gamma)$ can be obtained by finding values δ_- and δ_+ such that

$$\Pr[T(\delta_-) < \hat{\delta}] = 1-\gamma/2 \quad \text{and} \quad \Pr[T(\delta_+) < \hat{\delta}] = \gamma/2, \tag{3-12}$$

where $\hat{\delta} = \hat{\beta}Q_{xx}^{\frac{1}{2}}/\hat{\sigma}$. The SAS algorithms of Appendix B can be used to determine δ_- and δ_+. An interval estimate for β/σ is then given by

$$(\delta_- Q_{xx}^{-\frac{1}{2}} , \quad \delta_+ Q_{xx}^{-\frac{1}{2}}) . \tag{3-13}$$

Estimation of σ/β. An intuitive point estimate for σ/β is $\hat{\sigma}/\hat{\beta}$; it has infinite mean square error (like the estimate $\hat{X}$ in eq. (2-11)). An interval estimate for σ/β having coverage probability $(1-\gamma)$ is obtained by simply inverting the inverval end-points of (3-13):

$$(Q_{xx}^{\frac{1}{2}}/\delta_+, \; Q_{xx}^{\frac{1}{2}}/\delta_-), \tag{3-14}$$

where δ_+ and δ_- are determined via (3-12). This is an approximation that neglects the probability of negative estimates $\hat{\beta}$.

Estimation of $x_\ell(p,q)$. Interval estimation of the detection limit defined by (3-9) is accomplished by multiplying the interval end-points of (3-14) by $w_o\Delta(p,q)$ to produce the interval

$$(w_o\Delta(p,q)Q_{xx}^{\frac{1}{2}}/\delta_+,\quad w_o\Delta(p,q)Q_{xx}^{\frac{1}{2}}/\delta_-)\ . \tag{3-15}$$

This furnishes an interval estimate for $x_\ell(p,q)$ with coverage probability equal to $(1-\gamma)$ when δ_- and δ_+ are determined according to (3-12). A point estimate for $x_\ell(p,q)$ is obtained by substituting $\hat{\sigma}/\hat{\beta}$ for σ/β in (3-9).

Estimation of q(x). Specification of p and x (or several values of x) and substitution of $\hat{\beta}/M_v\hat{\sigma}$ for β/σ in (3-7) yields a point estimate $\hat{\Delta}$ of Δ. A point estimate for the false negative rate q(x) is then obtained as

$$\hat{q}(x) = \Pr[T(\hat{\Delta}) < t_p] \tag{3-16}$$

where $\hat{\Delta} = x\hat{\beta}/w_oM_v\hat{\sigma}$. An interval estimate for q(x) with coverage probability equal to $(1-\gamma)$ is obtained as follows:

1. Determine δ_- and δ_+ via (3-12).
2. Compute $\Delta_- = x\delta_-/w_oQ_{xx}^{\frac{1}{2}}$ and

   $$\Delta_+ = x\delta_+/w_oQ_{xx}^{\frac{1}{2}}\ .$$

   (Interval (Δ_-,Δ_+) provides an interval estimate for Δ having coverage probability equal to $(1-\gamma)$).
3. Determine $q_- = \Pr[T(\Delta_+) < t_p]$ and $q_+ = \Pr[T(\Delta_-) < t_p]$. (3-17)

Interval (q_-, q_+) provides an interval estimate for q(x) with coverage probability $(1-\gamma)$.

The algorithms in Appendix B can be used to determine $\hat{q}(x)$ in (3-16) and q_- and q_+ in (3-17).

3.4 EPA Procedures for Determining a "Method Detection Limit"

Appendix C provides the EPA guidelines for determining a "Method Detection Limit", which is defined as "the minimum concentration of a substance that can be measured and reported with 99% confidence that the true value, corresponding to a single measurement, is above zero". We first note the following:

1. The procedures for determining the "Method Detection Limit" presume that a calibration has been performed, since the calculations are made in terms of concentration units. The calibration is not necessarily confined to a range of "low" concentrations, however.
2. The procedures involve an (implicit) assumption that variances of "observed" concentrations are homogeneous, at least over a limited range in the vicinity of zero. For a straight-line calibration model, this implies that errors in the response scale (Y) are also expected to exhibit homogeneous variances.
3. The procedures assume that "observed" concentrations are normally distributed (since criteria are based upon t-statistics). For the linear calibration model, this implies that errors in the response scale are also normally distributed.

Three pertinent comments concerning the above points deserve mention:

a) For <u>curvilinear</u> calibration relationships, it is not clear why one would expect the "observed" concentrations, obtained via inverse interpolation of the estimated calibration model, to exhibit normality or homogeneous variances. It is more realistic, in our opinion, to consider that response measurements (on some scale) will exhibit these properties.
b) The assumptions required for the methods of Sections 3.2 and 3.3, at least for the linear calibration case, are the same as those inherent to the EPA procedure.
c) In most cases, the level of effort involved in generating the data needed for applying the methods of Sections 3.2 - 3.3 is not unrealistic in comparison to that required for the EPA method (including calibration).

Finally, we note that the EPA procedure suffers from the problem associated with "traditional detection limits" that was mentioned in Section 3.1. In particular, assume that a linear calibration model like (2-2) holds; let s_x denote the standard deviation of the k replicate "observed" concentrations involved in the EPA procedure, and let $t_{k-1,0.01}$ denote the Student's t-value used in that procedure (in Appendix D, s_x and k are denoted as S and n, respectively). Let $\hat{x}_m$ denote the estimated "Method Detection Limit" defined as

$$\hat{x}_m = t_{k-1,0.01}\, s_x \,.$$

If α and β are assumed known, we can rewrite this limit in the response scale as

$$\hat{y}_m = \alpha + \beta\hat{x}_m = \alpha + \beta t_{k-1,0.01}\, s_x$$
$$= \alpha + t_{k-1,0.01}\, s_y$$

where $s_y = \beta s_x$ is the estimated standard deviation of the k response values that led to the k "observed" concentrations.

For a given source matrix with zero concentration, let Y denote the observed response. Then

$$\Pr[Y > \hat{y}_m | X=0] = \Pr[\alpha + \sigma Z > \hat{y}_m]$$
$$= \Pr[\sigma Z > t_{k-1,0.01}\, \sigma S_{k-1}]$$
$$= \Pr[T_{k-1}(0) > t_{k-1,0.01}] = 0.01$$

Next assume that a given source matrix has concentration exactly equal to $\hat{x}_m$. Since the observed response, Y, is now given by $Y = \alpha + \beta x_m + \sigma Z$, we obtain

$$\Pr[Y > \hat{y}_m | X=\hat{x}_m] = \Pr[\alpha + \beta\hat{x}_m + \sigma Z > \alpha + \beta\hat{x}_m] = \Pr[\sigma Z > 0] = 0.50.$$

Thus, while providing the specified level of protection against false positives, the EPA procedure affords only 50 percent protection against false negatives when the true concentration is equal to the (estimated) "Method Detection Limit". The method also fails to recognize the effects that the calibration design and calibration model parameter estimates have on reported "detection limits", since estimation of the "Method Detection Limit" is treated separately from the calibration (which, in some cases, may involve an instrument rather than a method calibration) and since the calibration function is treated as though it is known (e.g., estimates of α and β are treated as known quantities).

4. AN EXPERIMENTAL EVALUATION OF THE RECOMMENDED APPROACH

A two-phased experiment was conducted to demonstrate and validate the methods described in Chapter 3. The first phase -- calibration -- involved generation of the calibration results and their statistical analysis: estimation of the model parameters and estimation of detection limits with specified assurance probabilities based upon the estimated power curve associated with the calibration design and chemical method. The statistical analysis involved in the first phase illustrates the types of statistical procedures that are needed in applications of the recommended approach. The second phase of the experiment -- validation -- involved generation of results at selected concentrations from which empirical estimates of detection rates could be derived and then compared to the theoretically derived estimates from the first phase.

4.1 Experimental Method.

A single sediment matrix was used as the source matrix for all determinations in the experiment. This sediment, collected from University Lake (Chapel Hill, North Carolina) was chosen since this source is regarded as an uncontaminated reservoir and the sediment is unlikely to contain any of the analytes of interest. A single large batch (approximately 1 kg) of the University Lake sediment was collected on December 6, 1984 for use in the experiment. After removing trash and homogenizing the sediment, 30 g aliquots were removed for analysis as required. When not in use, all sediment and sediment samples were stored in the dark at 4° C.

Standard solutions containing known concentrations of eight extractable analytes (in acetone) were prepared for fortifying the sediment samples. The eight chosen analytes, seven of which are EPA priority pollutants, were as follows:

2-chloronaphthalene
2,6-dinitrotoluene
dimethylphthalate
hexachlorobenzene
anthracene
phenanthrene
aldrin
fluoranthene

Anthracene was included since it is a common choice for an internal standard.

Sediment aliquots were processed according to the specific chemical protocol described in Appendix D. This protocol utilized an ultrasonic extraction technique, which was shown in a previous study (EPA Contract No. 68-03-3119) to be the "best" available extraction method for sediments. Except for this extraction method, all other components of the chemical methodology followed procedures outlined in EPA Test Method 625-B, as applicable. Each sample extract was fortified with pentadecane at a fixed, known concentration (.265 ppm, relative to the original sediment sample) immediately prior to the GC (gas chromatography) injection; this compound thus served as the internal standard for the method.

4.2 Phase I Design.

A single concentrated stock solution containing the eight analytes was first prepared. For Phase I of the experiment, the standard fortifying solutions were prepared by serial dilution of this stock solution to target concentrations of 0.2, 0.8, and 1.0 mg/kg sediment (i.e., ppm). Actual fortification levels for each analyte are shown in Table 4-1. The target levels were chosen on the basis of several preliminary runs, which were conducted primarily for instrument setup. These levels appeared satisfactory for all analytes except 2,6-dinitrotoluene, for which they were much too low. Based upon these same runs, it was also apparent that a background interference with aldrin existed. Hence, these two analytes were dropped from further consideration, although they remained in the standard solutions at the indicated concentrations. Although the problem with 2,6-dinitrotoluene could perhaps have been remedied by using higher concentrations, this was not done since it would have required reformulating the initial stock solution.

The Phase I design involved four sets of samples. Each set consisted of eight sediment samples and a control sample that were prepared and processed together (e.g., during extraction). Sediment samples were prepared by fortification of individual 30 g aliquots of sediment with appropriate levels of analytes (Table 4-1). Control samples were prepared in a similar manner without sediment. All control samples were

Table 4-1. Actual Analyte Concentrations (ppm) Used in Phase I of the Experiment

Analyte	Target Concentration (ppm)		
	0.2	0.8	1.0
2-chloronaphthalene	0.215	0.858	1.07
2,6-dinitrotoluene	0.205	0.818	1.02
dimethylphthalate	0.217	0.867	1.08
hexachlorobenzene	0.200	0.802	1.00
anthracene	0.217	0.870	1.07
phenanthrene	0.210	0.840	1.05
aldrin	0.203	0.815	1.02
fluoranthene	0.200	0.802	1.00

fortified with the intermediate level of analytes (Table 4-1). GC analyses for all samples in a set were performed on the same day. The table below indicates the design (each symbol "X" denotes an observation):

Type of Sample	Target Concentration (ppm)	Set Number 1	2	3	4	Number of Observations
Sediment	0.0 (blank)	XX	XX*	XX	XX	8*
	0.2	XX	XX	XX	XX	8
	0.8	XX	XX	XX	XX	8
	1.0	XX	XX	XX	XX	8
Control	0.8	X	X	X	X	4

(* one of these samples was not used)

In addition to the runs indicated above, a single GC check sample was analyzed along with each sample set. Check samples consisted of standard solutions of analytes at a level corresponding to analyte concentrations in sediment extracts fortified at the intermediate level (Table 4-1).

Since all sample preparations and chemical analyses were performed as close together in time as possible, "sets" were ignored in the statistical treatment of the results (i.e., the design was regarded as a CRD). Hence the design shown above corresponds to that designated as design number 4 in Table 2-1. If a linear calibration model can be assumed, we would therefore expect to achieve the properties shown for design number 4 with n=32 in Table 2-2. Figures 3-2 and 3-3 are also applicable to this design and model.

4.3 Phase I Data

Tables 4-2 through 4-7 provide listings of Phase I results for the various analytes (2,6-dinitrotoluene and aldrin are excluded due to the aforementioned problems). These tables indicate the sample type; the fortification level (X*) in ppm; the set, replicate (REP), and run numbers; and observed responses (peak-area counts) from the GC analysis for both the particular analyte and the internal standard (pentadecane). In addition, the tables contain the adjusted peak area, Y*, which is obtained as the ratio of analyte peak area to internal-standard peak

TABLE 4-2. LISTING OF CALIBRATION DATA FOR 2-CHLORONAPHTHALENE

SAMPLE TYPE	X*	X	SET NO.	REP	RUN NO.	GC AREAS FOR: ANALYTE	INT. STD.	Y*	Y
GC_CHK	0.858	0.6625	1	1	10	29001	18941	1.5311	1.2374
			2	1	20	31126	20420	1.5243	1.2346
			3	1	30	28778	18882	1.5241	1.2345
			4	1	40	26221	17198	1.5247	1.2348
CONTRL	0.858	0.6625	1	1	11	27549	19503	1.4126	1.1885
			2	1	21	25760	18239	1.4124	1.1884
			3	1	31	21511	17927	1.1999	1.0954
			4	1	41	21204	14656	1.4468	1.2028
UL SED	0.000	0.0000	1	1	12	3477	23652	0.1470	0.3834
			1	2	13	3532	25660	0.1376	0.3710
			2	2	29	1693	28403	0.0596	0.2441
			3	1	34	1381	24573	0.0562	0.2371
			3	2	38	2204	23558	0.0936	0.3059
			4	1	45	1191	22142	0.0538	0.2319
			4	2	44	2091	20175	0.1036	0.3219
UL SED	0.215	0.2450	1	1	15	10139	25075	0.4043	0.6359
			1	2	14	9306	25610	0.3634	0.6028
			2	1	22	8990	24944	0.3604	0.6003
			2	2	24	7881	24073	0.3274	0.5722
			3	1	37	6164	22436	0.2747	0.5242
			3	2	32	6384	27393	0.2331	0.4828
			4	1	43	7050	24045	0.2932	0.5415
			4	2	46	5209	20816	0.2502	0.5002
UL SED	0.858	0.6625	1	1	19	28628	26397	1.0845	1.0414
			1	2	18	25194	25244	0.9980	0.9990
			2	1	27	18480	21681	0.8524	0.9232
			2	2	28	24426	28036	0.8712	0.9334
			3	1	39	21226	23939	0.8867	0.9416
			3	2	35	22435	25198	0.8903	0.9436
			4	1	48	20760	24985	0.8309	0.9115
			4	2	47	20991	23978	0.8754	0.9356
UL SED	1.070	0.7654	1	1	16	33112	30710	1.0782	1.0384
			1	2	17	30837	22250	1.3859	1.1773
			2	1	23	32592	24641	1.3227	1.1501
			2	2	26	27094	23086	1.1736	1.0833
			3	1	33	28910	25820	1.1197	1.0581
			3	2	36	33094	27314	1.2116	1.1007
			4	1	42	27492	21783	1.2621	1.1234
			4	2	49	26072	22356	1.1662	1.0799

TABLE 4-3. LISTING OF CALIBRATION DATA FOR DIMETHYLPHTHALATE

SAMPLE TYPE	X*	X	SET NO.	REP	RUN NO.	GC AREAS FOR: ANALYTE	INT. STD.	Y*	Y
GC_CHK	0.867	0.6671	1	1	10	14944	18941	0.7890	0.8882
			2	1	20	11284	20420	0.5526	0.7434
			3	1	30	10658	18882	0.5645	0.7513
			4	1	40	9981	17198	0.5804	0.7618
CONTRL	0.867	0.6671	1	1	11	14709	19503	0.7542	0.8684
			2	1	21	10963	18239	0.6011	0.7753
			3	1	31	11815	17927	0.6591	0.8118
			4	1	41	10190	14656	0.6953	0.8338
UL SED	0.000	0.0000	1	1	12	1912	23652	0.0808	0.2843
			1	2	13	9199	25660	0.3585	0.5987
			2	2	29	2468	28403	0.0869	0.2948
			3	1	34	4040	24573	0.1644	0.4055
			3	2	38	2220	23558	0.0942	0.3070
			4	1	45	1635	22142	0.0738	0.2717
			4	2	44	1632	20175	0.0809	0.2844
UL SED	0.217	0.2468	1	1	15	3803	25075	0.1517	0.3894
			1	2	14	4790	25610	0.1870	0.4325
			2	1	22	3492	24944	0.1400	0.3742
			2	2	24	3193	24073	0.1326	0.3642
			3	1	37	2568	22436	0.1145	0.3383
			3	2	32	4832	27393	0.1764	0.4200
			4	1	43	4573	24045	0.1902	0.4361
			4	2	46	2974	20816	0.1429	0.3780
UL SED	0.867	0.6671	1	1	19	9636	26397	0.3650	0.6042
			1	2	18	9009	25244	0.3569	0.5974
			2	1	27	6302	21681	0.2907	0.5391
			2	2	28	13558	28036	0.4836	0.6954
			3	1	39	9664	23939	0.4037	0.6354
			3	2	35	12059	25198	0.4786	0.6918
			4	1	48	13998	24985	0.5603	0.7485
			4	2	47	9757	23978	0.4069	0.6379
UL SED	1.080	0.7701	1	1	16	14440	30710	0.4702	0.6857
			1	2	17	12537	22250	0.5635	0.7506
			2	1	23	14028	24641	0.5693	0.7545
			2	2	26	11553	23086	0.5004	0.7074
			3	1	33	15105	25820	0.5850	0.7649
			3	2	36	15119	27314	0.5535	0.7440
			4	1	42	13799	21783	0.6335	0.7959
			4	2	49	11798	22356	0.5277	0.7265

TABLE 4-4. LISTING OF CALIBRATION DATA FOR HEXACHLOROBENZENE

SAMPLE TYPE	X*	X	SET NO.	REP	RUN NO.	GC AREAS FOR: ANALYTE	INT. STD.	Y*	Y
GC_CHK	0.802	0.6335	1	1	10	9599	18941	0.5068	0.7119
			2	1	20	9968	20420	0.4881	0.6987
			3	1	30	9304	18882	0.4927	0.7020
			4	1	40	8496	17198	0.4940	0.7029
CONTRL	0.802	0.6335	1	1	11	9143	19503	0.4688	0.6847
			2	1	21	9122	18239	0.5001	0.7072
			3	1	31	8490	17927	0.4736	0.6882
			4	1	41	7389	14656	0.5042	0.7100
UL SED	0.000	0.0000	1	1	12	1090	23652	0.0461	0.2147
			1	2	13	1282	25660	0.0500	0.2235
			2	2	29	1744	28403	0.0614	0.2478
			3	1	34	1038	24573	0.0422	0.2055
			3	2	38	448	23558	0.0190	0.1379
			4	1	45	604	22142	0.0273	0.1652
			4	2	44	235	20175	0.0116	0.1079
UL SED	0.200	0.2315	1	1	15	3280	25075	0.1308	0.3617
			1	2	14	2781	25610	0.1086	0.3295
			2	1	22	3274	24944	0.1313	0.3623
			2	2	24	3068	24073	0.1274	0.3570
			3	1	37	2762	22436	0.1231	0.3509
			3	2	32	2592	27393	0.0946	0.3076
			4	1	43	2676	24045	0.1113	0.3336
			4	2	46	2462	20816	0.1183	0.3439
UL SED	0.802	0.6335	1	1	19	11401	26397	0.4319	0.6572
			1	2	18	10302	25244	0.4081	0.6388
			2	1	27	8525	21681	0.3932	0.6271
			2	2	28	10722	28036	0.3824	0.6184
			3	1	39	9074	23939	0.3790	0.6157
			3	2	35	9616	25198	0.3816	0.6178
			4	1	48	9733	24985	0.3896	0.6241
			4	2	47	9495	23978	0.3960	0.6293
UL SED	1.000	0.7326	1	1	16	14415	30710	0.4694	0.6851
			1	2	17	12612	22250	0.5668	0.7529
			2	1	23	14877	24641	0.6037	0.7770
			2	2	26	13644	23086	0.5910	0.7688
			3	1	33	13249	25820	0.5131	0.7163
			3	2	36	14541	27314	0.5324	0.7296
			4	1	42	11472	21783	0.5266	0.7257
			4	2	49	11685	22356	0.5227	0.7230

TABLE 4-5. LISTING OF CALIBRATION DATA FOR ANTHRACENE

SAMPLE TYPE	X*	X	SET NO.	REP	RUN NO.	GC AREAS FOR: ANALYTE	INT. STD.	Y*	Y
GC_CHK	0.870	0.6687	1	1	10	32015	18941	1.6902	1.3001
			2	1	20	35261	20420	1.7268	1.3141
			3	1	30	32812	18882	1.7377	1.3182
			4	1	40	29719	17198	1.7280	1.3146
CONTRL	0.870	0.6687	1	1	11	33959	19503	1.7412	1.3196
			2	1	21	32472	18239	1.7804	1.3343
			3	1	31	30676	17927	1.7112	1.3081
			4	1	41	26382	14656	1.8001	1.3417
UL SED	0.000	0.0000	1	1	12	740	23652	0.0313	0.1769
			1	2	13	1580	25660	0.0616	0.2481
			2	2	29	997	28403	0.0351	0.1874
			3	1	34	811	24573	0.0330	0.1817
			3	2	38	1092	23558	0.0464	0.2153
			4	1	45	982	22142	0.0444	0.2106
			4	2	44	796	20175	0.0395	0.1986
UL SED	0.217	0.2468	1	1	15	9023	25075	0.3598	0.5999
			1	2	14	7948	25610	0.3103	0.5571
			2	1	22	9371	24944	0.3757	0.6129
			2	2	24	7815	24073	0.3246	0.5698
			3	1	37	7717	22436	0.3440	0.5865
			3	2	32	7751	27393	0.2830	0.5319
			4	1	43	8109	24045	0.3372	0.5807
			4	2	46	6926	20816	0.3327	0.5768
UL SED	0.870	0.6687	1	1	19	37040	26397	1.4032	1.1846
			1	2	18	32371	25244	1.2823	1.1324
			2	1	27	25715	21681	1.1861	1.0891
			2	2	28	31503	28036	1.1237	1.0600
			3	1	39	28626	23939	1.1958	1.0935
			3	2	35	29883	25198	1.1859	1.0890
			4	1	48	30437	24985	1.2182	1.1037
			4	2	47	29597	23978	1.2343	1.1110
UL SED	1.070	0.7654	1	1	16	48641	30710	1.5839	1.2585
			1	2	17	38841	22250	1.7457	1.3212
			2	1	23	48621	24641	1.9732	1.4047
			2	2	26	36312	23086	1.5729	1.2542
			3	1	33	42071	25820	1.6294	1.2765
			3	2	36	47124	27314	1.7253	1.3135
			4	1	42	37929	21783	1.7412	1.3196
			4	2	49	37667	22356	1.6849	1.2980

TABLE 4-6. LISTING OF CALIBRATION DATA FOR PHENANTHRENE

SAMPLE TYPE	X*	X	SET NO.	REP	RUN NO.	GC AREAS FOR: ANALYTE	INT. STD.	Y*	Y
GC_CHK	0.840	0.6533	1	1	10	29108	18941	1.5368	1.2397
			2	1	20	32214	20420	1.5776	1.2560
			3	1	30	30030	18882	1.5904	1.2611
			4	1	40	27423	17198	1.5945	1.2628
CONTRL	0.840	0.6533	1	1	11	32030	19503	1.6423	1.2815
			2	1	21	31459	18239	1.7248	1.3133
			3	1	31	30365	17927	1.6938	1.3015
			4	1	41	25711	14656	1.7543	1.3245
UL SED	0.000	0.0000	1	1	12	1472	23652	0.0622	0.2495
			1	2	13	1999	25660	0.0779	0.2791
			2	2	29	1352	28403	0.0476	0.2182
			3	1	34	1904	24573	0.0775	0.2784
			3	2	38	1272	23558	0.0540	0.2324
			4	1	45	1139	22142	0.0514	0.2268
			4	2	44	1592	20175	0.0789	0.2809
UL SED	0.210	0.2405	1	1	15	9918	25075	0.3955	0.6289
			1	2	14	8603	25610	0.3359	0.5796
			2	1	22	10349	24944	0.4149	0.6441
			2	2	24	8963	24073	0.3723	0.6102
			3	1	37	8301	22436	0.3700	0.6083
			3	2	32	8740	27393	0.3191	0.5649
			4	1	43	8842	24045	0.3677	0.6064
			4	2	46	6904	20816	0.3317	0.5759
UL SED	0.840	0.6533	1	1	19	40871	26397	1.5483	1.2443
			1	2	18	36950	25244	1.4637	1.2098
			2	1	27	29973	21681	1.3825	1.1758
			2	2	28	35652	28036	1.2717	1.1277
			3	1	39	33499	23939	1.3993	1.1829
			3	2	35	34803	25198	1.3812	1.1752
			4	1	48	34852	24985	1.3949	1.1811
			4	2	47	33846	23978	1.4115	1.1881
UL SED	1.050	0.7562	1	1	16	53361	30710	1.7376	1.3182
			1	2	17	42845	22250	1.9256	1.3877
			2	1	23	52966	24641	2.1495	1.4661
			2	2	26	45976	23086	1.9915	1.4112
			3	1	33	48360	25820	1.8730	1.3686
			3	2	36	52988	27314	1.9400	1.3928
			4	1	42	41945	21783	1.9256	1.3877
			4	2	49	43104	22356	1.9281	1.3886

TABLE 4-7. LISTING OF CALIBRATION DATA FOR FLUORANTHENE

SAMPLE TYPE	X*	X	SET NO.	REP	RUN NO.	GC AREAS FOR: ANALYTE	INT. STD.	Y*	Y
GC_CHK	0.802	0.6335	1	1	10	27177	18941	1.4348	1.1978
			2	1	20	30911	20420	1.5138	1.2303
			3	1	30	28982	18882	1.5349	1.2389
			4	1	40	26794	17198	1.5580	1.2482
CONTRL	0.802	0.6335	1	1	11	30307	19503	1.5540	1.2466
			2	1	21	29592	18239	1.6225	1.2738
			3	1	31	29262	17927	1.6323	1.2776
			4	1	41	24250	14656	1.6546	1.2863
UL SED	0.000	0.0000	1	1	12	1533	23652	0.0648	0.2546
			1	2	13	1748	25660	0.0681	0.2610
			2	2	29	1222	28403	0.0430	0.2074
			3	1	34	1554	24573	0.0632	0.2515
			3	2	38	1008	23558	0.0428	0.2069
			4	1	45	1440	22142	0.0650	0.2550
			4	2	44	1256	20175	0.0623	0.2495
UL SED	0.200	0.2315	1	1	15	9209	25075	0.3673	0.6060
			1	2	14	7991	25610	0.3120	0.5586
			2	1	22	9206	24944	0.3691	0.6075
			2	2	24	7334	24073	0.3047	0.5520
			3	1	37	7500	22436	0.3343	0.5782
			3	2	32	7340	27393	0.2680	0.5176
			4	1	43	9963	24045	0.4143	0.6437
			4	2	46	8778	20816	0.4217	0.6494
UL SED	0.802	0.6335	1	1	19	36563	26397	1.3851	1.1769
			1	2	18	33091	25244	1.3108	1.1449
			2	1	27	24972	21681	1.1518	1.0732
			2	2	28	34499	28036	1.2305	1.1093
			3	1	39	28485	23939	1.1899	1.0908
			3	2	35	29774	25198	1.1816	1.0870
			4	1	48	31888	24985	1.2763	1.1297
			4	2	47	31998	23978	1.3345	1.1552
UL SED	1.000	0.7326	1	1	16	41284	30710	1.3443	1.1594
			1	2	17	38348	22250	1.7235	1.3128
			2	1	23	51414	24641	2.0865	1.4445
			2	2	26	42443	23086	1.8385	1.3559
			3	1	33	44229	25820	1.7130	1.3088
			3	2	36	45825	27314	1.6777	1.2953
			4	1	42	40518	21783	1.8601	1.3638
			4	2	49	37264	22356	1.6668	1.2911

area. Finally, the tables contain values of two additional quantities that are used in subsequent sections:

$$X = \sqrt{X^* + .1} - \sqrt{.1} \quad \text{and}$$

$$Y = \sqrt{Y^*} \ .$$

4.4 Determination of the Calibration Function.

As indicated in Section 2.2, several steps are involved in determining a suitable form for the calibration model and estimating its parameters. We first plotted the observed analyte responses (adjusted peak areas, designated as Y* in Tables 4-2 through 4-7) versus concentration (designated as X* in these tables). These plots are shown in Figures 4-1 through 4-6 for the various analytes and suggest the following:

(1) At least one outlier appears to be present for dimethylphthalate (Figure 4-2) -- Y* = .3585 for X* = 0. This is listed as run number 13 in Table 4-3. All subsequent plots and calculations exclude this observation. Hence n=30 for this analyte and n=31 for all others.

(2) With the possible exception of dimethylphthalate, variation in observed responses tends to increase with increasing concentration level.

(3) Although Y* tends to increase with X*, the relationship is not a simple straight line for most analytes.

This curvilinearity, and the tendency for the response variation to increase with increasing concentration level, are further exhibited, respectively, by the means and standard deviations shown in Table 4-8. Tests for homogeneous response variation across the concentration levels yielded the following results:

FIGURE 4-1

CALIBRATION DATA: Y* VERSUS X*
ANALYTE=2-CHLORONAPHTHALENE

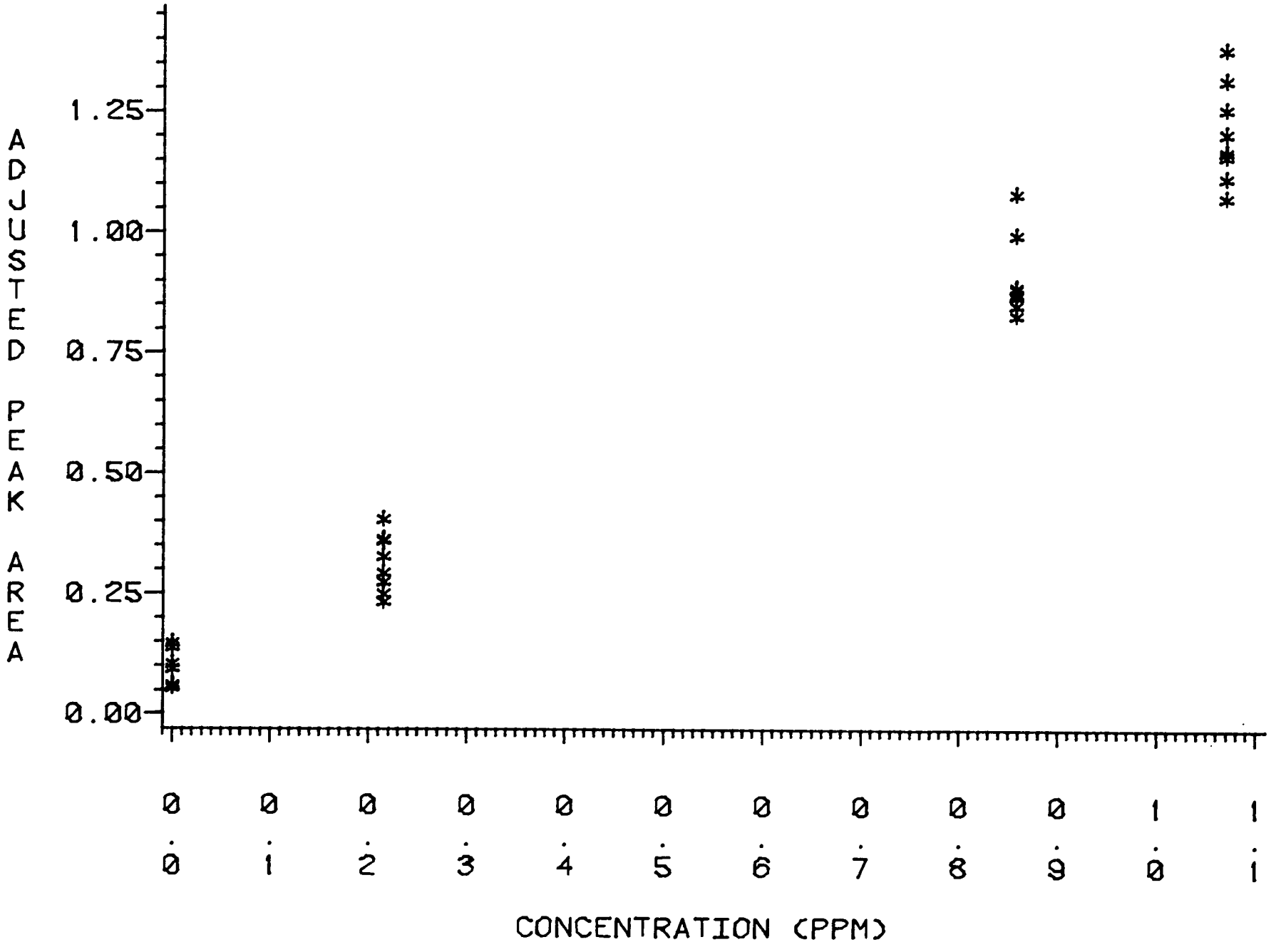


FIGURE 4-2.

CALIBRATION DATA: Y* VERSUS X*
ANALYTE=DIMETHYLPHTHALATE

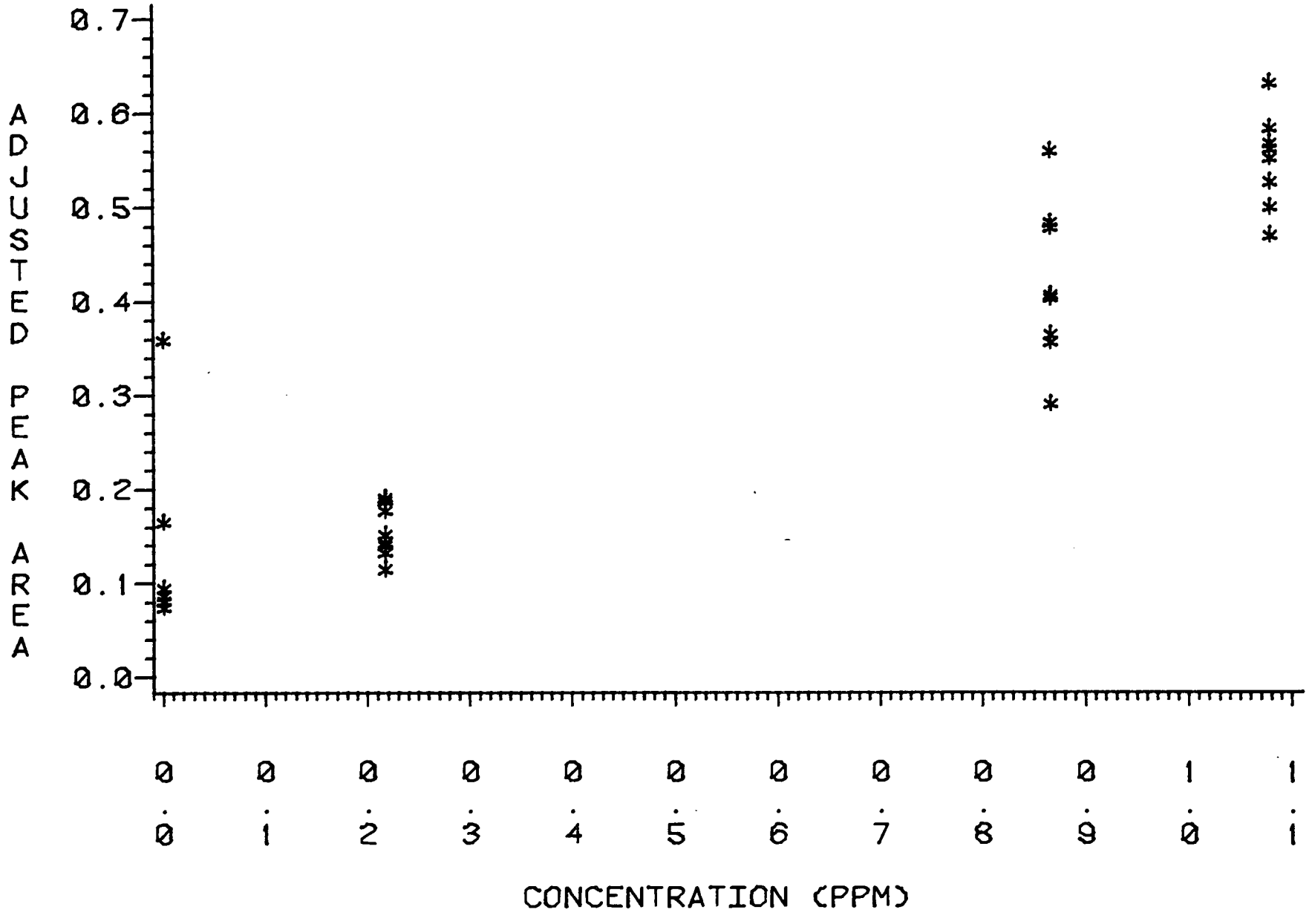


FIGURE 4-3.

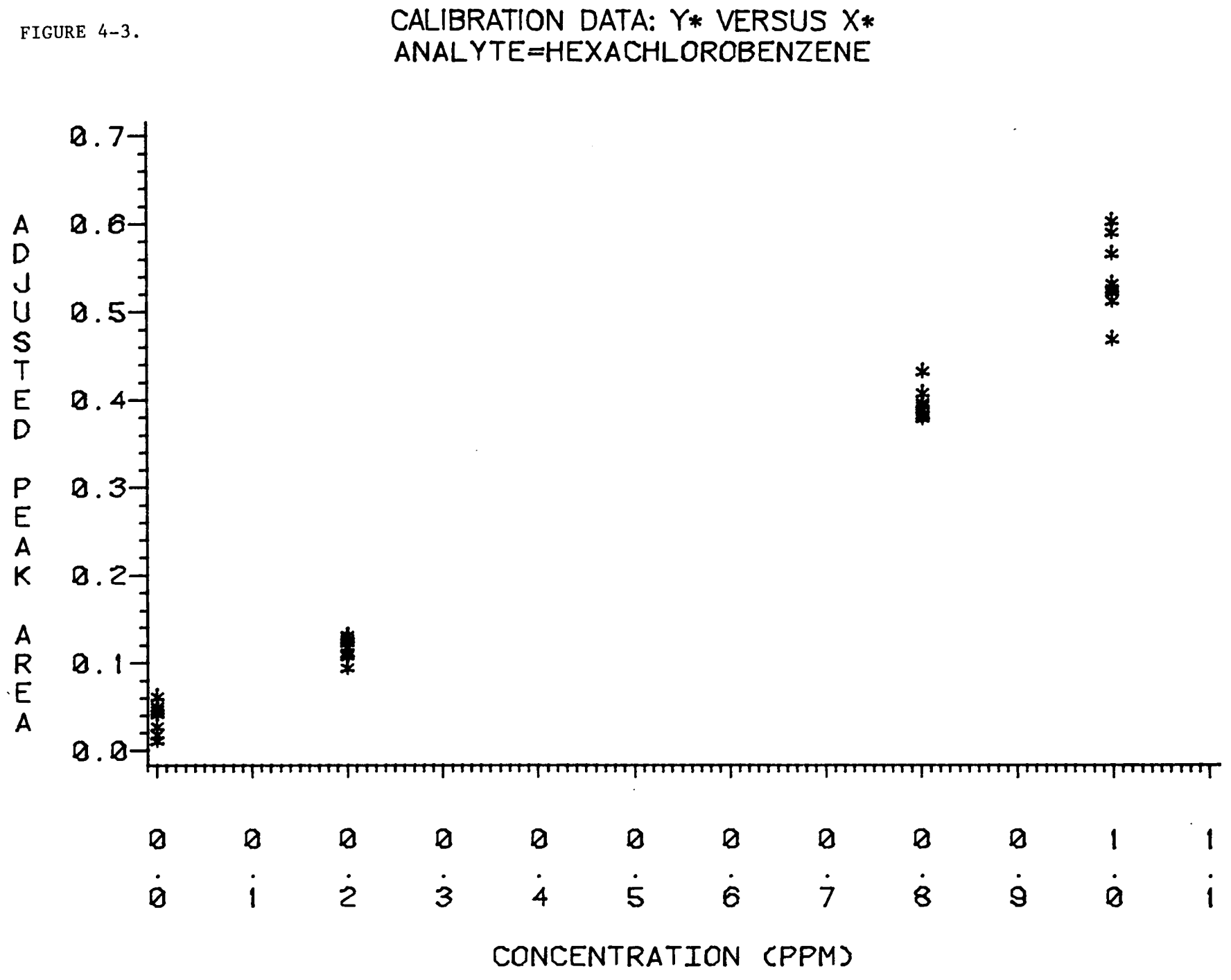

CALIBRATION DATA: Y* VERSUS X*
ANALYTE=HEXACHLOROBENZENE
ADJUSTED PEAK AREA
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
CONCENTRATION (PPM)

FIGURE 4-4.

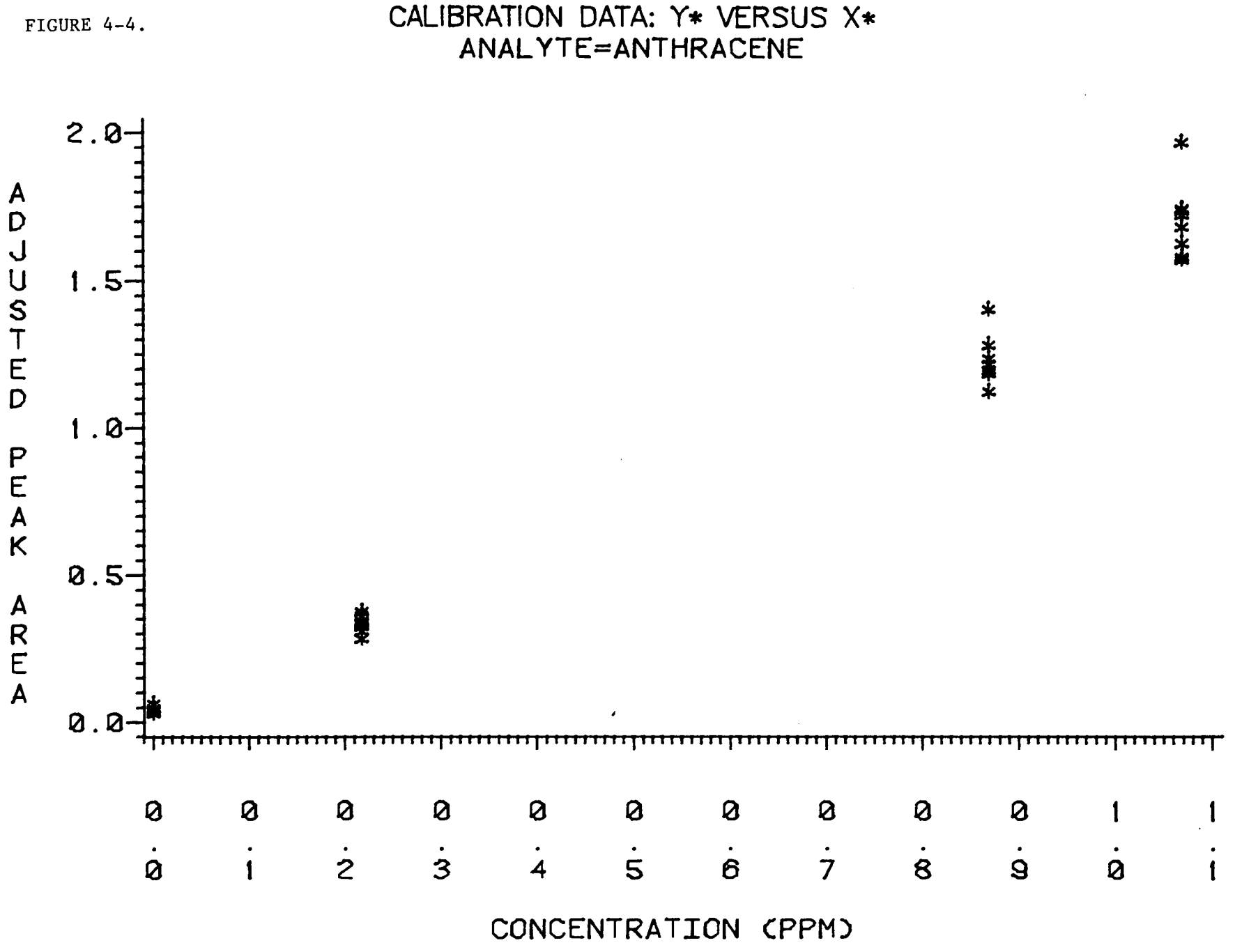

CALIBRATION DATA: Y* VERSUS X*
ANALYTE=ANTHRACENE
ADJUSTED PEAK AREA
2.0
1.5
1.0
0.5
0.0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
CONCENTRATION (PPM)

FIGURE 4-5.

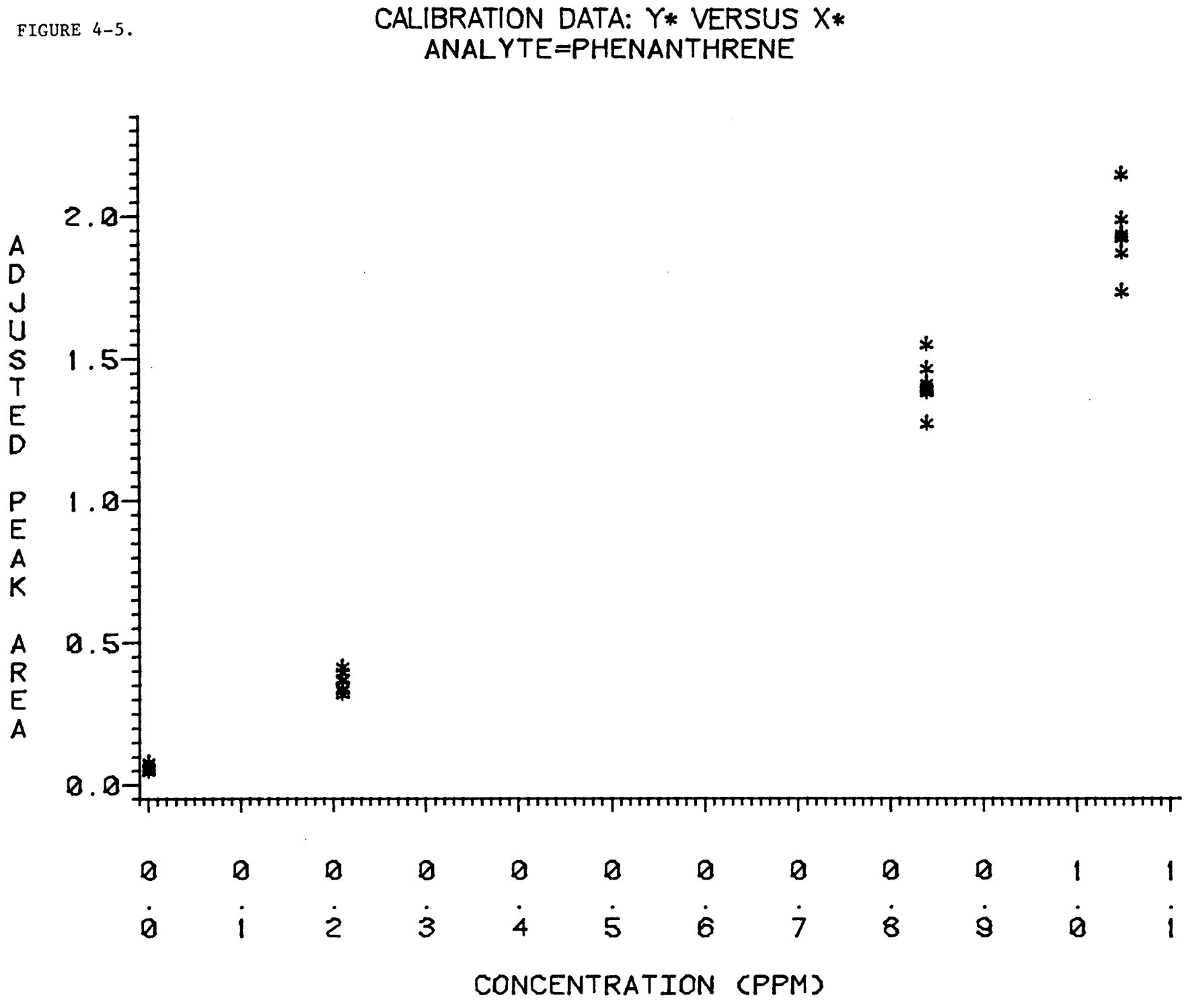

CALIBRATION DATA: Y* VERSUS X*
ANALYTE=PHENANTHRENE
ADJUSTED PEAK AREA
2.0
1.5
1.0
0.5
0.0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
CONCENTRATION (PPM)

FIGURE 4-6.

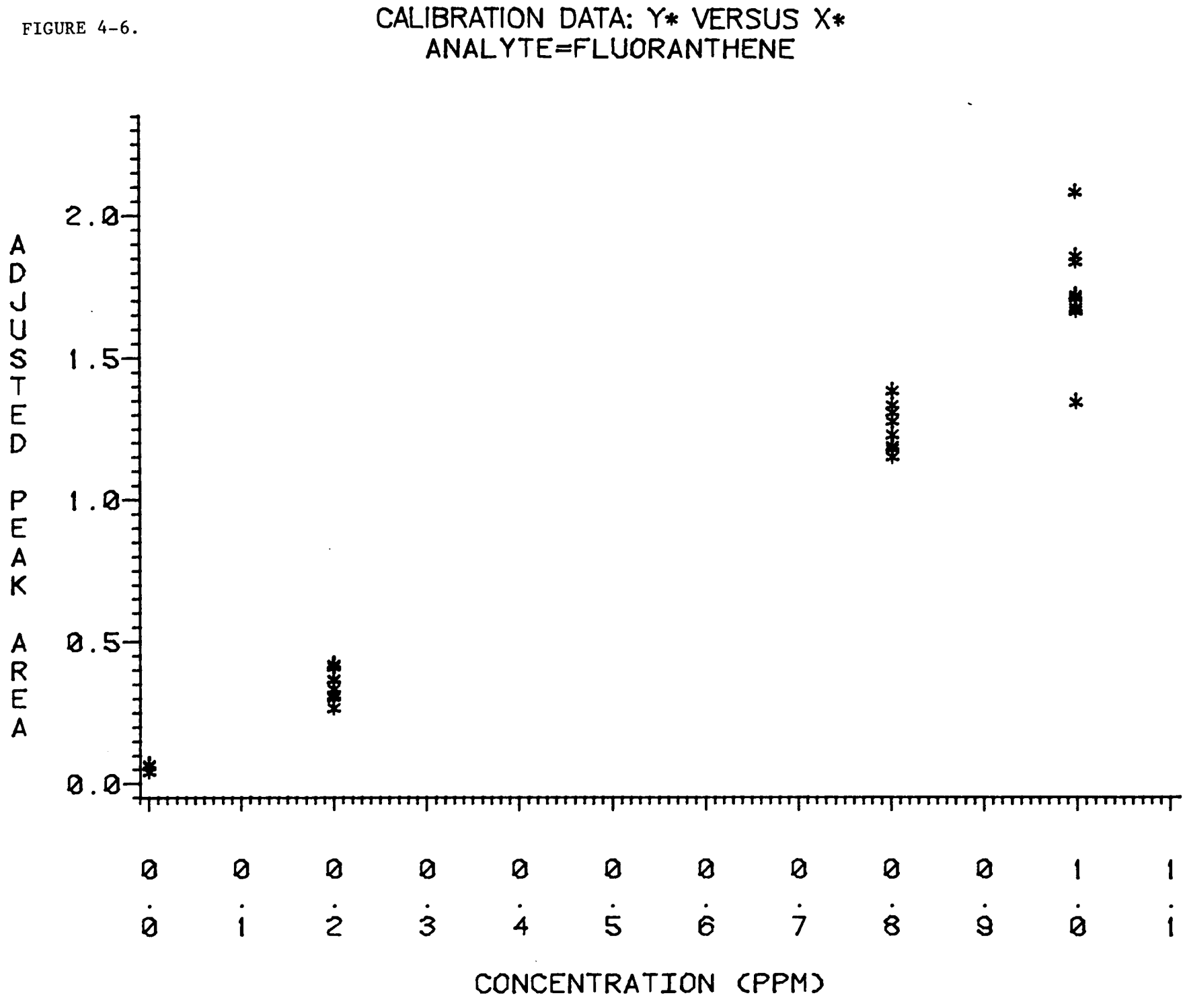

CALIBRATION DATA: Y* VERSUS X*
ANALYTE=FLUORANTHENE
ADJUSTED PEAK AREA
2.0
1.5
1.0
0.5
0.0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
CONCENTRATION (PPM)

Table 4.8. Means and Standard Deviations of Adjusted Peak Areas, by Analyte and Concentration Level

Analyte	Standard Conc. j	Standard Conc. X*(ppm)	n_j	Adjusted Peak Area (Y*) Mean	Adjusted Peak Area (Y*) Std. Deviation
2-chloronaphthalene	1	0.000	7	0.093	0.039
	2	0.215	8	0.313	0.060
	3	0.858	8	0.911	0.086
	4	1.070	8	1.215	0.103
dimethylphthalate	1	0.000	6	0.097	0.034
	2	0.217	8	0.154	0.027
	3	0.867	8	0.418	0.086
	4	1.080	8	0.550	0.051
hexachlorobenzene	1	0.000	7	0.037	0.018
	2	0.200	8	0.118	0.013
	3	0.802	8	0.395	0.018
	4	1.000	8	0.541	0.044
anthracene	1	0.000	7	0.042	0.010
	2	0.217	8	0.333	0.029
	3	0.870	8	1.229	0.084
	4	1.070	8	1.707	0.127
phenanthrene	1	0.000	7	0.064	0.014
	2	0.210	8	0.363	0.033
	3	0.840	8	1.407	0.078
	4	1.050	8	1.934	0.115
fluoranthene	1	0.000	7	0.058	0.011
	2	0.200	8	0.349	0.054
	3	0.802	8	1.258	0.082
	4	1.000	8	1.739	0.211

Analyte	Chi-square value for Bartlett's Test	F-value for Levene's Test
2-chloronaphthalene	5.77	1.97
dimethylphthalate	9.47*	3.39*
hexachlorobenzene	12.62**	5.05**
anthracene	29.21**	3.82*
phenanthrene	22.53**	1.76
fluoranthene	34.66**	4.01*

* = exceeds critical value at .05 level.
** = exceeds critical value at .01 level.

We considered several transformations of the observed responses in an attempt to remedy the variance heterogeneity (see, for example, Kempthorne (1952) and Snedecor and Cochran (1980)). Three of the candidate transformations were

$$\ell n\ (Y^* + C), \quad \sqrt{Y^* + C}, \quad \text{and} \quad Y^*/(X^* + C)$$

where C denotes some constant (various values of C were considered). Although the performance of the various transformations varied across the analytes, the square root transformation, with C=0, appeared to provide reasonably stable variances over the concentration levels. We therefore adopted the transformation

$$Y = \sqrt{Y^*},$$

where Y* is the adjusted peak area. Values of both Y* and Y are included in Tables 4.2 through 4.7. Table 4.9 contains the means and standard deviations of the transformed variate (Y), by analyte and concentration level.

On this transformed scale, results of the variance homogeneity tests are as follows:

Table 4.9. Means and Standard Deviations of Transformed Responses (Square Root of Adjusted Peak Areas), by Analyte and Concentration Level

Analyte	Standard Conc. j	Standard Conc. X*(ppm)	n_j	$Y = \sqrt{\text{Adjusted Peak Area}}$ Mean	$Y = \sqrt{\text{Adjusted Peak Area}}$ Std. Deviation
2-chloronaphthalene	1	0.000	7	0.299	0.064
	2	0.215	8	0.557	0.054
	3	0.858	8	0.954	0.044
	4	1.070	8	1.101	0.047
dimethylphthalate	1	0.000	6	0.308	0.049
	2	0.217	8	0.392	0.035
	3	0.867	8	0.644	0.066
	4	1.080	8	0.741	0.034
hexachlorobenzene	1	0.000	7	0.186	0.050
	2	0.200	8	0.343	0.019
	3	0.802	8	0.629	0.014
	4	1.000	8	0.735	0.030
anthracene	1	0.000	7	0.203	0.025
	2	0.217	8	0.577	0.025
	3	0.870	8	1.108	0.037
	4	1.070	8	1.306	0.048
phenanthrene	1	0.000	7	0.252	0.027
	2	0.210	8	0.602	0.027
	3	0.840	8	1.186	0.033
	4	1.050	8	1.390	0.041
fluoranthene	1	0.000	7	0.241	0.023
	2	0.200	8	0.589	0.046
	3	0.802	8	1.121	0.037
	4	1.000	8	1.316	0.081

Analyte	Chi-square value for Bartlett's Test	F-value for Levene's Test
2-chloronaphthalene	1.03	0.88
dimethylphthalate	3.99	1.32
hexachlorobenzene	11.58**	6.56**
anthracene	3.98	0.83
phenanthrene	1.56	0.05
fluoranthene	9.60*	1.46

* = exceeds critical value at .05 level.
** = exceeds critical value at .01 level.

With the possible exception of hexachlorobenzene, the transformed scale is clearly preferable to the original scale in terms of variance stability over concentration levels. Even for hexachlorobenzene, there does not appear to be a systematic trend in the variation of responses with concentration level (see Table 4.9).

We then prepared plots of $Y \equiv \sqrt{Y^*}$ versus concentration (X^*), as shown in Figures 4-7 through 4-12. In an effort to determine a simple (i.e., one or two parameters) relationship between Y and X^*, we considered a number of monotonic transformations of X^*. The most promising of these was the following formulation:

$$Y = A + B\sqrt{X^* + C} + \varepsilon \quad (C \geq 0). \tag{4-1}$$

This model was fitted by nonlinear least squares for each analyte separately.

Examination of the parameter estimates $\hat{A}$, $\hat{B}$, and $\hat{C}$, which are highly intercorrelated, revealed that the residual sum of squares was very insensitive to the $\hat{C}$ value. Based on these results, a value of C = 0.1 appeared to be a reasonable choice for all analytes. Using this value of C, the model (4-1) becomes a two-parameter model that can be written in the form

$$Y = A + BX' + \varepsilon \tag{4-2}$$

where $X' = \sqrt{X^* + 0.1}$. This can be rewritten in the form of model (2-2), which requires $f(0,\beta)$ = zero, by choosing

$$x = (X' - \sqrt{0.1}\,) = (\sqrt{X^* + 0.1} - \sqrt{0.1}\,) \tag{4-3}$$

FIGURE 4-7.

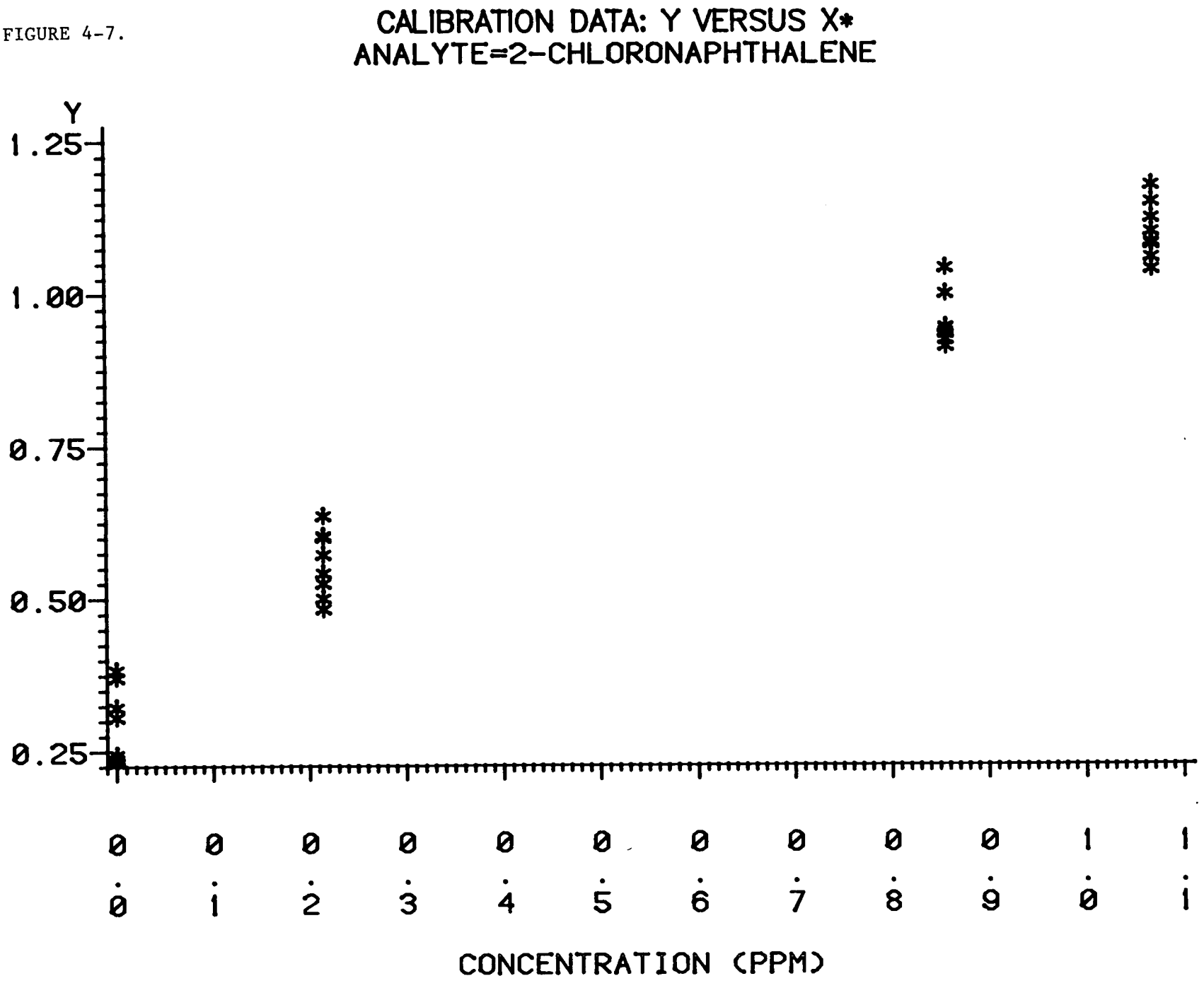

CALIBRATION DATA: Y VERSUS X*
ANALYTE=2-CHLORONAPHTHALENE
Y
1.25
1.00
0.75
0.50
0.25
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
CONCENTRATION (PPM)

FIGURE 4-8.

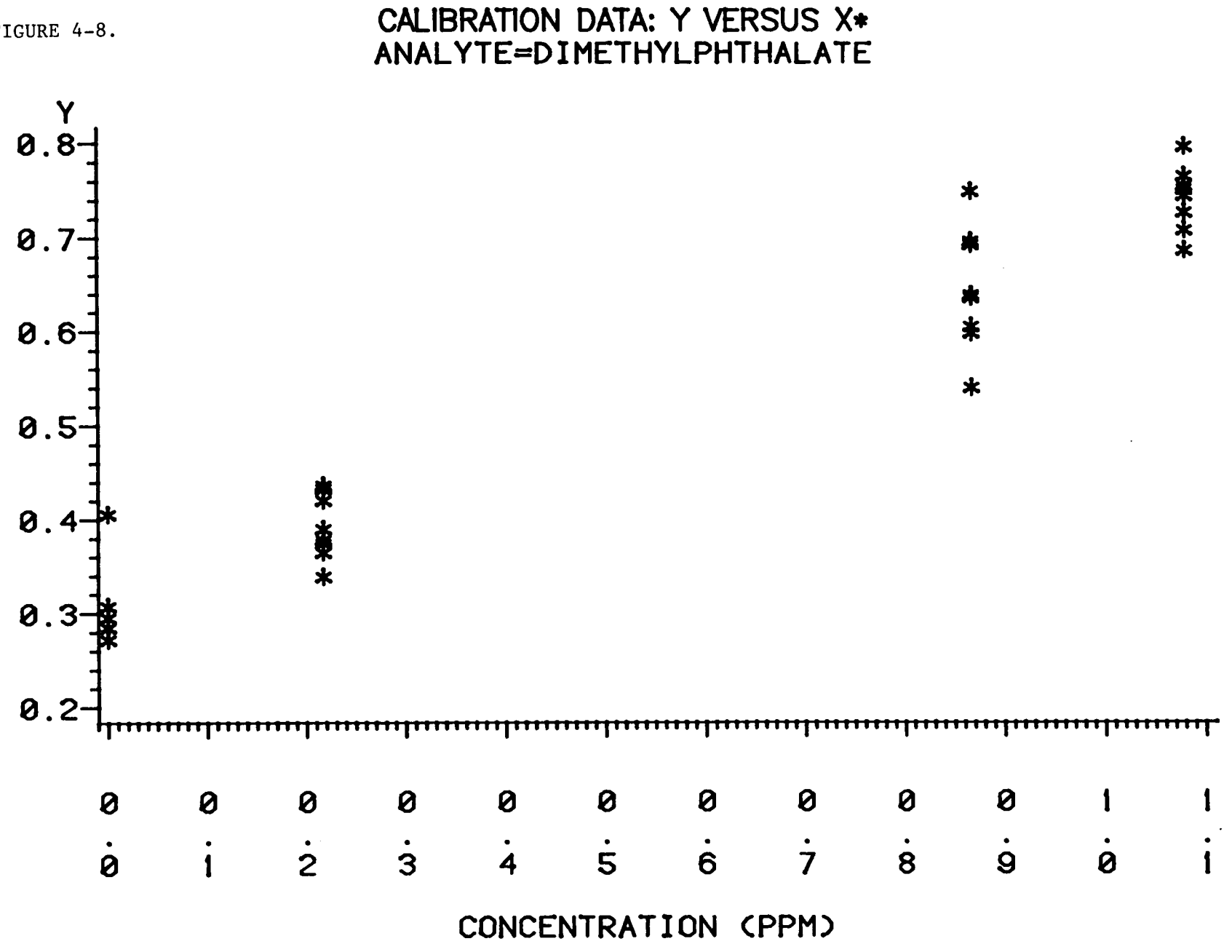

CALIBRATION DATA: Y VERSUS X*
ANALYTE=DIMETHYLPHTHALATE
Y
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
CONCENTRATION (PPM)

FIGURE 4-9.

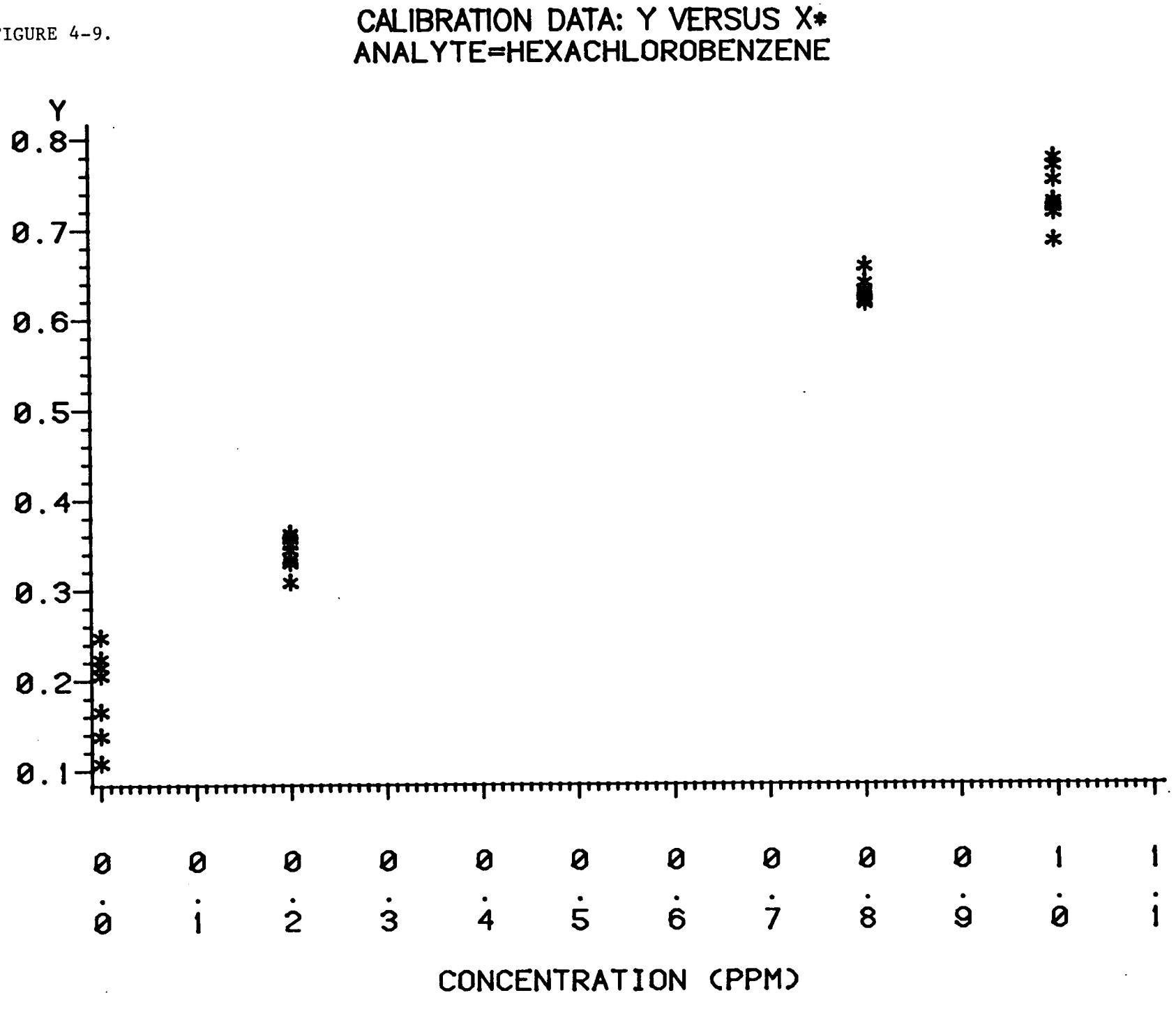

CALIBRATION DATA: Y VERSUS X*
ANALYTE=HEXACHLOROBENZENE
Y
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
CONCENTRATION (PPM)

FIGURE 4-10.

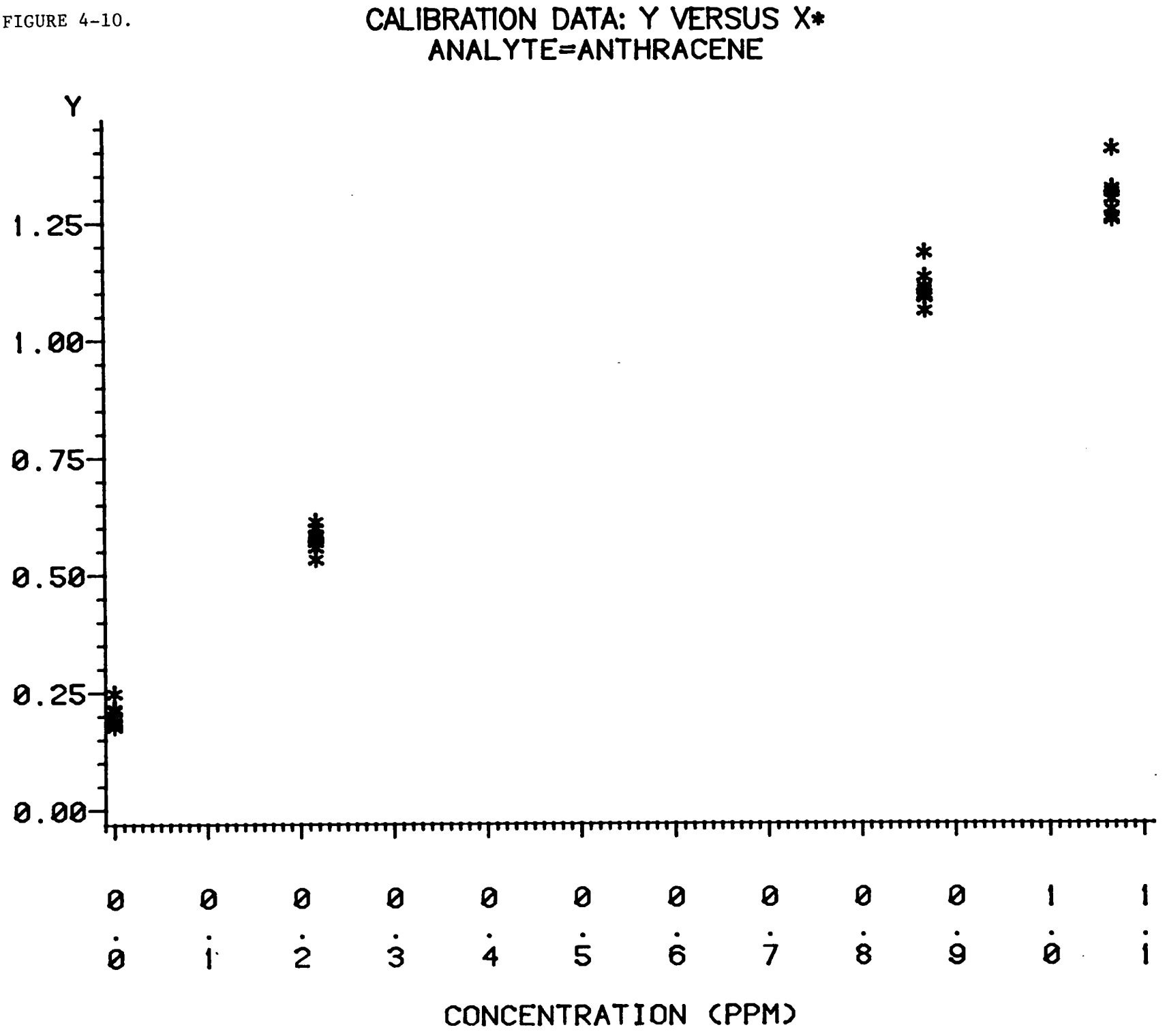

CALIBRATION DATA: Y VERSUS X*
ANALYTE=ANTHRACENE
Y
1.25
1.00
0.75
0.50
0.25
0.00
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
CONCENTRATION (PPM)

FIGURE 4-11.

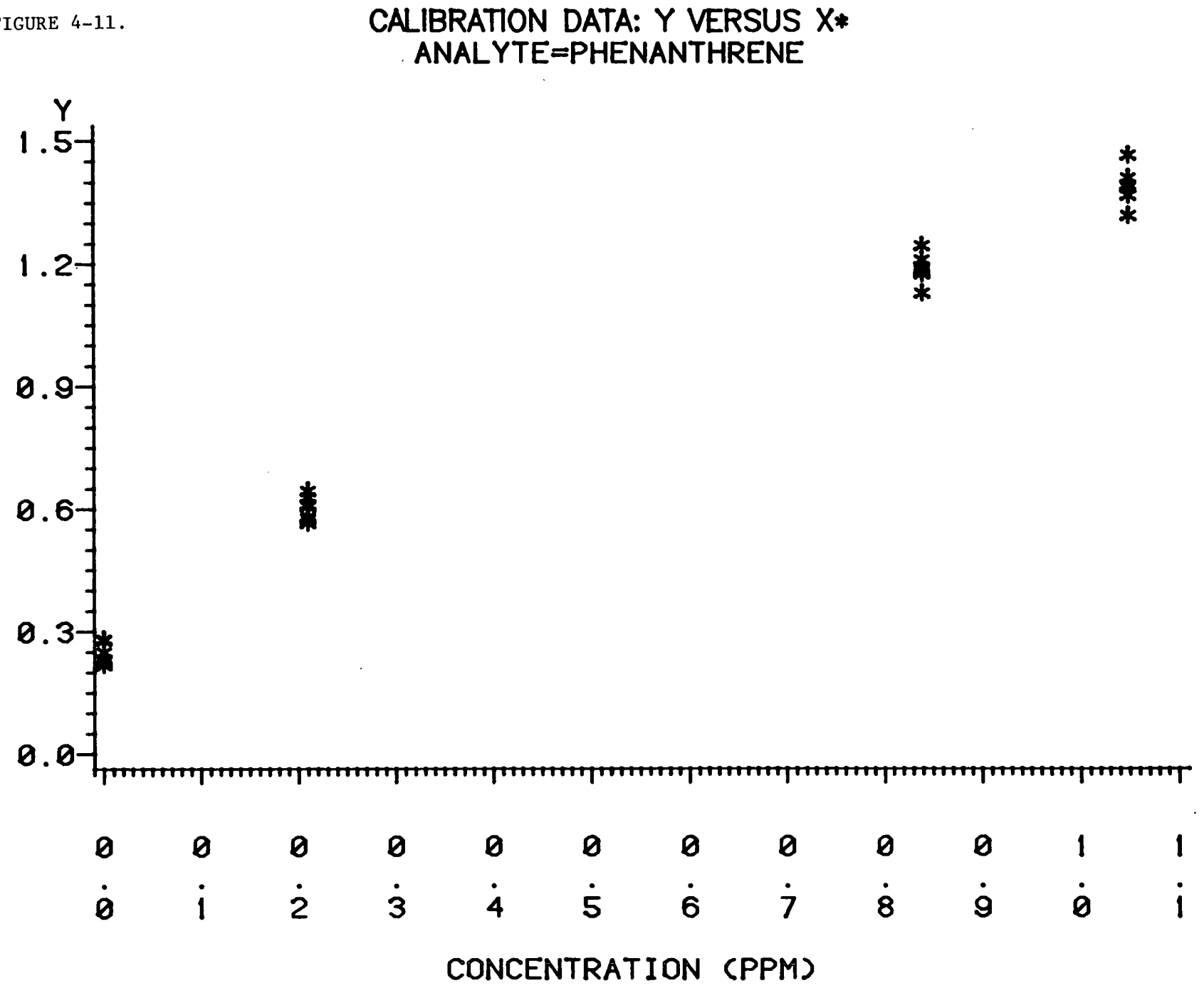
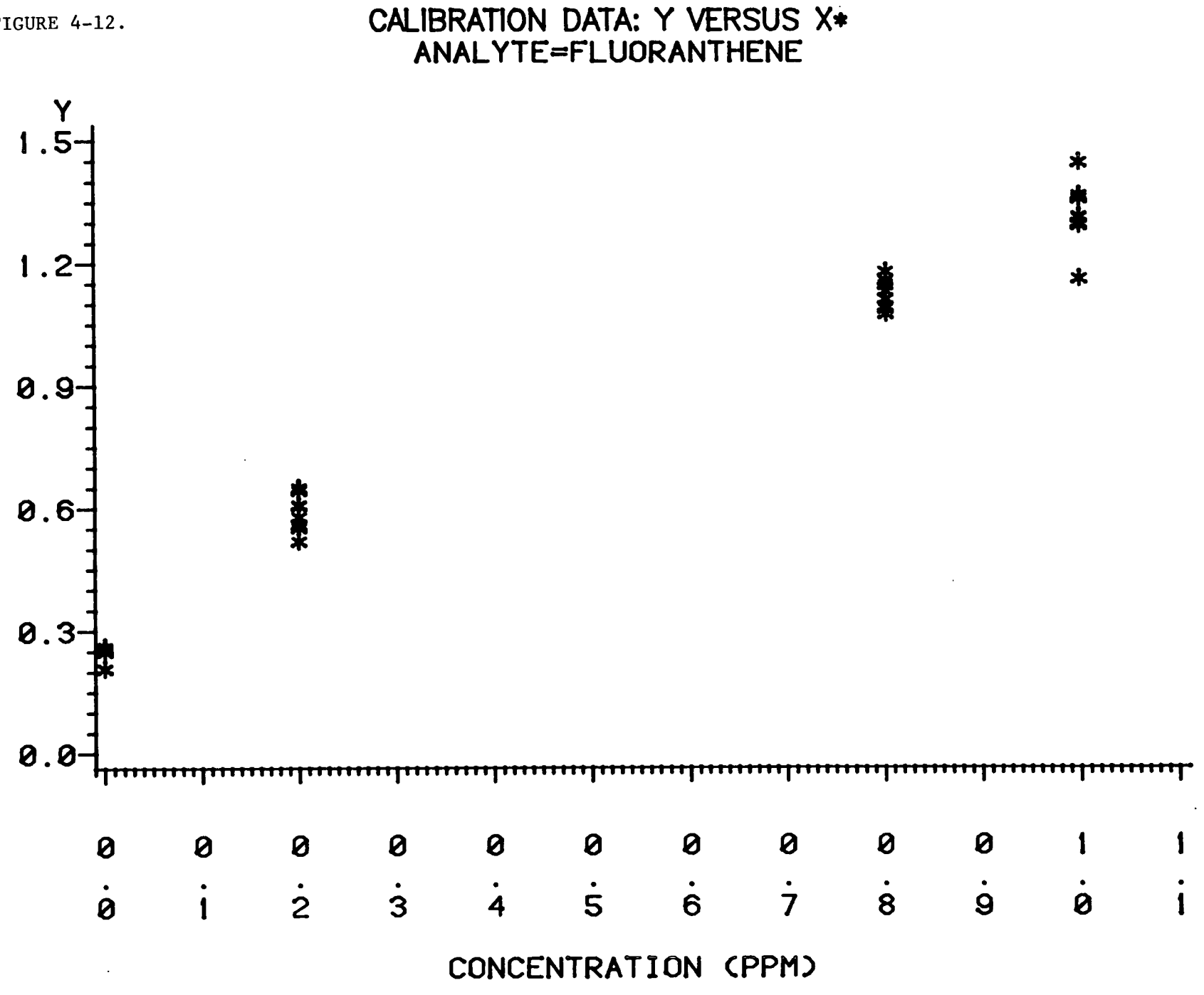

CALIBRATION DATA: Y VERSUS X*
ANALYTE=PHENANTHRENE
Y
1.5
1.2
0.9
0.6
0.3
0.0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
CONCENTRATION (PPM)

FIGURE 4-12.


CALIBRATION DATA: Y VERSUS X*
ANALYTE=FLUORANTHENE
Y
1.5
1.2
0.9
0.6
0.3
0.0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
1.1
CONCENTRATION (PPM)

and letting $\beta = B$ and $\alpha = A + B\sqrt{0.1}$. Values of x defined by (4-3) are included in Tables 4.2 through 4.7. The selected model form is thus identical to the linear calibration model (2-2):

$$Y_x = \alpha + \beta x + \varepsilon \qquad (4\text{-}4)$$

where α and β are the unknown model parameters, and where

Y_x = square root {observed peak area adjusted for the peak area of the internal standard},

$x = \sqrt{X^* + 0.1} - \sqrt{0.1}$ where X^* is the standard concentration,

ε = observational error, which is assumed (on the transformed scale) to be normally distributed with zero mean and variance σ^2.

Plots of the transformed responses, Y_x, versus the transformed concentrations, x, are shown in Figures 4-13 through 4-18.

These figures also show (1) the fitted model (solid line), based on least squares estimates $\hat{\alpha}$ and $\hat{\beta}$ (eq. (2-6) and (2-7)), and (2) bands constructed from interval estimates expected to contain 99% of individual (future) observations (dashed lines):

$$\hat{\alpha} + \hat{\beta}x \pm t_{v,.005}\, w_x \hat{\sigma}$$

where

$t_{v,.005}$ is the upper 0.5 percentage point of Student's t-distribution with v degrees of freedom (v=29 for most analytes),

w_x is defined by (2-12) with r=1, and

$\hat{\sigma}$ is the estimate of σ derived from the residual variance.

We examined model adequacy in two ways. First, we conducted an overall lack of fit test, as described under Step 5 in Section 2.2. The table below shows the pooled variance $\tilde{\sigma}^2$, the residual variance $\hat{\sigma}^2$, and the F-statistic (eq. 2-3) for testing lack of fit:

Analyte	$\tilde{\sigma}^2$	$\hat{\sigma}^2$	F-Statistic
2-chloronaphthalene	.0027180	.0027966	1.42
dimethylphthalate	.0022934	.0027582	3.84*
hexachlorobenzene	.0009418	.0010524	2.70
anthracene	.0012537	.0018670	8.09**
phenanthrene	.0010790	.0013905	5.19*
fluoranthene	.0027212	.0029900	2.43

FIGURE 4-13.

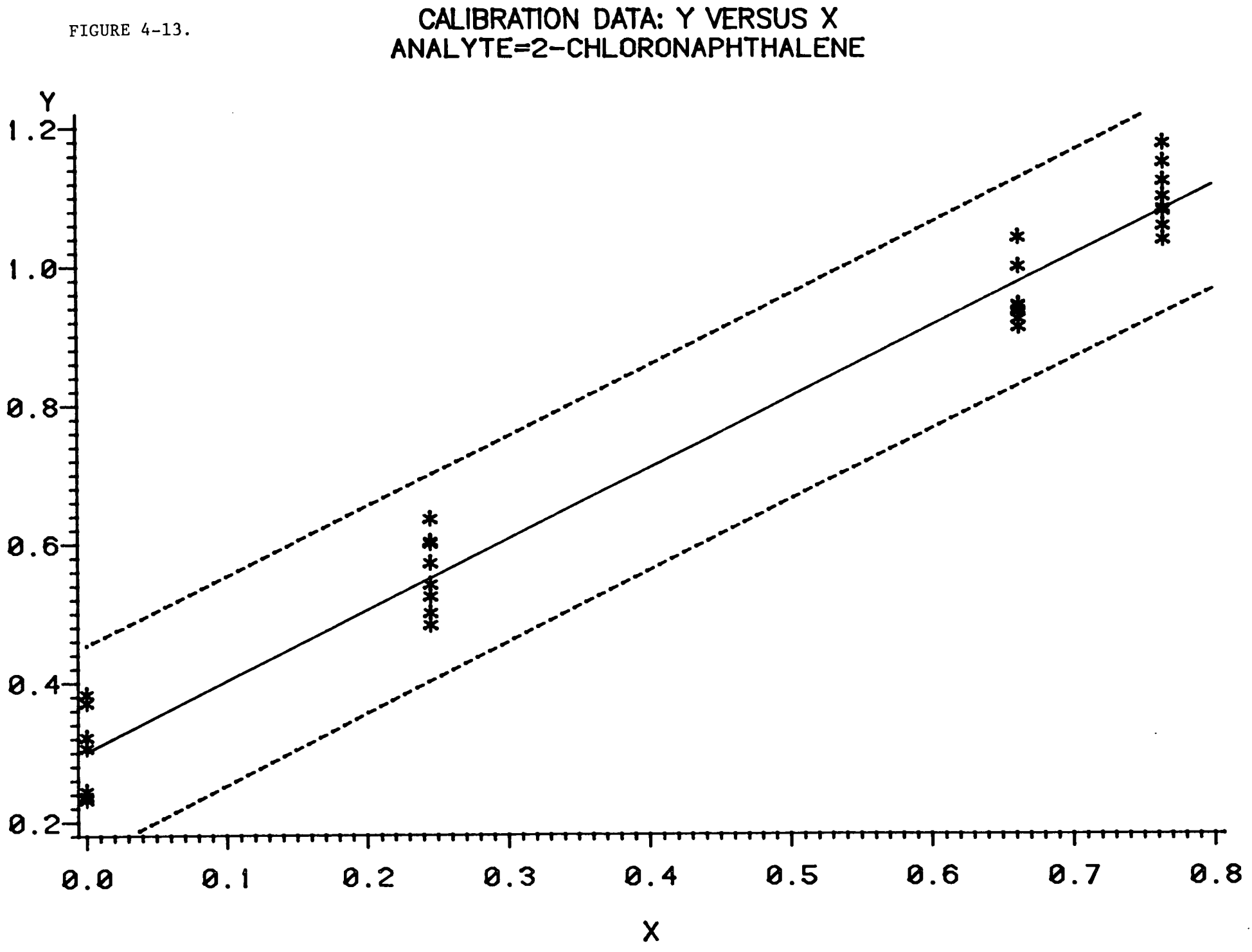

CALIBRATION DATA: Y VERSUS X
ANALYTE=2-CHLORONAPHTHALENE
Y
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
X

FIGURE 4-14.

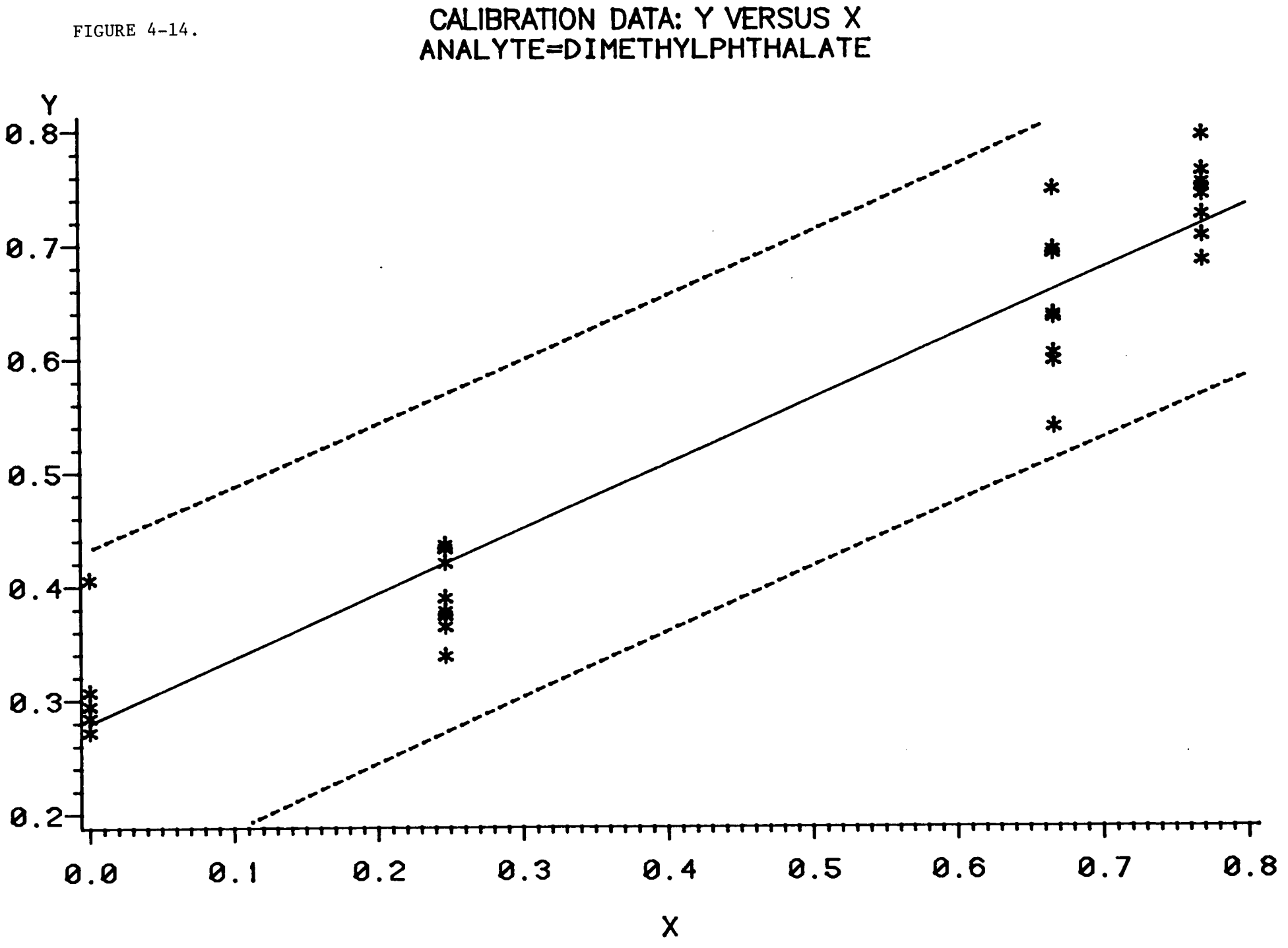

CALIBRATION DATA: Y VERSUS X
ANALYTE=DIMETHYLPHTHALATE
Y
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
X

FIGURE 4-15.

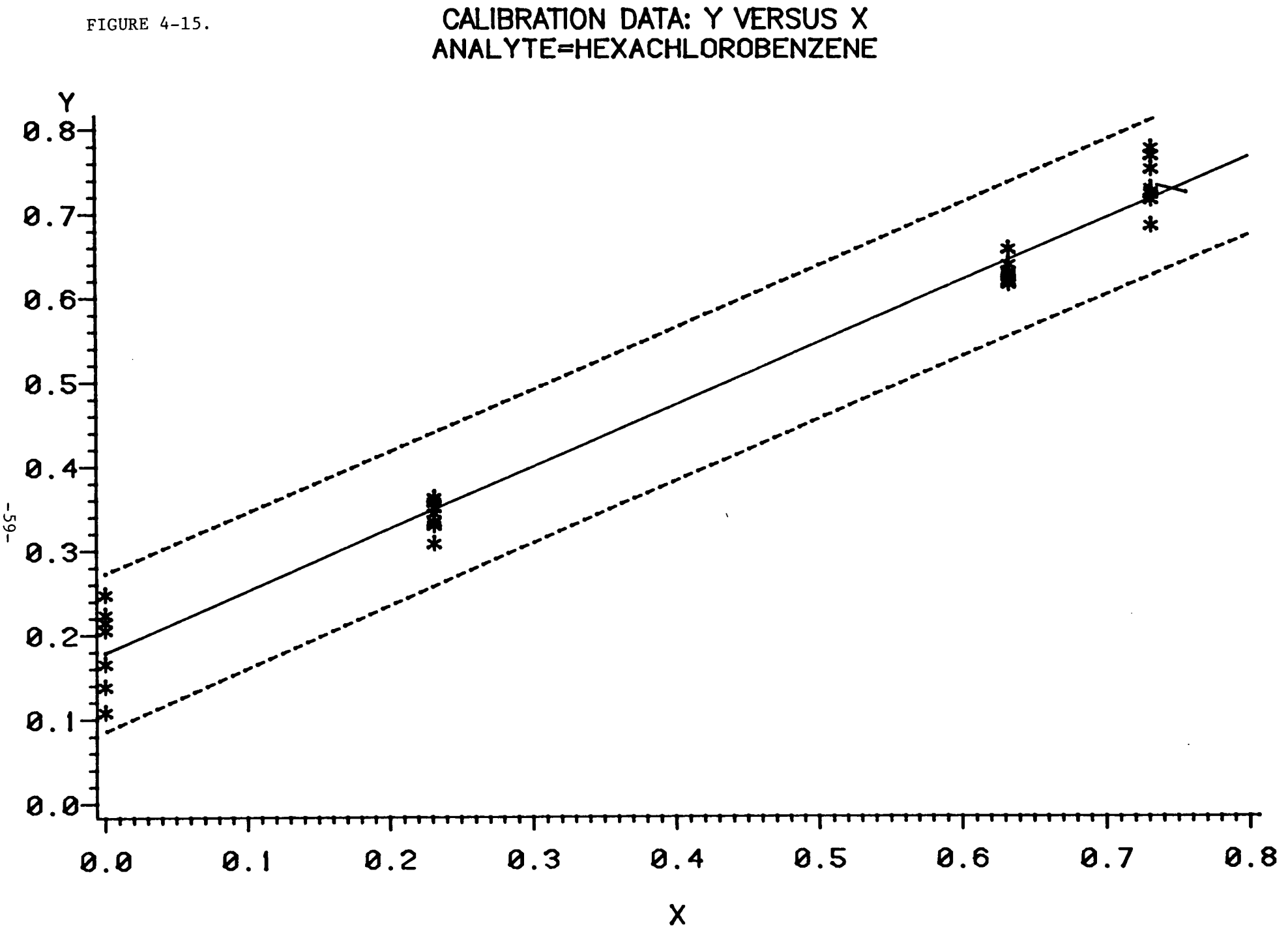

CALIBRATION DATA: Y VERSUS X
ANALYTE=HEXACHLOROBENZENE
Y
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
X

FIGURE 4-16.

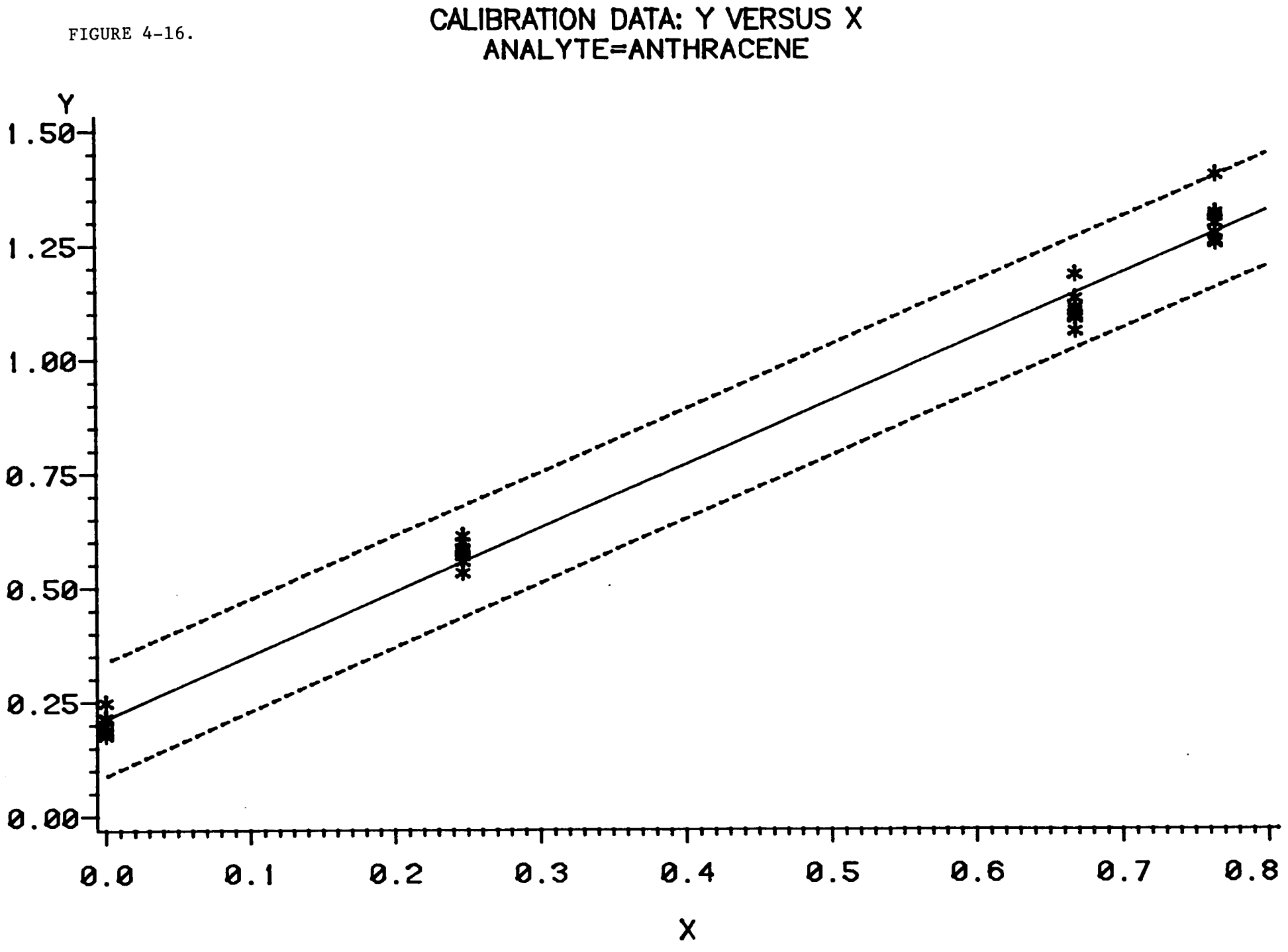

CALIBRATION DATA: Y VERSUS X
ANALYTE=ANTHRACENE
Y
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
X

FIGURE 4-17.

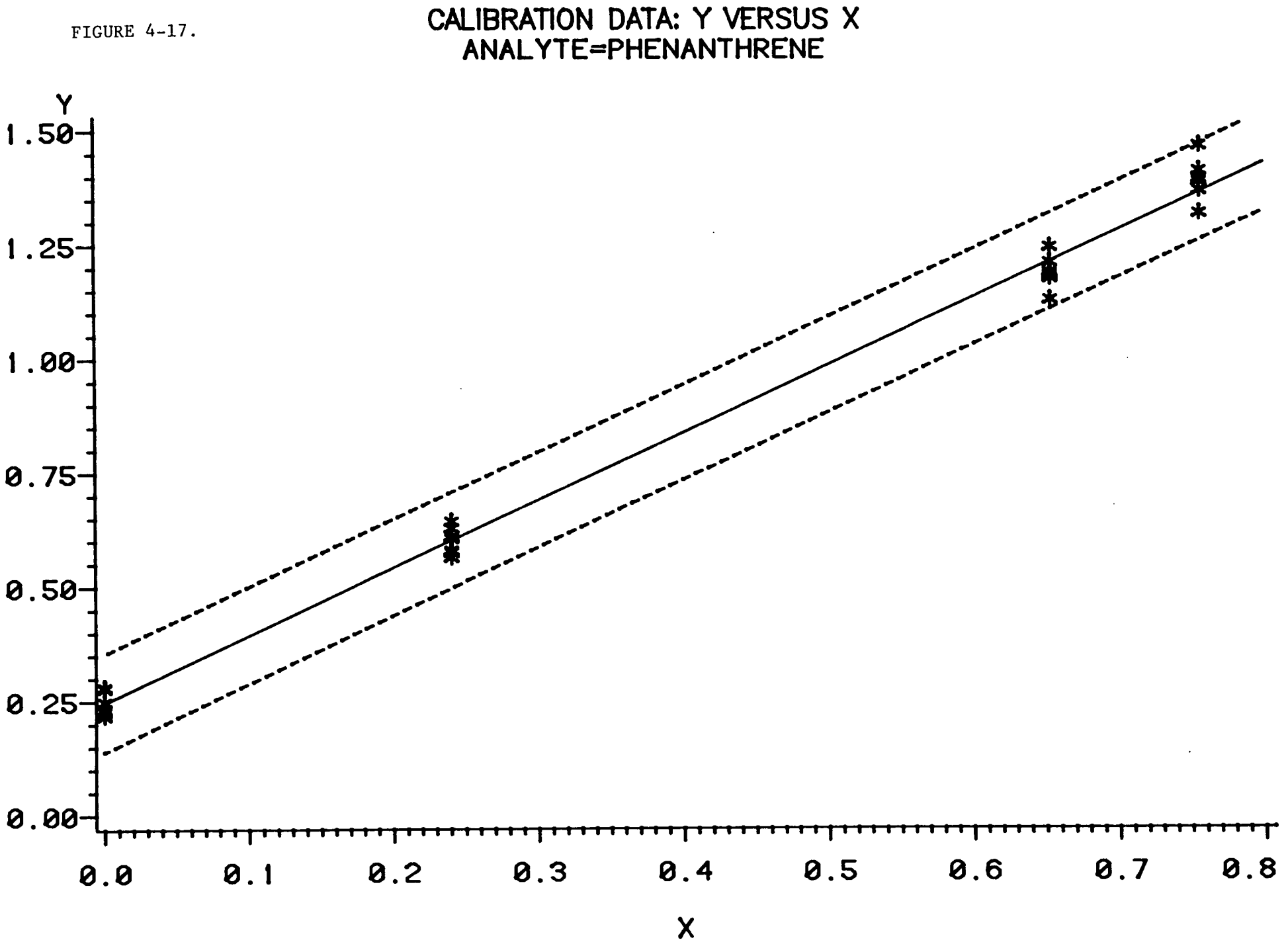
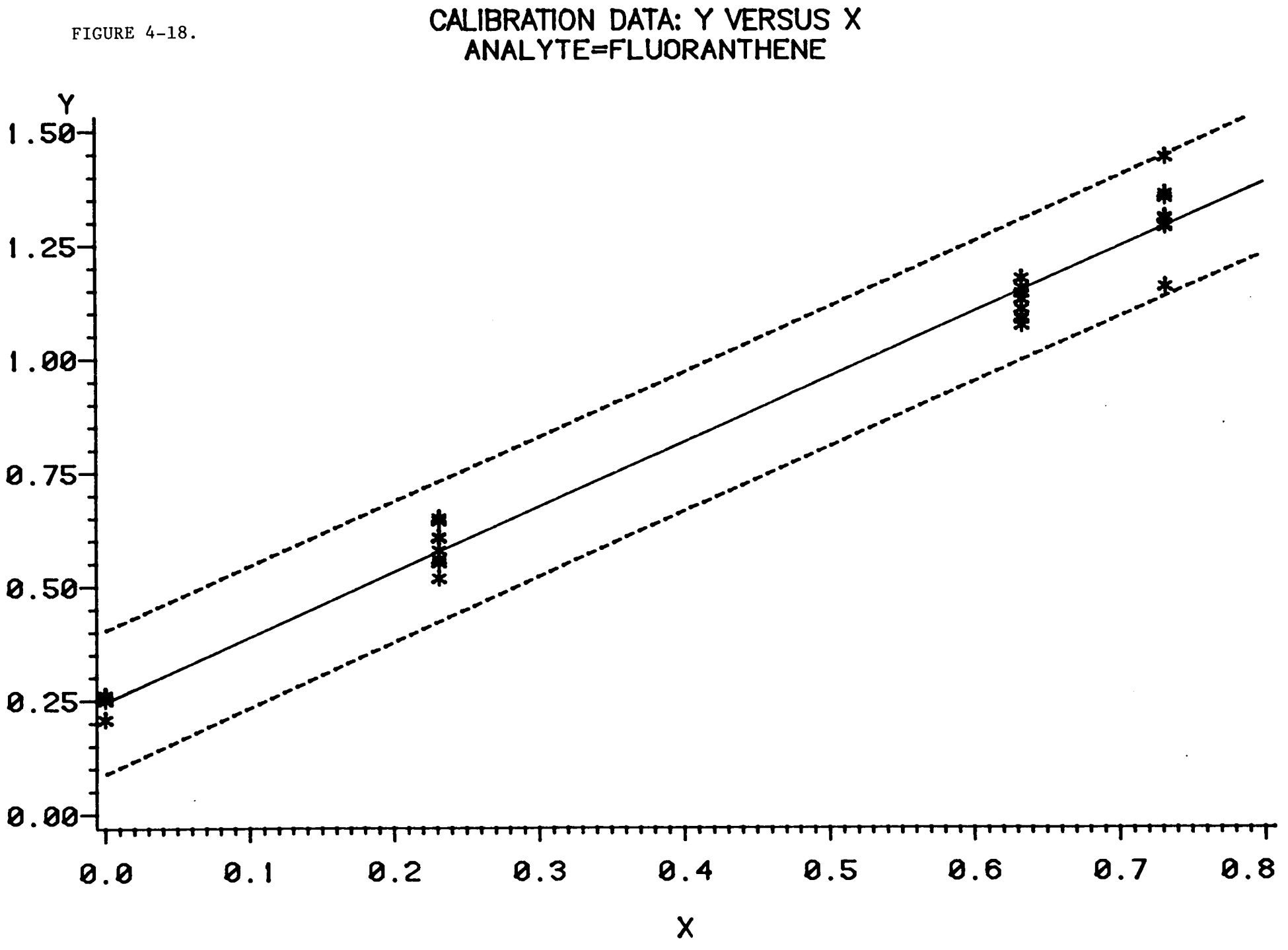

CALIBRATION DATA: Y VERSUS X
ANALYTE=PHENANTHRENE
Y
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
X

FIGURE 4-18.


CALIBRATION DATA: Y VERSUS X
ANALYTE=FLUORANTHENE
Y
1.50
1.25
1.00
0.75
0.50
0.25
0.00
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
X

The F-statistics, based on 2 and 27 degrees of freedom (2 and 26 for dimethylphthalate), provide evidence of lack of fit for anthracene, and perhaps for dimethylphthalate and phenanthrene.

We also tested the straight line model against the specific alternative of a quadratic model. With the exception of dimethylphthalate (for which some erratic behavior has been noted previously), there was no evidence that the inclusion of a quadratic term was necessary.

We adopted the model form (4-4) for all analytes; on the transformed scales, all of the methods described for the linear calibration in Chapters 2 and 3 are applicable therefore.

Table 4-10 contains the relevant calibration design characteristics, and Table 4-11 contains estimates of the parameters. The notation used in these tables is defined in Chapter 2.

4.5 Estimation of Detection Limits

After estimating the parameters for each analyte, we proceeded in the manner described in Section 3.2. This involved:

(1) specification of a detection rule;

(2) construction of the threshold response value $\hat{y}_p$;

(3) estimation of β/σ (Section 3.3); and

(4) estimation of detection limits with specified false positive and false negative rates.

These four steps are addressed in the subsections below.

Specification of a detection rule. In accordance with the formulation in Chapter 3, the analyte is to be declared detected whenever the mean of r replicate response observations from the same source matrix exceeds a response threshold value, which is a function of the chosen rate p for false positives. In practice, we would therefore choose a particular value of p and a single value r as a part of the detection protocol. For purposes of this report we considered two p values ($p = 0.01$ and $p = 0.05$) and three r values ($r = 1$, 2, and 3) in order to provide a more comprehensive description of detectability. Values of t_p corresponding to the two chosen p values are shown below (values from Table 1 of Appendix A):

Table 4-10. Calibration Design Parameters, By Analyte

Analyte	n	$\bar{x}$	Q_{xx}	w_o Values For r=1	r=2	r=3
2-chloronaphthalene	31	.43174	2.90072	1.04715	0.77235	0.65563
Dimethylphthalate	30	.44906	2.74193	1.05208	0.77902	0.66349
Hexachlorobenzene	31	.41228	2.66357	1.04694	0.77206	0.65529
Anthracene	31	.43378	2.91816	1.04725	0.77249	0.65580
Phenanthrene	31	.42581	2.83083	1.04705	0.77221	0.65547
Fluoranthene	31	.41228	2.66357	1.04694	0.77206	0.65529

Table 4-11. Calibration Model Parameter Estimates, By Analyte

Analyte	n	$\hat{\alpha}$	$\hat{\beta}$	$\hat{\sigma}$	Standard Error of $\hat{\alpha}$	Standard Error of $\hat{\beta}$
2-Chloronaphthalene	31	0.300676	1.02173	0.052883	0.016429	0.03105
Dimethylphthalate	30	0.279346	0.57002	0.052519	0.017170	0.03172
Hexachlorobenzene	31	0.178793	0.73651	0.032441	0.010055	0.01988
Anthracene	31	0.212840	1.39402	0.043208	0.013439	0.02529
Phenanthrene	31	0.247232	1.47915	0.037289	0.011572	0.02216
Fluoranthene	31	0.245677	1.43041	0.054681	0.016949	0.03350

Degrees of Freedom	False Positive Rate p = 0.01	p = 0.05
v = n-2 = 29*	2.46202	1.69913
v = n-2 = 28	2.46714	1.70113

* Applicable to all analytes except dimethylphthalate.

Values of w_o corresponding to r = 1, 2, and 3, which also depend on the calibration design, were given previously in Table 4-10.

Determination of the threshold response value. Threshold response values for each p and r combination, given the calibration design, were determined via eq. (3-6) and are given in Table 4-12. These values of $\hat{y}_p$ apply to the transformed scale associated with model (4-4).

Estimation of β/σ. We next used the results of Table 4-11 to produce point estimates of β/σ and of σ/β. These estimates, derived in Section 3.3, are given below:

Analyte	M_v	$\hat{\beta}/M_v\hat{\sigma}$	$\hat{\sigma}/\hat{\beta}$
2-chloronaphthalene	1.02683	18.81575	0.051758
dimethylphthalate	1.02782	10.55982	0.092135
hexachlorobenzene	1.02683	22.10985	0.044047
anthracene	1.02683	31.42001	0.030995
phenanthrene	1.02683	38.63073	0.025210
fluoranthene	1.02683	25.47567	0.038228

Estimation of detection limits. Point estimates of $x_\ell(p,q)$ were determined for each analyte as

$$\hat{x}_\ell(p,q) = w_o\Delta(p,q)\hat{\sigma}/\hat{\beta} \qquad (4\text{-}5)$$

where q was allowed to vary over the range (p,1). For each such q, the Δ value satisfying (3-8) was determined using the algorithms of Appendix B. Estimated power (i.e., detection) curves, such as those shown in Figures 4-19 through 4-24, were produced by this means. The curves shown in these figures correspond to p = 0.01; each figure contains a curve for each of r = 1, 2, or 3. A common horizontal scale (i.e., the transformed concentration scale) is used for all six figures in order to

Table 4-12. Threshold Response Values $\hat{y}_p$, By Analyte, for p=0.01 and 0.05, and for r=1, 2, and 3

Analyte	r=1		r=2		r=3	
	p=0.01	p=0.05	p=0.01	p=0.05	p=0.01	p=0.05
2-Chloronaphthalene	0.43701	0.39477	0.40123	0.37008	0.38604	0.35959
Dimethylphthalate	0.41567	0.37334	0.38029	0.34895	0.36531	0.33862
Hexachlorobenzene	0.26241	0.23650	0.24046	0.22135	0.23113	0.21491
Anthracene	0.32425	0.28973	0.29502	0.26955	0.28260	0.26099
Phenanthrene	0.34336	0.31357	0.31813	0.29616	0.30741	0.28876
Fluoranthene	0.38662	0.34295	0.34962	0.31741	0.33390	0.30656

FIGURE 4-19.

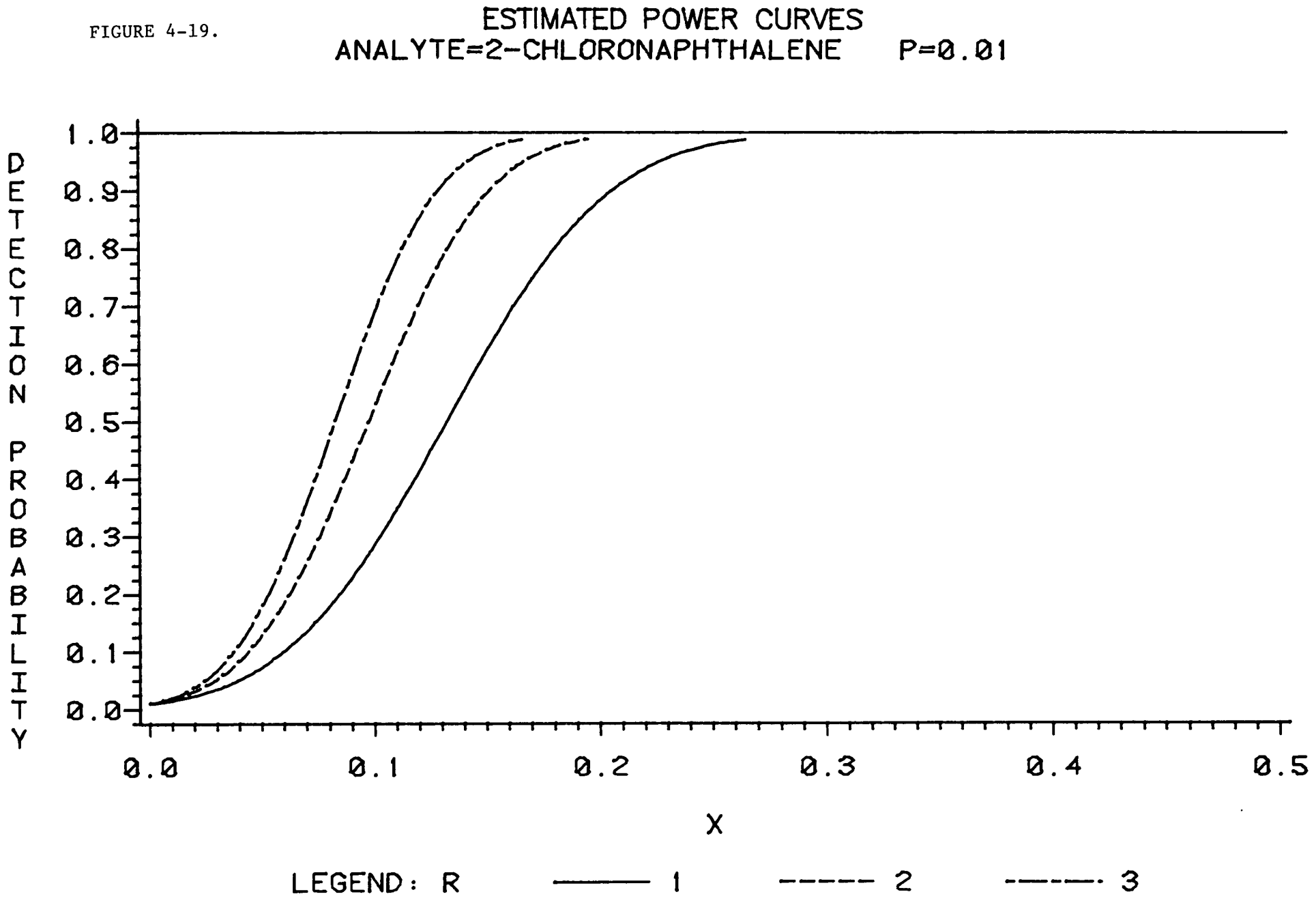

ESTIMATED POWER CURVES
ANALYTE=2-CHLORONAPHTHALENE P=0.01
DETECTION PROBABILITY
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.0
0.1
0.2
0.3
0.4
0.5
X
LEGEND: R
1
2
3

FIGURE 4-20.

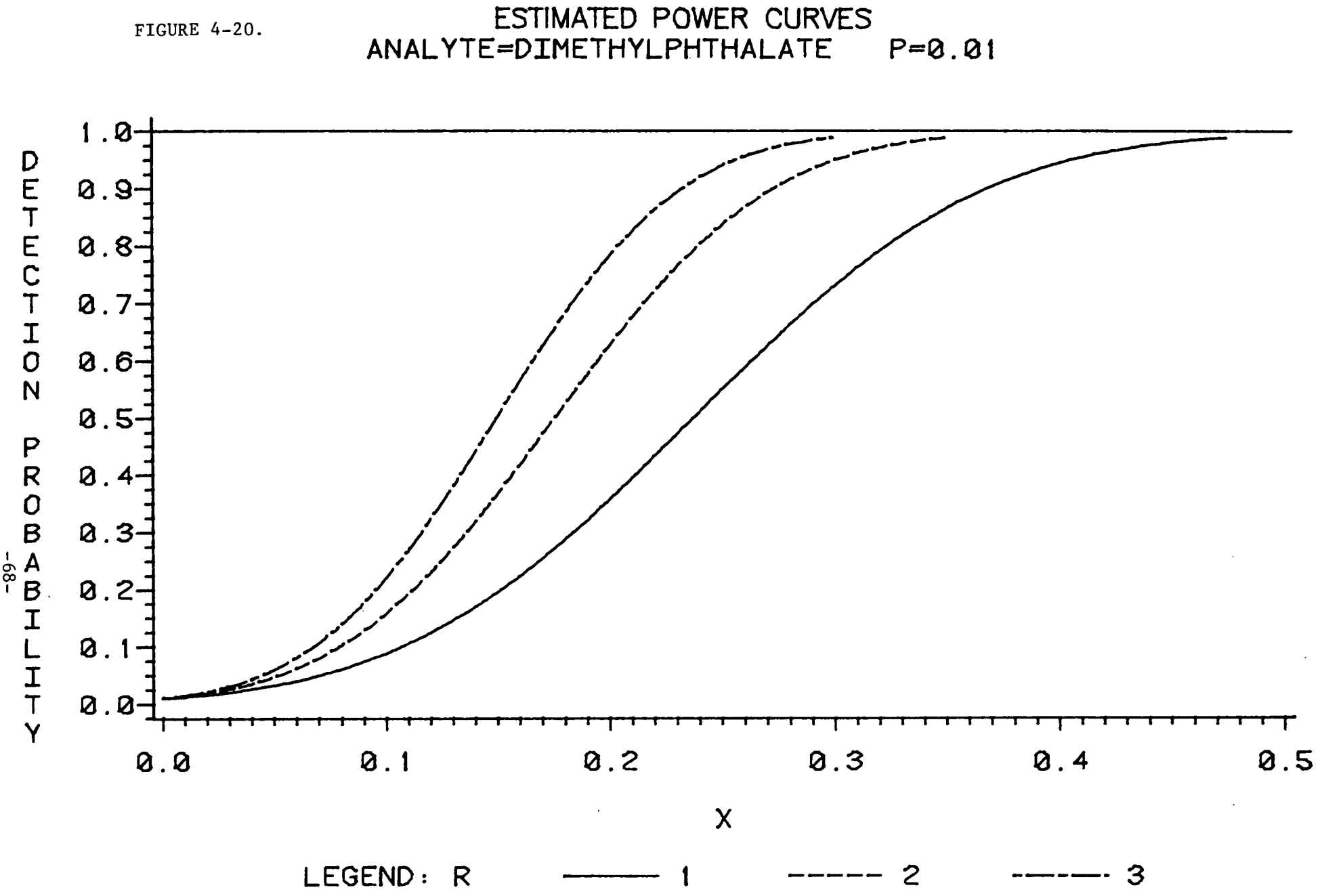

ESTIMATED POWER CURVES
ANALYTE=DIMETHYLPHTHALATE P=0.01
DETECTION PROBABILITY
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.0
0.1
0.2
0.3
0.4
0.5
X
LEGEND: R
1
2
3

FIGURE 4-21.

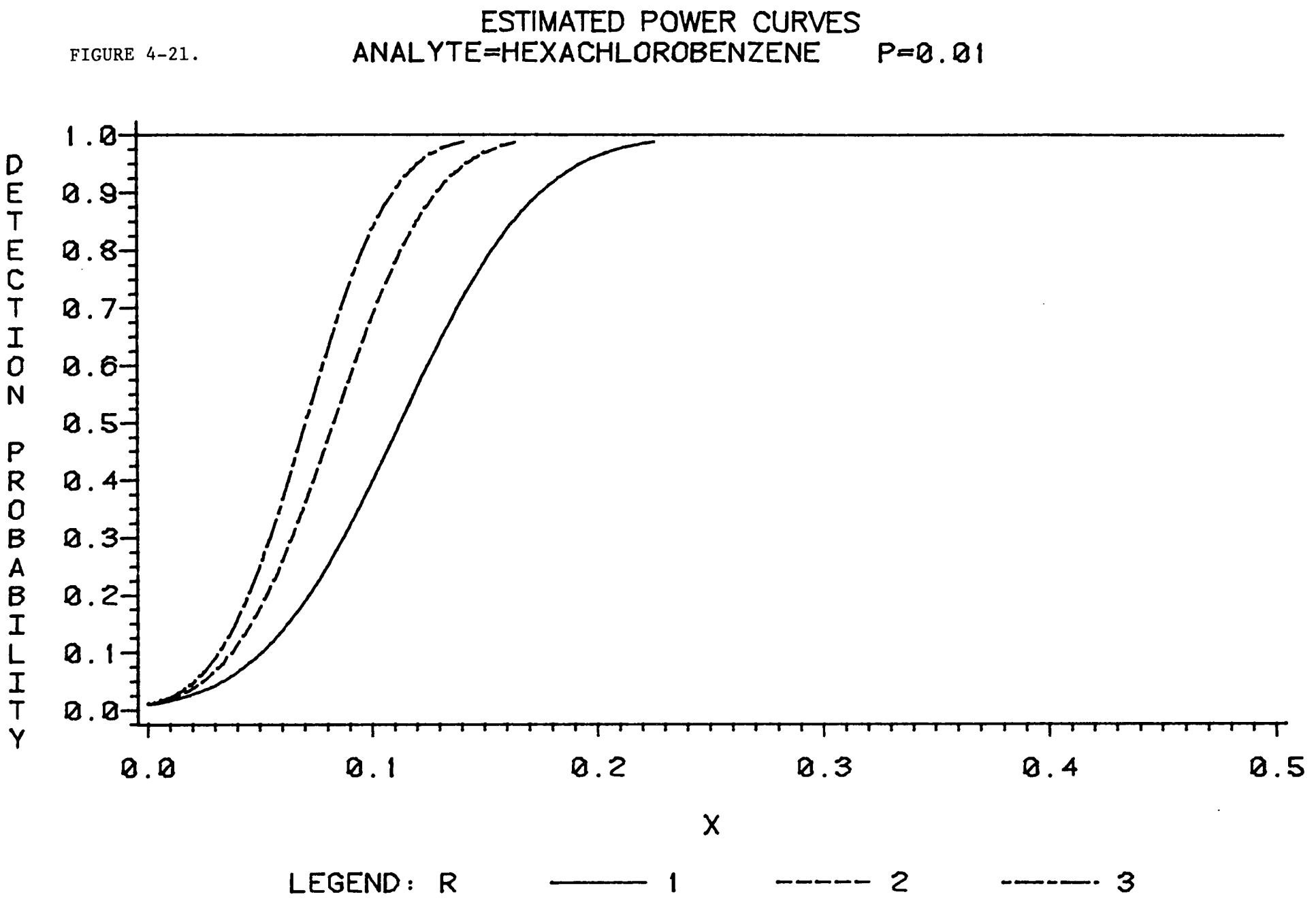

ESTIMATED POWER CURVES
ANALYTE=HEXACHLOROBENZENE P=0.01
DETECTION PROBABILITY
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.0
0.1
0.2
0.3
0.4
0.5
X
LEGEND: R
1
2
3

FIGURE 4-22.

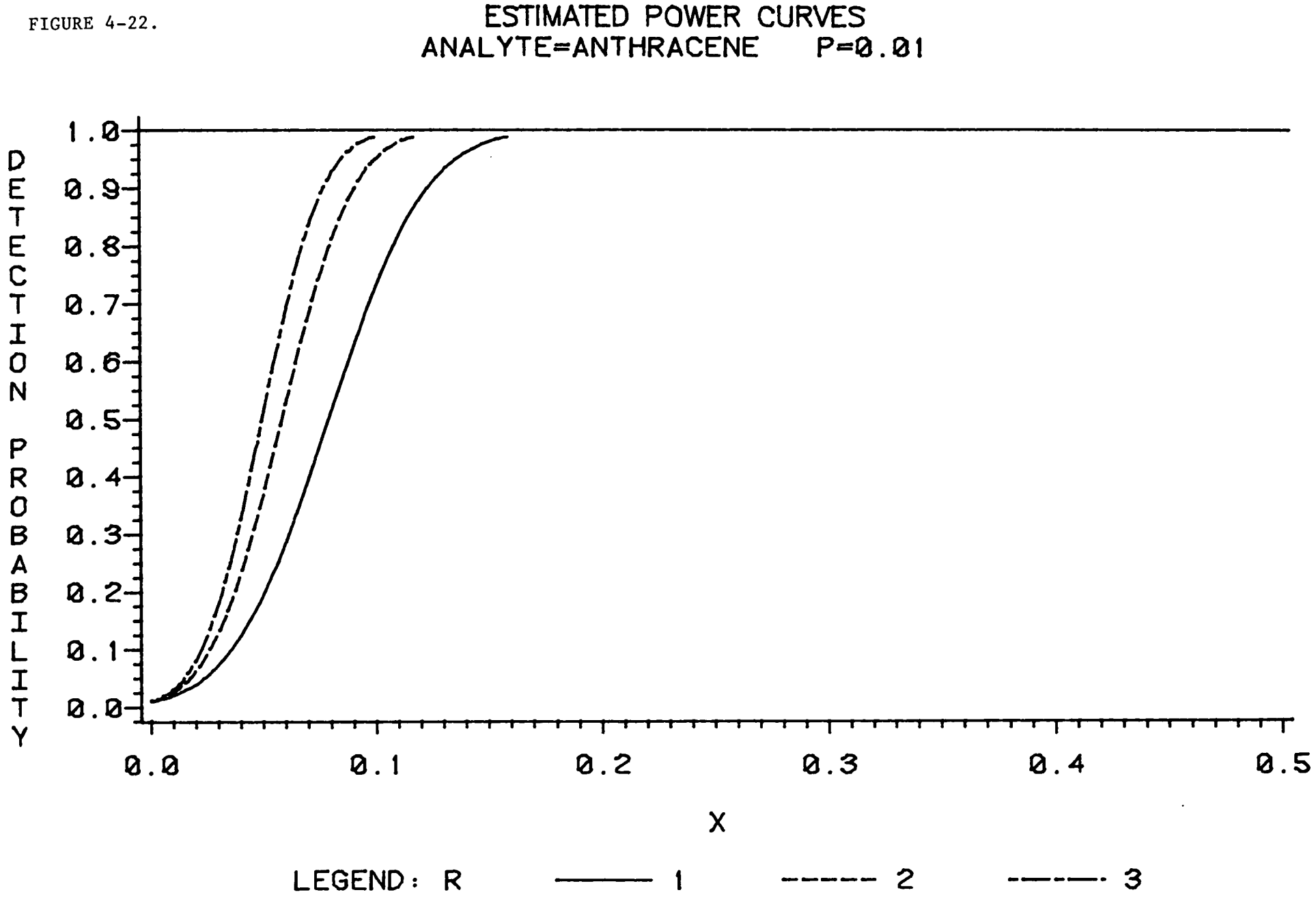

ESTIMATED POWER CURVES
ANALYTE=ANTHRACENE P=0.01
DETECTION PROBABILITY
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.0
0.1
0.2
0.3
0.4
0.5
X
LEGEND: R
1
2
3

FIGURE 4-23.

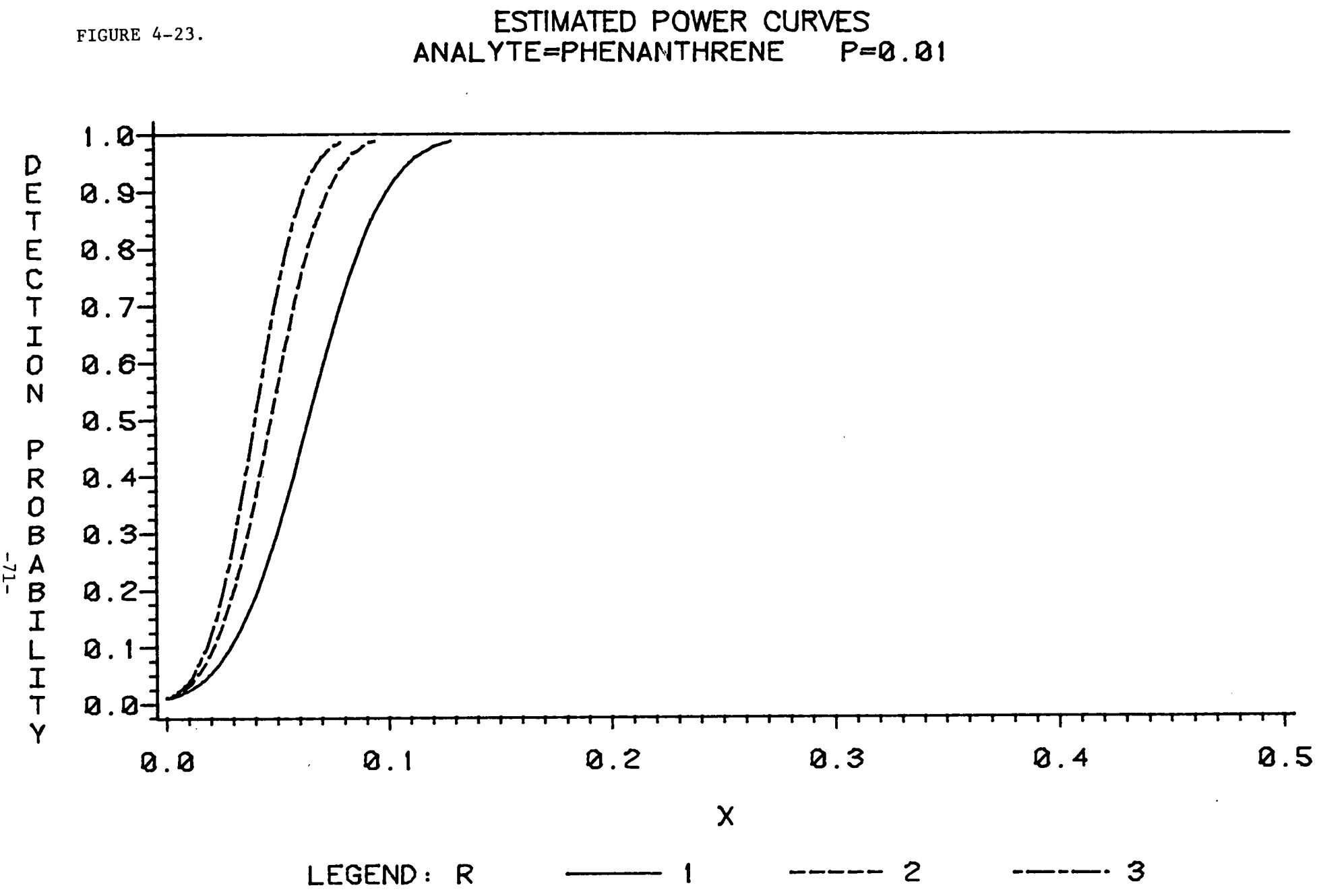
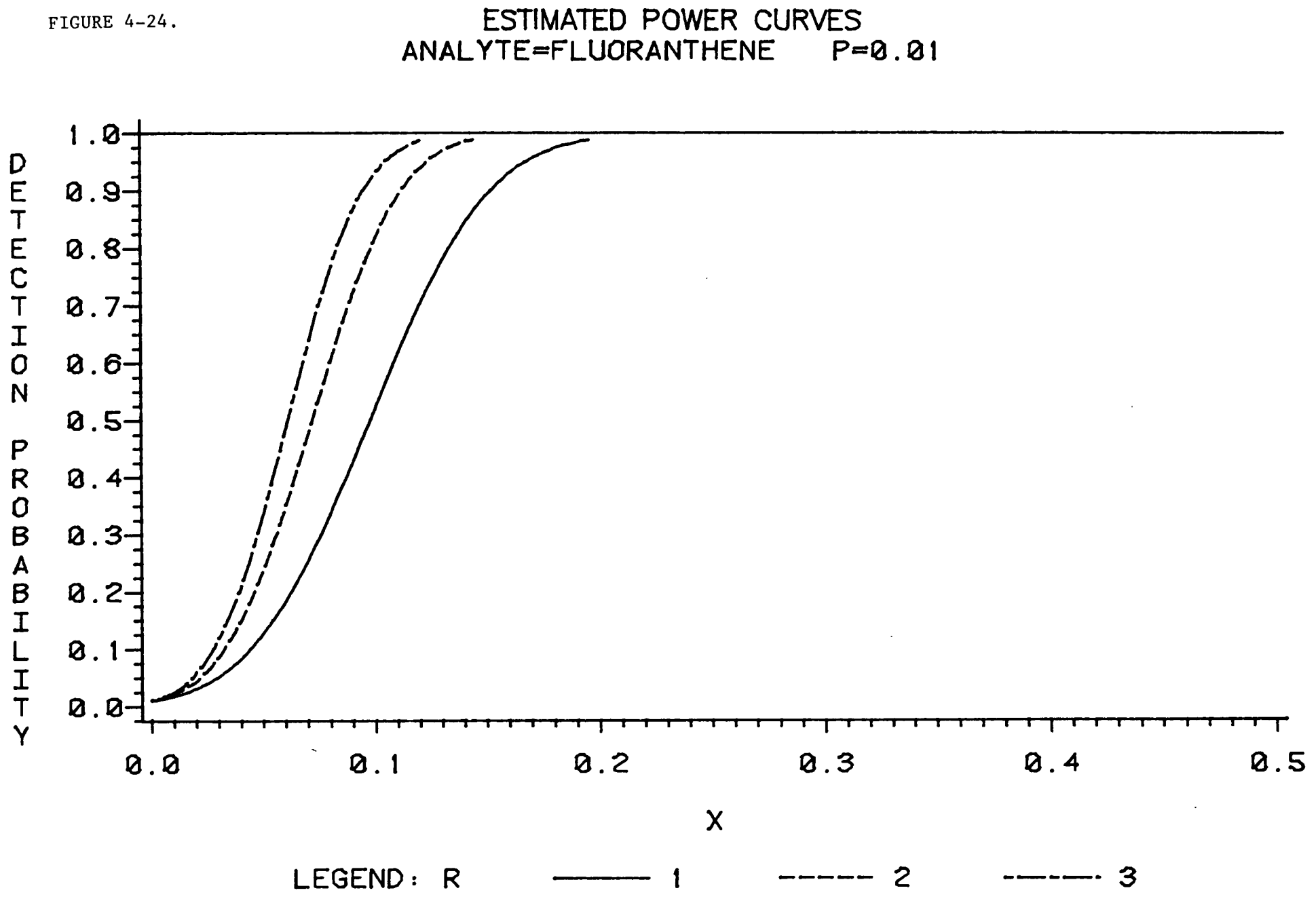

ESTIMATED POWER CURVES
ANALYTE=PHENANTHRENE P=0.01
DETECTION PROBABILITY
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.0
0.1
0.2
0.3
0.4
0.5
X
LEGEND: R
1
2
3

FIGURE 4-24.


ESTIMATED POWER CURVES
ANALYTE=FLUORANTHENE P=0.01
DETECTION PROBABILITY
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.0
0.1
0.2
0.3
0.4
0.5
X
LEGEND: R
1
2
3

demonstrate the different detectabililty characteristics of the analytes. For example, for a fixed q, dimethylphthalate (Figure 4-20) will clearly have a larger detection limit (less sensitive detection) than any of the other analytes.

We note that while the horizontal scale used in Figures 4-19 through 4-24 is given in terms of x, a similar set of curves could just as readily have been produced in the original concentration units (ppm) by determining $\hat{x}_\ell$ values according to (4-5) and then using the relationship

$$\hat{X}^*_\ell = \hat{x}_\ell(\hat{x}_\ell + 2\sqrt{0.1}) \qquad (4\text{-}6)$$

$$= \hat{x}_\ell(\hat{x}_\ell + 0.632456)$$

Tables 4-13, 4-14, and 4-15 contain estimated detection limits for r = 1, 2, and 3, respectively; point estimates $\hat{x}(p,q)$ are given for two p values (p = 0.01 and 0.05) and two q values (q = 0.01 and q = 0.05). These tables also provide the corresponding interval estimates for $x_\ell(p,q)$, as determined via (3-15) with coverage probabilities $(1-\gamma)$ = 0.95 or 0.99. Again, all of the results are given in terms of the transformed scale associated with model (4-4), but could be translated to ppm through eq. (4-6). For example, for the first line of Table 4-13 (analyte = 2-chloronaphthalene, r=1, p=0.01, q=0.05), we obtain:

$\hat{X}^*_\ell$ = .194 ppm,

Interval for X^*_ℓ = (0.145, 0.288) with $(1-\gamma)$ = 0.95, and

Interval for X^*_ℓ = (0.134, 0.332) with $(1-\gamma)$ = 0.99.

4.6 Phase II Design

The first phase of the experiment -- calibration -- provides the mechanism for estimating detection limits with specified assurance probabilities. Phase II of the experiment was designed to demonstrate and validate the Phase I formulation. Phase I results were generated during December 1984, while Phase II was conducted during February 1985. Experimental methods and the source matrix for both phases were identical (see Section 4.1).

The design for Phase II involved fortifying aliquots of the University Lake sediment at three distinct concentration values (x^*_1, x^*_2, and x^*_3) for each analyte and conducting twelve independent "blind"

TABLE 4-13. DETECTION LIMIT ESTIMATES FOR SELECTED p AND q VALUES, FOR $\tau=1$

ANALYTE	p	q	DETECTION LIMIT ESTIMATES POINT ESTIMATE	95% CONF. LIMITS LOW	95% CONF. LIMITS HIGH	99% CONF. LIMITS LOW	99% CONF. LIMITS HIGH
2-CHLORONAPHTHALENE	0.01	0.05	0.22601	0.17907	0.30684	0.16721	0.34108
		0.01	0.26491	0.20990	0.35965	0.19599	0.39979
	0.05	0.05	0.18264	0.14472	0.24796	0.13513	0.27564
		0.01	0.22051	0.17472	0.29938	0.16314	0.33279
DIMETHYLPHTHALATE	0.01	0.05	0.40495	0.31616	0.56550	0.29427	0.63748
		0.01	0.47467	0.37060	0.66286	0.34493	0.74723
	0.05	0.05	0.32695	0.25526	0.45657	0.23758	0.51468
		0.01	0.39474	0.30819	0.55124	0.28685	0.62141
HEXACHLOROBENZENE	0.01	0.05	0.19230	0.15253	0.26053	0.14245	0.28929
		0.01	0.22539	0.17878	0.30537	0.16697	0.33909
	0.05	0.05	0.15540	0.12326	0.21054	0.11512	0.23379
		0.01	0.18762	0.14882	0.25420	0.13899	0.28226
ANTHRACENE	0.01	0.05	0.13536	0.10760	0.18261	0.10054	0.20232
		0.01	0.15866	0.12612	0.21404	0.11784	0.23714
	0.05	0.05	0.10939	0.08696	0.14757	0.08125	0.16350
		0.01	0.13207	0.10499	0.17817	0.09809	0.19740
PHENANTHRENE	0.01	0.05	0.11007	0.08755	0.14833	0.08181	0.16424
		0.01	0.12902	0.10262	0.17386	0.09589	0.19251
	0.05	0.05	0.08895	0.07075	0.11987	0.06611	0.13273
		0.01	0.10739	0.08542	0.14472	0.07982	0.16025
FLUORANTHENE	0.01	0.05	0.16689	0.13251	0.22568	0.12378	0.25034
		0.01	0.19562	0.15531	0.26452	0.14508	0.29343
	0.05	0.05	0.13487	0.10708	0.18238	0.10003	0.20231
		0.01	0.16283	0.12929	0.22019	0.12077	0.24426

TABLE 4-14. DETECTION LIMIT ESTIMATES FOR SELECTED p AND q VALUES, FOR r=2

ANALYTE	p	q	DETECTION LIMIT ESTIMATES POINT ESTIMATE	95% CONF. LIMITS LOW	95% CONF. LIMITS HIGH	99% CONF. LIMITS LOW	99% CONF. LIMITS HIGH
2-CHLORONAPHTHALENE	0.01	0.05	0.16670	0.13208	0.22631	0.12333	0.25157
		0.01	0.19539	0.15481	0.26527	0.14455	0.29487
	0.05	0.05	0.13471	0.10674	0.18289	0.09966	0.20330
		0.01	0.16264	0.12887	0.22081	0.12033	0.24546
DIMETHYLPHTHALATE	0.01	0.05	0.29985	0.23411	0.41873	0.21789	0.47203
		0.01	0.35147	0.27441	0.49082	0.25540	0.55329
	0.05	0.05	0.24209	0.18901	0.33807	0.17592	0.38110
		0.01	0.29229	0.22820	0.40817	0.21240	0.46013
HEXACHLOROBENZENE	0.01	0.05	0.14181	0.11248	0.19213	0.10505	0.21334
		0.01	0.16622	0.13184	0.22520	0.12313	0.25006
	0.05	0.05	0.11460	0.09090	0.15526	0.08489	0.17241
		0.01	0.13836	0.10975	0.18746	0.10250	0.20815
ANTHRACENE	0.01	0.05	0.09984	0.07937	0.13470	0.07416	0.14924
		0.01	0.11703	0.09303	0.15788	0.08692	0.17492
	0.05	0.05	0.08069	0.06414	0.10885	0.05993	0.12060
		0.01	0.09742	0.07744	0.13142	0.07236	0.14561
PHENANTHRENE	0.01	0.05	0.08118	0.06457	0.10939	0.06034	0.12113
		0.01	0.09515	0.07568	0.12822	0.07072	0.14198
	0.05	0.05	0.06560	0.05218	0.08840	0.04876	0.09789
		0.01	0.07920	0.06300	0.10673	0.05887	0.11819
FLUORANTHENE	0.01	0.05	0.12307	0.09772	0.16643	0.09128	0.18461
		0.01	0.14426	0.11454	0.19507	0.10699	0.21639
	0.05	0.05	0.09946	0.07897	0.13449	0.07376	0.14919
		0.01	0.12008	0.09534	0.16238	0.08906	0.18013

TABLE 4-15. DETECTION LIMIT ESTIMATES FOR SELECTED p AND q VALUES, FOR r=3

ANALYTE	p	q	DETECTION LIMIT ESTIMATES POINT ESTIMATE	95% CONF. LIMITS LOW	95% CONF. LIMITS HIGH	99% CONF. LIMITS LOW	99% CONF. LIMITS HIGH
2-CHLORONAPHTHALENE	0.01	0.05	0.14151	0.11212	0.19211	0.10469	0.21356
		0.01	0.16586	0.13142	0.22518	0.12271	0.25031
	0.05	0.05	0.11435	0.09061	0.15525	0.08460	0.17258
		0.01	0.13807	0.10940	0.18744	0.10215	0.20836
DIMETHYLPHTHALATE	0.01	0.05	0.25538	0.19939	0.35663	0.18558	0.40202
		0.01	0.29935	0.23371	0.41802	0.21752	0.47123
	0.05	0.05	0.20619	0.16098	0.28793	0.14983	0.32458
		0.01	0.24894	0.19436	0.34763	0.18090	0.39188
HEXACHLOROBENZENE	0.01	0.05	0.12036	0.09547	0.16307	0.08916	0.18107
		0.01	0.14108	0.11190	0.19114	0.10451	0.21224
	0.05	0.05	0.09727	0.07715	0.13178	0.07205	0.14633
		0.01	0.11744	0.09315	0.15911	0.08699	0.17667
ANTHRACENE	0.01	0.05	0.08476	0.06738	0.11435	0.06296	0.12669
		0.01	0.09935	0.07898	0.13403	0.07379	0.14850
	0.05	0.05	0.06850	0.05445	0.09241	0.05088	0.10238
		0.01	0.08270	0.06574	0.11157	0.06143	0.12361
PHENANTHRENE	0.01	0.05	0.06891	0.05481	0.09286	0.05122	0.10282
		0.01	0.08077	0.06424	0.10884	0.06003	0.12052
	0.05	0.05	0.05568	0.04429	0.07504	0.04139	0.08309
		0.01	0.06723	0.05348	0.09060	0.04997	0.10032
FLUORANTHENE	0.01	0.05	0.10446	0.08294	0.14125	0.07747	0.15669
		0.01	0.12244	0.09721	0.16557	0.09081	0.18366
	0.05	0.05	0.08442	0.06702	0.11415	0.06261	0.12663
		0.01	0.10192	0.08092	0.13782	0.07559	0.15288

replicate response determinations at each concentration. The lowest concentration value was chosen initially so that, on the basis of Phase I results and the theoretical formulation of detectability, approximately 10 percent of the observations at x_1^* would be expected to be detected (i.e., exceed the threshold $\hat{y}_p$) when p=0.01 and r=1. For 2-chloronaphthalene, for instance, Figure 4-19 indicates that choosing $x \approx .06$, or $x^* \approx .042$ ppm, will yield a detection rate approximately equal to 0.10. The highest concentration value, x_3^*, was selected initially in a similar manner to yield an approximate detection rate of 0.90 when p=0.05 and r=1. The middle concentration value was chosen to be the mean of the highest and lowest concentrations.

We then examined the initially-specified concentrations and adjusted them slightly so that a single stock solution of all analytes could be diluted serially. This resulted in choosing a Phase II design that consisted of three equally-spaced concentration levels (apart from rounding); for each analyte, these are given in Table 4-16 in the column labeled X* (ppm).

Table 4-16 also shows, by analyte, the following quantities: w_o values, selected p values (p=0.01 and p=0.05), associated t_p values, and the corresponding threshold response values $\hat{y}_p$ (denoted Yp). For each analyte and for each Phase II concentration level, this table also provides:

X, the transformed concentration level;

DELTA, the estimated noncentrality parameter $\hat{\Delta}$ associated with X (computed via eq. (3-7) with β/σ replaced with its estimate from eq. (3-11));

the estimated detection rate, $1-\hat{q}(x)$, corresponding to X (computed via eq. (3-16)); and

interval estimates for $1-q(x)$, as determined by eq. (3-17) with $(1-\gamma) = 0.95$ and 0.99.

All of the estimated quantities shown in this table depend solely on the Phase I results. Tables 4-17 and 4-18 provide comparable results for r=2 and r=3, respectively.

The purposes of Phase II were (1) to generate empirical estimates of the detection rates at each of the selected concentration levels, and

TABLE 4-16. ESTIMATED PHASE I DETECTION RATES ASSOCIATED WITH EACH PHASE II CONCENTRATION LEVEL, FOR r=1

ANALYTE	Wo	p	Tp	Yp	X*	X	DELTA	ESTIMATE OF 1-q	CONFIDENCE INTERVALS FOR 1-q 95 % LIMITS		99 % LIMITS	
2-CHLORONAPHTHALENE	1.04715	0.01000	2.46202	0.43701	0.043	0.06193	1.11272	0.10299	0.06385	0.17086	0.05437	0.19676
					0.086	0.11505	2.06727	0.36126	0.20187	0.58992	0.16242	0.65846
					0.129	0.16231	2.91651	0.67482	0.41172	0.89866	0.33213	0.93685
		0.05000	1.69913	0.39477	0.043	0.06193	1.11272	0.28840	0.20530	0.40651	0.18264	0.44566
					0.086	0.11505	2.06727	0.64573	0.45307	0.83420	0.39314	0.87624
					0.129	0.16231	2.91651	0.88542	0.69465	0.97955	0.61521	0.98945
DIMETHYLPHTHALATE	1.05208	0.01000	2.46714	0.41567	0.087	0.11621	1.16638	0.11211	0.06571	0.19374	0.05471	0.22498
					0.173	0.20627	2.07031	0.36107	0.19070	0.60524	0.14940	0.67709
					0.260	0.28377	2.84824	0.64933	0.37038	0.89232	0.28914	0.93366
		0.05000	1.70113	0.37334	0.087	0.11621	1.16638	0.30620	0.20982	0.44174	0.18363	0.48600
					0.173	0.20627	2.07031	0.64618	0.43724	0.84467	0.37228	0.88716
					0.260	0.28377	2.84824	0.87150	0.65558	0.97789	0.56733	0.98881
HEXACHLOROBENZENE	1.04694	0.01000	2.46202	0.26241	0.036	0.05255	1.10987	0.10251	0.06380	0.16960	0.05442	0.19520
					0.072	0.09850	2.08021	0.36589	0.20539	0.59505	0.16554	0.66342
					0.108	0.13984	2.95328	0.68731	0.42378	0.90581	0.34304	0.94207
		0.05000	1.69913	0.23650	0.036	0.05255	1.10987	0.28745	0.20519	0.40453	0.18275	0.44338
					0.072	0.09850	2.08021	0.65042	0.45812	0.83755	0.39812	0.87906
					0.108	0.13984	2.95328	0.89222	0.70565	0.98156	0.62685	0.99063
ANTHRACENE	1.04725	0.01000	2.46202	0.32425	0.024	0.03591	1.07734	0.09707	0.06131	0.15894	0.05261	0.18257
					0.047	0.06718	2.01550	0.34296	0.19405	0.56173	0.15733	0.62909
					0.071	0.09729	2.91904	0.67568	0.41763	0.89712	0.33940	0.93538
		0.05000	1.69913	0.28973	0.024	0.03591	1.07734	0.27672	0.19936	0.38753	0.17828	0.42454
					0.047	0.06718	2.01550	0.62676	0.44169	0.81516	0.38491	0.85894
					0.071	0.09729	2.91904	0.88590	0.70007	0.97911	0.62299	0.98911
PHENANTHRENE	1.04705	0.01000	2.46202	0.34336	0.019	0.02874	1.06021	0.09430	0.05993	0.15370	0.05154	0.17640
					0.038	0.05526	2.03866	0.35110	0.19917	0.57235	0.16157	0.63984
					0.058	0.08126	2.99824	0.70229	0.44112	0.91315	0.35983	0.94721
		0.05000	1.69913	0.31357	0.019	0.02874	1.06021	0.27115	0.19607	0.37899	0.17562	0.41511
					0.038	0.05526	2.03866	0.63529	0.44916	0.82245	0.39176	0.86540
					0.058	0.08126	2.99824	0.90013	0.72104	0.98355	0.64427	0.99176
FLUORANTHENE	1.04694	0.01000	2.46202	0.38662	0.030	0.04433	1.07864	0.09729	0.06121	0.15972	0.05243	0.18357
					0.060	0.08377	2.03848	0.35104	0.19770	0.57403	0.15982	0.64192
					0.090	0.11966	2.91181	0.67321	0.41366	0.89631	0.33520	0.93488
		0.05000	1.69913	0.34295	0.030	0.04433	1.07864	0.27715	0.19910	0.38879	0.17784	0.42606
					0.060	0.08377	2.03848	0.63522	0.44703	0.82360	0.38895	0.86663
					0.090	0.11966	2.91181	0.88453	0.69643	0.97887	0.61850	0.98899

TABLE 4-17. ESTIMATED PHASE I DETECTION RATES ASSOCIATED WITH EACH PHASE II CONCENTRATION LEVEL, FOR r=2

ANALYTE	Wo	p	Tp	Yp	X*	X	DELTA	ESTIMATE OF 1-q	CONFIDENCE INTERVALS FOR 1-q 95 % LIMITS		99 % LIMITS	
2-CHLORONAPHTHALENE	0.77235	0.01000	2.46202	0.40123	0.043	0.06193	1.50862	0.18746	0.10791	0.32207	0.08900	0.37079
					0.086	0.11505	2.80281	0.63498	0.38018	0.87159	0.30583	0.91604
					0.129	0.16231	3.95421	0.92504	0.69980	0.99463	0.59412	0.99811
		0.05000	1.69913	0.37008	0.043	0.06193	1.50862	0.43191	0.29791	0.60423	0.26034	0.65533
					0.086	0.11505	2.80281	0.86250	0.66463	0.97134	0.58605	0.98431
					0.129	0.16231	3.95421	0.98661	0.89883	0.99960	0.83694	0.99990
DIMETHYLPHTHALATE	0.77902	0.01000	2.46714	0.38029	0.087	0.11621	1.57521	0.20429	0.11084	0.36228	0.08904	0.41852
					0.173	0.20627	2.79598	0.63077	0.35666	0.87975	0.27815	0.92415
					0.260	0.28377	3.84658	0.90840	0.64331	0.99351	0.52538	0.99775
		0.05000	1.70113	0.34895	0.087	0.11621	1.57521	0.45707	0.30382	0.64741	0.26070	0.70154
					0.173	0.20627	2.79598	0.86050	0.64167	0.97411	0.55421	0.98651
					0.260	0.28377	3.84658	0.98242	0.86797	0.99950	0.78965	0.99987
HEXACHLOROBENZENE	0.77206	0.01000	2.46202	0.24046	0.036	0.05255	1.50502	0.18654	0.10785	0.31976	0.08912	0.36803
					0.072	0.09850	2.82083	0.64141	0.38674	0.87542	0.31197	0.91893
					0.108	0.13984	4.00475	0.93161	0.71436	0.99544	0.60989	0.99844
		0.05000	1.69913	0.22135	0.036	0.05255	1.50502	0.43053	0.29778	0.60169	0.26059	0.65257
					0.072	0.09850	2.82083	0.86633	0.67103	0.97256	0.59300	0.98506
					0.108	0.13984	4.00475	0.98821	0.90630	0.99968	0.84706	0.99992
ANTHRACENE	0.77249	0.01000	2.46202	0.29502	0.024	0.03591	1.46054	0.17538	0.10281	0.29903	0.08551	0.34424
					0.047	0.06718	2.73238	0.60952	0.36570	0.84993	0.29590	0.89829
					0.071	0.09729	3.95730	0.92546	0.70682	0.99444	0.60447	0.99801
		0.05000	1.69913	0.26955	0.024	0.03591	1.46054	0.41353	0.28805	0.57826	0.25307	0.62811
					0.047	0.06718	2.73238	0.84682	0.65023	0.96411	0.57463	0.97944
					0.071	0.09729	3.95730	0.98672	0.90247	0.99959	0.84362	0.99989
PHENANTHRENE	0.77221	0.01000	2.46202	0.31813	0.019	0.02874	1.43755	0.16978	0.10006	0.28893	0.08342	0.33269
					0.038	0.05526	2.76424	0.62110	0.37528	0.85843	0.30423	0.90510
					0.058	0.08126	4.06535	0.93888	0.73423	0.99619	0.63312	0.99873
		0.05000	1.69913	0.29616	0.019	0.02874	1.43755	0.40482	0.28265	0.56648	0.24869	0.61580
					0.038	0.05526	2.76424	0.85405	0.65980	0.96701	0.58423	0.98136
					0.058	0.08126	4.06535	0.98991	0.91611	0.99975	0.86138	0.99994
FLUORANTHENE	0.77206	0.01000	2.46202	0.34962	0.030	0.04433	1.46267	0.17590	0.10264	0.30071	0.08519	0.34632
					0.060	0.08377	2.76424	0.62110	0.37265	0.85979	0.30090	0.90642
					0.090	0.11966	3.94850	0.92428	0.70231	0.99436	0.59870	0.99798
		0.05000	1.69913	0.31741	0.030	0.04433	1.46267	0.41435	0.28772	0.58019	0.25241	0.63029
					0.060	0.08377	2.76424	0.85405	0.65719	0.96747	0.58041	0.98173
					0.090	0.11966	3.94850	0.98642	0.90014	0.99958	0.83991	0.99989

TABLE 4-18. ESTIMATED PHASE I DETECTION RATES ASSOCIATED WITH EACH PHASE II CONCENTRATION LEVEL, FOR r=3

ANALYTE	Wo	p	Tp	Yp	X*	X	DELTA	ESTIMATE OF 1-q	CONFIDENCE INTERVALS FOR 1-q 95 % LIMITS		99 % LIMITS	
2-CHLORONAPHTHALENE	0.65563	0.01000	2.46202	0.38604	0.043	0.06193	1.77719	0.26389	0.14823	0.44809	0.12046	0.51002
					0.086	0.11505	3.30177	0.79380	0.52164	0.95979	0.42685	0.97916
					0.129	0.16231	4.65813	0.98250	0.84852	0.99968	0.75609	0.99993
		0.05000	1.69913	0.35959	0.043	0.06193	1.77719	0.53612	0.36991	0.72708	0.32137	0.77743
					0.086	0.11505	3.30177	0.94276	0.78624	0.99432	0.70841	0.99765
					0.129	0.16231	4.65813	0.99814	0.96362	0.99999	0.92637	1.00000
DIMETHYLPHTHALATE	0.66349	0.01000	2.46714	0.36531	0.087	0.11621	1.84952	0.28586	0.15152	0.49634	0.11976	0.56473
					0.173	0.20627	3.28287	0.78703	0.48930	0.96258	0.38740	0.98152
					0.260	0.28377	4.51643	0.97535	0.79824	0.99955	0.68404	0.99991
		0.05000	1.70113	0.33862	0.087	0.11621	1.84952	0.56345	0.37581	0.76745	0.32045	0.81783
					0.173	0.20627	3.28287	0.94028	0.76188	0.99491	0.67232	0.99803
					0.260	0.28377	4.51643	0.99710	0.94491	0.99998	0.89094	1.00000
HEXACHLOROBENZENE	0.65529	0.01000	2.46202	0.23113	0.036	0.05255	1.77320	0.26265	0.14818	0.44522	0.12067	0.50672
					0.072	0.09850	3.32348	0.79964	0.52984	0.96169	0.43504	0.98029
					0.108	0.13984	4.71835	0.98483	0.86003	0.99975	0.77107	0.99995
		0.05000	1.69913	0.21491	0.036	0.05255	1.77320	0.53457	0.36982	0.72460	0.32175	0.77489
					0.072	0.09850	3.32348	0.94515	0.79233	0.99468	0.71570	0.99782
					0.108	0.13984	4.71835	0.99846	0.96755	0.99999	0.93308	1.00000
ANTHRACENE	0.65580	0.01000	2.46202	0.28260	0.024	0.03591	1.72042	0.24652	0.14071	0.41781	0.11532	0.47655
					0.047	0.06718	3.21857	0.77052	0.50362	0.94876	0.41366	0.97196
					0.071	0.09729	4.66144	0.98264	0.85400	0.99966	0.76581	0.99993
		0.05000	1.69913	0.26099	0.024	0.03591	1.72042	0.51406	0.35720	0.70024	0.31188	0.75096
					0.047	0.06718	3.21857	0.93283	0.77250	0.99209	0.69644	0.99651
					0.071	0.09729	4.66144	0.99816	0.96551	0.99999	0.93075	1.00000
PHENANTHRENE	0.65547	0.01000	2.46202	0.30741	0.019	0.02874	1.69358	0.23853	0.13671	0.40450	0.11230	0.46188
					0.038	0.05526	3.25655	0.78133	0.51565	0.95325	0.42479	0.97484
					0.058	0.08126	4.78939	0.98723	0.87498	0.99981	0.79219	0.99996
		0.05000	1.69913	0.28876	0.019	0.02874	1.69358	0.50361	0.35031	0.68794	0.30623	0.73881
					0.038	0.05526	3.25655	0.93752	0.78172	0.99302	0.70657	0.99698
					0.058	0.08126	4.78939	0.99877	0.97242	0.99999	0.94210	1.00000
FLUORANTHENE	0.65529	0.01000	2.46202	0.33390	0.030	0.04433	1.72331	0.24739	0.14053	0.42021	0.11490	0.47939
					0.060	0.08377	3.25680	0.78140	0.51247	0.95398	0.42046	0.97540
					0.090	0.11966	4.65209	0.98225	0.85062	0.99966	0.76058	0.99993
		0.05000	1.69913	0.30656	0.030	0.04433	1.72331	0.51518	0.35688	0.70242	0.31111	0.75327
					0.060	0.08377	3.25680	0.93755	0.77930	0.99317	0.70265	0.99707
					0.090	0.11966	4.65209	0.99811	0.96435	0.99999	0.92840	1.00000

(2) to compare these with the estimated detection rates derived from Phase I that appear in Tables 4-16 through 4-18.

4.7 Phase II Data

Results from this validation phase of the experiment are given in Tables 4-19 through 4-24. Each table contains the results for a particular analyte. The notation is identical to that used previously for the Phase I data.

Some problems with dimethylphthalate were encountered again (see Table 4-20). The GC peak areas for this analyte, which had been small even in Phase I, tended to be indistinct for virtually all of the Phase II runs. For four of the runs, no peaks were discernable at all. Figure 4-25 shows a pair of chromatograms illustrating the problem with dimethylphthalate. The first chromatogram (Figure 4-25a) shows a typical Phase II run for which the dimethylphthalate fortification level was 0.173 ppm. The second chromatogram (Figure 4-25b) illustrates a typical Phase I run with a 0.217 ppm fortification level for this analyte. The lower peak response for dimethylphthalate in Phase II resulted from a deterioration of chromatographic performance for this analyte following repeated injections of sediment extract. This produced wider and correspondingly shorter peaks which were more difficult to distinguish from matrix background at the low concentration levels used in the study.

4.8 Phase II Detectability

Each of the individual Phase II response values Y was compared to the predetermined threshold response values (i.e., the $\hat{y}_p$ values corresponding to r=1 in Table 4-12). An observation was considered to yield a "detection" if $Y > \hat{y}_p$. The count of detections occurring for each analyte and concentration level was then divided by the number of observations (equal to 12 in most cases) to yield an empirical estimate of the detection rate associated with that x* and p value (p=0.01 or 0.05). The results are summarized in Table 4-25.

This table shows the false-positive rate p and the associated $\hat{y}_p$ value for each analyte. In addition, for each concentration level (X*), it provides:

TABLE 4-19 . LISTING OF VALIDATION DATA FOR 2-CHLORONAPHTHALENE

SAMPLE TYPE	X*	X	SET NO.	REP	RUN NO.	GC AREAS FOR: ANALYTE	INT.STD.	Y*	Y
GC_CHK	0.086	0.1150	1	1	0	3979	19568	0.2033	0.4509
			1	1	0	3455	19265	0.1793	0.4235
			1	1	0	2652	14495	0.1830	0.4277
			2	1	0	2987	15244	0.1959	0.4427
			3	1	0	2941	15830	0.1858	0.4310
			3	1	0	3498	18498	0.1891	0.4349
			4	1	0	2606	14507	0.1796	0.4238
CONTRL	0.086	0.1150	1	1	9	3016	22948	0.1314	0.3625
			2	1	2	2248	18803	0.1196	0.3458
			3	1	5	2100	16186	0.1297	0.3602
			4	1	4	2483	17394	0.1428	0.3778
UL SED	0.043	0.0619	1	1	7	2749	25053	0.1097	0.3313
			1	2	10	2955	25022	0.1181	0.3437
			1	3	3	1830	25475	0.0718	0.2680
			2	1	5	2202	23748	0.0927	0.3045
			2	2	8	3172	22759	0.1394	0.3733
			2	3	4	2242	24107	0.0930	0.3050
			3	1	3	2542	21019	0.1209	0.3478
			3	2	8	2630	25130	0.1047	0.3235
			3	3	4	2188	22372	0.0978	0.3127
			4	1	2	2358	23894	0.0987	0.3141
			4	2	3	2756	23440	0.1176	0.3429
			4	3	1	2794	17986	0.1553	0.3941
UL SED	0.086	0.1150	1	1	8	3417	24472	0.1396	0.3737
			1	2	6	3563	25188	0.1415	0.3761
			1	3	4	2972	24126	0.1232	0.3510
			2	1	9	3578	22026	0.1624	0.4030
			2	2	10	4602	24494	0.1879	0.4335
			2	3	1	4491	25142	0.1786	0.4226
			3	1	1	3858	24269	0.1590	0.3987
			3	2	10	2234	19750	0.1131	0.3363
			3	3	6	3006	23035	0.1305	0.3612
			4	1	6	3284	19824	0.1657	0.4070
			4	2	9	2279	16828	0.1354	0.3680
			4	3	5	3686	20592	0.1790	0.4231
UL SED	0.129	0.1623	1	1	5	4970	25046	0.1984	0.4455
			1	2	2	5292	26892	0.1968	0.4436
			1	3	1	4944	25277	0.1956	0.4423
			2	1	7	4758	22335	0.2130	0.4616
			2	2	6	6524	24962	0.2614	0.5112
			2	3	3	4941	26404	0.1871	0.4326
			3	1	2	4202	19308	0.2176	0.4665
			3	2	9	3742	21292	0.1757	0.4192
			3	3	7	4396	21132	0.2080	0.4561
			4	1	7	3407	21958	0.1552	0.3939
			4	2	8	2485	18091	0.1374	0.3706
			4	3	10	1820	13498	0.1348	0.3672

TABLE 4-20 . LISTING OF VALIDATION DATA FOR DIMETHYLPHTHALATE

SAMPLE TYPE	X*	X	SET NO.	REP	RUN NO.	GC AREAS FOR: ANALYTE	INT.STD.	Y*	Y
GC_CHK	0.173	0.2063	1	1	0	920	19568	0.0470	0.2168
			1	1	0	1320	19265	0.0685	0.2618
			1	1	0	930	14495	0.0642	0.2533
			2	1	0	776	15244	0.0509	0.2256
			3	1	0	825	15830	0.0521	0.2283
			3	1	0	832	18498	0.0450	0.2121
			4	1	0	1226	14507	0.0845	0.2907
CONTRL	0.173	0.2063	1	1	9	951	22948	0.0414	0.2036
			2	1	2	1098	18803	0.0584	0.2417
			3	1	5	1005	16186	0.0621	0.2492
			4	1	4	1272	17394	0.0731	0.2704
UL SED	0.087	0.1162	1	1	7	884	25053	0.0353	0.1878
			1	2	10	798	25022	0.0319	0.1786
			1	3	3	790	25475	0.0310	0.1761
			2	1	5	528	23748	0.0222	0.1491
			2	2	8	850	22759	0.0373	0.1933
			2	3	4	.	24107	.	.
			3	1	3	776	21019	0.0369	0.1921
			3	2	8	1174	25130	0.0467	0.2161
			3	3	4	448	22372	0.0200	0.1415
			4	1	2	792	23894	0.0331	0.1821
			4	2	3	.	23440	.	.
			4	3	1	.	17986	.	.
UL SED	0.173	0.2063	1	1	8	513	24472	0.0210	0.1448
			1	2	6	940	25188	0.0373	0.1932
			1	3	4	664	24126	0.0275	0.1659
			2	1	9	1334	22026	0.0606	0.2461
			2	2	10	2288	24494	0.0934	0.3056
			2	3	1	1114	25142	0.0443	0.2105
			3	1	1	516	24269	0.0213	0.1458
			3	2	10	1056	19750	0.0535	0.2312
			3	3	6	1588	23035	0.0689	0.2626
			4	1	6	588	19824	0.0297	0.1722
			4	2	9	1022	16828	0.0607	0.2464
			4	3	5	1662	20592	0.0807	0.2841
UL SED	0.260	0.2838	1	1	5	1283	25046	0.0512	0.2263
			1	2	2	1469	26892	0.0546	0.2337
			1	3	1	1183	25277	0.0468	0.2163
			2	1	7	1229	22335	0.0550	0.2346
			2	2	6	1632	24962	0.0654	0.2557
			2	3	3	1252	26404	0.0474	0.2178
			3	1	2	1612	19308	0.0835	0.2889
			3	2	9	1088	21292	0.0511	0.2261
			3	3	7	1617	21132	0.0765	0.2766
			4	1	7	.	21958	.	.
			4	2	8	1286	18091	0.0711	0.2666
			4	3	10	1693	13498	0.1254	0.3542

TABLE 4-21 . LISTING OF VALIDATION DATA FOR HEXACHLOROBENZENE

SAMPLE TYPE	X*	X	SET NO.	REF	RUN NO.	GC AREAS FOR: ANALYTE	INT.STD.	Y*	Y
GC_CHK	0.072	0.0985	1	1	0	787	19568	0.0402	0.2005
			1	1	0	929	19265	0.0482	0.2196
			1	1	0	642	14495	0.0443	0.2105
			2	1	0	767	15244	0.0503	0.2243
			3	1	0	803	15830	0.0507	0.2252
			3	1	0	978	18498	0.0529	0.2299
			4	1	0	786	14507	0.0542	0.2328
CONTRL	0.072	0.0985	1	1	9	1413	22948	0.0616	0.2481
			2	1	2	922	18803	0.0490	0.2214
			3	1	5	754	16186	0.0466	0.2158
			4	1	4	870	17394	0.0500	0.2236
UL SED	0.036	0.0526	1	1	7	1859	25053	0.0742	0.2724
			1	2	10	1730	25022	0.0691	0.2629
			1	3	3	1002	25475	0.0393	0.1983
			2	1	5	770	23748	0.0324	0.1801
			2	2	8	1322	22759	0.0581	0.2410
			2	3	4	2246	24107	0.0932	0.3052
			3	1	3	1164	21019	0.0554	0.2353
			3	2	8	1078	25130	0.0429	0.2071
			3	3	4	857	22372	0.0383	0.1957
			4	1	2	1005	23894	0.0421	0.2051
			4	2	3	826	23440	0.0352	0.1877
			4	3	1	1035	17986	0.0575	0.2399
UL SED	0.072	0.0985	1	1	8	1395	24472	0.0570	0.2388
			1	2	6	1438	25188	0.0571	0.2389
			1	3	4	1638	24126	0.0679	0.2606
			2	1	9	1695	22026	0.0770	0.2774
			2	2	10	2613	24494	0.1067	0.3266
			2	3	1	1870	25142	0.0744	0.2727
			3	1	1	1214	24269	0.0500	0.2237
			3	2	10	878	19750	0.0445	0.2108
			3	3	6	1536	23035	0.0667	0.2582
			4	1	6	1432	19824	0.0722	0.2688
			4	2	9	887	16828	0.0527	0.2296
			4	3	5	1524	20592	0.0740	0.2720
UL SED	0.108	0.1398	1	1	5	1914	25046	0.0764	0.2764
			1	2	2	1899	26892	0.0706	0.2657
			1	3	1	1608	25277	0.0636	0.2522
			2	1	7	1555	22335	0.0696	0.2639
			2	2	6	2070	24962	0.0829	0.2880
			2	3	3	1537	26404	0.0582	0.2413
			3	1	2	1969	19308	0.1020	0.3193
			3	2	9	1661	21292	0.0780	0.2793
			3	3	7	1754	21132	0.0830	0.2881
			4	1	7	1732	21958	0.0789	0.2809
			4	2	8	1032	18091	0.0570	0.2388
			4	3	10	659	13498	0.0488	0.2210

TABLE 4-22 . LISTING OF VALIDATION DATA FOR ANTHRACENE

SAMPLE TYPE	X*	X	SET NO.	REP	RUN NO.	GC AREAS FOR: ANALYTE	INT.STD.	Y*	Y
GC_CHK	0.047	0.0672	1	1	0	1577	19568	0.0806	0.2839
			1	1	0	1629	19265	0.0846	0.2908
			1	1	0	1105	14495	0.0762	0.2761
			2	1	0	1326	15244	0.0870	0.2949
			3	1	0	1397	15830	0.0883	0.2971
			3	1	0	1738	18498	0.0940	0.3065
			4	1	0	1392	14507	0.0960	0.3098
CONTRL	0.047	0.0672	1	1	9	2677	22948	0.1167	0.3415
			2	1	2	1650	18803	0.0878	0.2962
			3	1	5	1473	16186	0.0910	0.3017
			4	1	4	1567	17394	0.0901	0.3001
UL SED	0.024	0.0359	1	1	7	2482	25053	0.0991	0.3148
			1	2	10	2345	25022	0.0937	0.3061
			1	3	3	1724	25475	0.0677	0.2601
			2	1	5	1369	23748	0.0576	0.2401
			2	2	8	2101	22759	0.0923	0.3038
			2	3	4	2100	24107	0.0871	0.2951
			3	1	3	1797	21019	0.0855	0.2924
			3	2	8	2255	25130	0.0897	0.2996
			3	3	4	1351	22372	0.0604	0.2457
			4	1	2	2035	23894	0.0852	0.2918
			4	2	3	1548	23440	0.0660	0.2570
			4	3	1	1791	17986	0.0996	0.3156
UL SED	0.047	0.0672	1	1	8	2468	24472	0.1008	0.3176
			1	2	6	2376	25188	0.0943	0.3071
			1	3	4	2286	24126	0.0948	0.3078
			2	1	9	2400	22026	0.1090	0.3301
			2	2	10	3927	24494	0.1603	0.4004
			2	3	1	2540	25142	0.1010	0.3178
			3	1	1	2469	24269	0.1017	0.3190
			3	2	10	1832	19750	0.0928	0.3046
			3	3	6	2127	23035	0.0923	0.3039
			4	1	6	2092	19824	0.1055	0.3249
			4	2	9	1538	16828	0.0914	0.3023
			4	3	5	2047	20592	0.0994	0.3153
UL SED	0.071	0.0973	1	1	5	3399	25046	0.1357	0.3684
			1	2	2	4213	26892	0.1567	0.3958
			1	3	1	3584	25277	0.1418	0.3765
			2	1	7	3033	22335	0.1358	0.3685
			2	2	6	4110	24962	0.1647	0.4058
			2	3	3	2960	26404	0.1121	0.3348
			3	1	2	2751	19308	0.1425	0.3775
			3	2	9	2766	21292	0.1299	0.3604
			3	3	7	2793	21132	0.1322	0.3636
			4	1	7	3446	21958	0.1569	0.3962
			4	2	8	2189	18091	0.1210	0.3478
			4	3	10	1465	13498	0.1085	0.3294

TABLE 4-23 . LISTING OF VALIDATION DATA FOR PHENANTHRENE

SAMPLE TYPE	X*	X	SET NO.	REP	RUN NO.	GC AREAS FOR: ANALYTE	INT.STD.	Y*	Y
GC_CHK	0.038	0.0553	1	1	0	1829	19568	0.0935	0.3057
			1	1	0	1887	19265	0.0979	0.3130
			1	1	0	1365	14495	0.0942	0.3069
			2	1	0	1558	15244	0.1022	0.3197
			3	1	0	1677	15830	0.1059	0.3255
			3	1	0	2004	18498	0.1083	0.3291
			4	1	0	1561	14507	0.1076	0.3280
CONTRL	0.038	0.0553	1	1	9	2682	22948	0.1169	0.3419
			2	1	2	1801	18803	0.0958	0.3095
			3	1	5	1526	16186	0.0943	0.3070
			4	1	4	1806	17394	0.1038	0.3222
UL SED	0.019	0.0287	1	1	7	954	25053	0.0381	0.1951
			1	2	10	1070	25022	0.0428	0.2068
			1	3	3	1014	25475	0.0398	0.1995
			2	1	5	944	23748	0.0398	0.1994
			2	2	8	1237	22759	0.0544	0.2331
			2	3	4	2204	24107	0.0914	0.3024
			3	1	3	1494	21019	0.0711	0.2666
			3	2	8	2702	25130	0.1075	0.3279
			3	3	4	1282	22372	0.0573	0.2394
			4	1	2	2780	23894	0.1163	0.3411
			4	2	3	2618	23440	0.1117	0.3342
			4	3	1	2397	17986	0.1333	0.3651
UL SED	0.038	0.0553	1	1	8	1630	24472	0.0666	0.2581
			1	2	6	1721	25188	0.0683	0.2614
			1	3	4	1694	24126	0.0702	0.2650
			2	1	9	1796	22026	0.0815	0.2856
			2	2	10	2035	24494	0.0831	0.2882
			2	3	1	1898	25142	0.0755	0.2748
			3	1	1	1872	24269	0.0771	0.2777
			3	2	10	1660	19750	0.0841	0.2899
			3	3	6	1484	23035	0.0644	0.2538
			4	1	6	2740	19824	0.1382	0.3718
			4	2	9	2184	16828	0.1298	0.3603
			4	3	5	3052	20592	0.1482	0.3850
UL SED	0.058	0.0813	1	1	5	2424	25046	0.0968	0.3111
			1	2	2	3257	26892	0.1211	0.3480
			1	3	1	3036	25277	0.1201	0.3466
			2	1	7	2367	22335	0.1060	0.3255
			2	2	6	3486	24962	0.1397	0.3737
			2	3	3	2682	26404	0.1016	0.3187
			3	1	2	2114	19308	0.1095	0.3309
			3	2	9	2703	21292	0.1269	0.3563
			3	3	7	3798	21132	0.1797	0.4239
			4	1	7	3755	21958	0.1710	0.4135
			4	2	8	2931	18091	0.1620	0.4025
			4	3	10	1846	13498	0.1368	0.3698

TABLE 4-24 . LISTING OF VALIDATION DATA FOR FLUORANTHENE

SAMPLE TYPE	X*	X	SET NO.	REP	RUN NO.	GC AREAS FOR: ANALYTE	INT.STD.	Y*	Y
GC_CHK	0.060	0.0838	1	1	0	1988	19568	0.1016	0.3187
			1	1	0	2316	19265	0.1202	0.3467
			1	1	0	1702	14495	0.1174	0.3427
			2	1	0	1928	15244	0.1265	0.3556
			3	1	0	2102	15830	0.1328	0.3644
			3	1	0	2680	18498	0.1449	0.3806
			4	1	0	2068	14507	0.1426	0.3776
CONTRL	0.060	0.0838	1	1	9	4281	22948	0.1866	0.4319
			2	1	2	2613	18803	0.1390	0.3728
			3	1	5	2206	16186	0.1363	0.3692
			4	1	4	2265	17394	0.1302	0.3609
UL SED	0.030	0.0443	1	1	7	2386	25053	0.0952	0.3086
			1	2	10	2343	25022	0.0936	0.3060
			1	3	3	2082	25475	0.0817	0.2859
			2	1	5	1849	23748	0.0779	0.2790
			2	2	8	2225	22759	0.0978	0.3127
			2	3	4	2476	24107	0.1027	0.3205
			3	1	3	2312	21019	0.1100	0.3317
			3	2	8	2136	25130	0.0850	0.2915
			3	3	4	1968	22372	0.0880	0.2966
			4	1	2	2218	23894	0.0928	0.3047
			4	2	3	2396	23440	0.1022	0.3197
			4	3	1	1680	17986	0.0934	0.3056
UL SED	0.060	0.0838	1	1	8	3383	24472	0.1382	0.3718
			1	2	6	3430	25188	0.1362	0.3690
			1	3	4	3054	24126	0.1266	0.3558
			2	1	9	2990	22026	0.1357	0.3684
			2	2	10	3284	24494	0.1341	0.3662
			2	3	1	3408	25142	0.1356	0.3682
			3	1	1	3128	24269	0.1289	0.3590
			3	2	10	3032	19750	0.1535	0.3918
			3	3	6	3709	23035	0.1610	0.4013
			4	1	6	3124	19824	0.1576	0.3970
			4	2	9	1341	16828	0.0797	0.2823
			4	3	5	3614	20592	0.1755	0.4189
UL SED	0.090	0.1197	1	1	5	3430	25046	0.1369	0.3701
			1	2	2	4183	26892	0.1555	0.3944
			1	3	1	4307	25277	0.1704	0.4128
			2	1	7	3981	22335	0.1782	0.4222
			2	2	6	4892	24962	0.1960	0.4427
			2	3	3	4847	26404	0.1836	0.4285
			3	1	2	3687	19308	0.1910	0.4370
			3	2	9	3695	21292	0.1735	0.4166
			3	3	7	4675	21132	0.2212	0.4703
			4	1	7	3904	21958	0.1778	0.4217
			4	2	8	4734	18091	0.2617	0.5115
			4	3	10	2040	13498	0.1511	0.3888

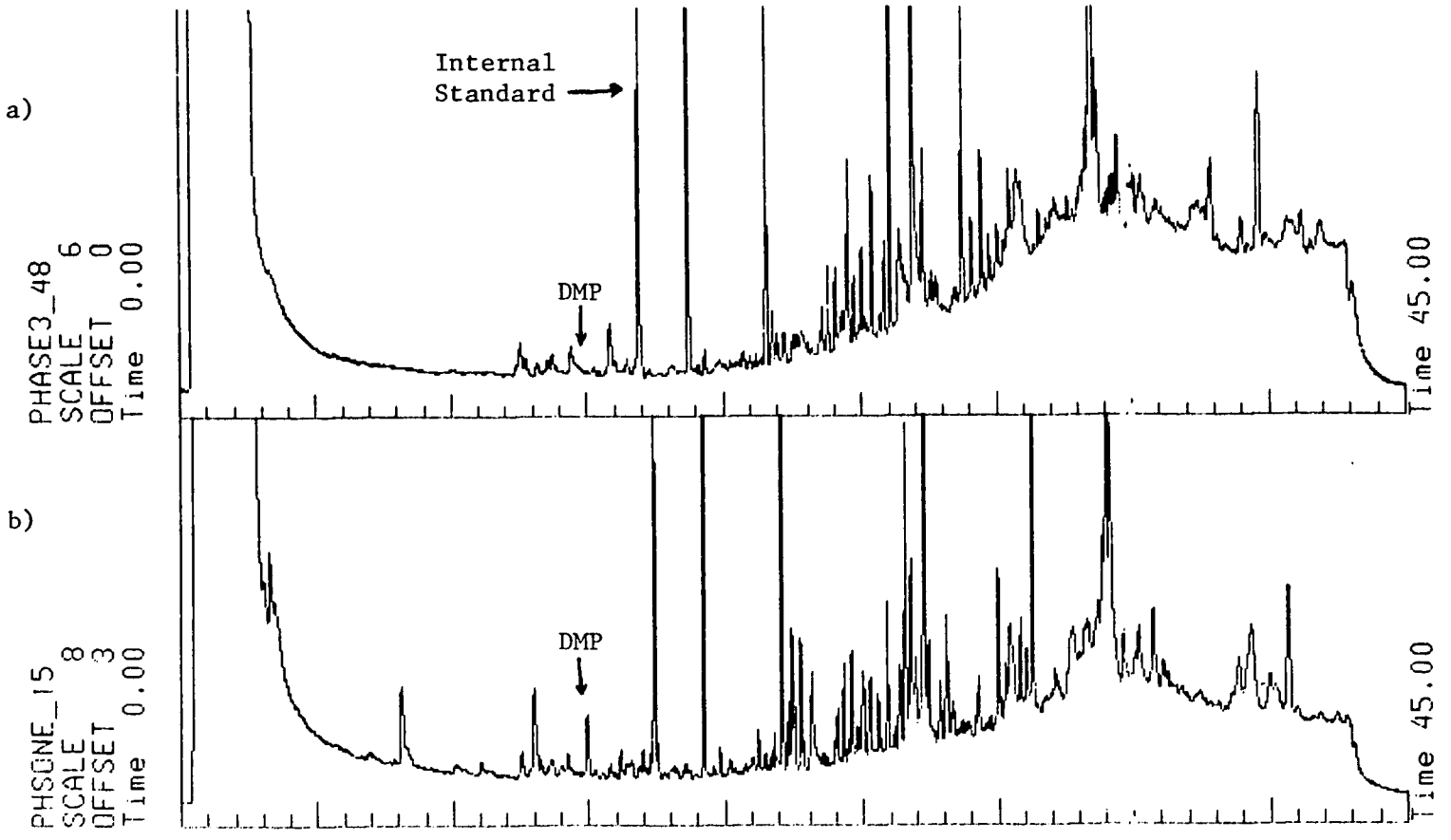


FIGURE 4-25. Representative Chromatograms For Analysis of Fortified Sediment Samples,
a) Phase II Sample, Dimethylphthalate (DMP) Fortified at 0.173 ppm.
b) Phase I Sample, Dimethylphthalate (DMP) Fortified at 0.217 ppm.

TABLE 4-25. COMPARISON OF PHASE I AND PHASE II DETECTION RATE ESTIMATES, BY ANALYTE, FOR r=1

ANALYTE	p	Yp	X*	ESTIMATE OF 1-q	NO. DETECTED	NO. CASES	PROPORTN DETECTED
2-CHLORONAPHTHALENE	0.01	0.43701	0.043	0.10299	0	12	0.00000
			0.086	0.36126	0	12	0.00000
			0.129	0.67482	7	12	0.58333
	0.05	0.39477	0.043	0.28840	0	12	0.00000
			0.086	0.64573	6	12	0.50000
			0.129	0.88542	9	12	0.75000
DIMETHYLPHTHALATE	0.01	0.41567	0.087	0.11211	0	9	0.00000
			0.173	0.36107	0	12	0.00000
			0.260	0.64933	0	11	0.00000
	0.05	0.37334	0.087	0.30620	0	9	0.00000
			0.173	0.64618	0	12	0.00000
			0.260	0.87150	0	11	0.00000
HEXACHLOROBENZENE	0.01	0.26241	0.036	0.10251	3	12	0.25000
			0.072	0.36589	5	12	0.41667
			0.108	0.68731	8	12	0.66667
	0.05	0.23650	0.036	0.28745	5	12	0.41667
			0.072	0.65042	9	12	0.75000
			0.108	0.89222	11	12	0.91667
ANTHRACENE	0.01	0.32425	0.024	0.09707	0	12	0.00000
			0.047	0.34296	3	12	0.25000
			0.071	0.67568	12	12	1.00000
	0.05	0.28973	0.024	0.27672	8	12	0.66667
			0.047	0.62676	12	12	1.00000
			0.071	0.88590	12	12	1.00000
PHENANTHRENE	0.01	0.34336	0.019	0.09430	1	12	0.08333
			0.038	0.35110	3	12	0.25000
			0.058	0.70229	8	12	0.66667
	0.05	0.31357	0.019	0.27115	4	12	0.33333
			0.038	0.63529	3	12	0.25000
			0.058	0.90013	11	12	0.91667
FLUORANTHENE	0.01	0.38662	0.030	0.09729	0	12	0.00000
			0.060	0.35104	4	12	0.33333
			0.090	0.67321	11	12	0.91667
	0.05	0.34295	0.030	0.27715	0	12	0.00000
			0.060	0.63522	11	12	0.91667
			0.090	0.88453	12	12	1.00000

(i) the Phase I point estimate of the detection rate;

(ii) the number of Phase II observations Y for which $Y > \hat{y}_p$;

(iii) the number of Phase II observations; and

(iv) the proportion of cases [(ii) ÷ (iii)] for which a detection was noted (i.e., the empirical estimate of 1-q(x)).

Table 4-26 shows a set of similar results for r=2. In this case, the twelve observed responses for each concentration level were paired together to form six $\bar{Y}$ values. The observations comprising each pair were chosen by sequential grouping of the first two runs, then the next two runs, etc. (The order of the runs is defined by set number and by run number within set number, as given in Tables 4-19 through 4-24).

A similar sequential grouping of the 12 individual responses was used to form a mean of triplicate observations (i.e., r=3). Four such means were therefore available for each concentration level and each analyte. Table 4-27 summarizes these results for the case r=3.

Results given in Tables 4-25, 4-26, and 4-27 indicate the following:

1. No detections were counted for dimethylphthalate -- attributed to the aforementioned problem with this analyte.
2. For the remaining analytes, the observed proportion of cases detected is, with several exceptions, in general agreement with the estimated detection rates from Phase I; the Phase II proportions are quite variable, however, due to the small sample sizes (especially when r=3).
3. With one exception (anthracene, p=0.05, r=1), the agreement between Phase I and Phase II rates tended to be better for the high concentration level than for the two lower levels.

Because of the deliberate manner in which the Phase II fortification levels were chosen, estimated detection rates from Phase I for the lowest Phase II concentration level were essentially the same for <u>all</u> analytes (e.g., $1-\hat{q}(x) \approx 0.10$ when p=0.01 and r=1). Similarly, the chosen fortification levels for Phase II guaranteed that this property (i.e., approximate constancy of the estimated, Phase I detection rates over analytes) would apply also to the other fortification levels, to other choices of p, and to other choices of r. The approximate Phase I

TABLE 4-26. COMPARISON OF PHASE I AND PHASE II DETECTION RATE ESTIMATES, BY ANALYTE, FOR r=2

ANALYTE	p	Yp	X*	ESTIMATE OF 1-q	NO. DETECTED	NO. CASES	PROPORTN DETECTED
2-CHLORONAPHTHALENE	0.01	0.40123	0.043	0.18746	0	6	0.00000
			0.086	0.63498	1	6	0.16667
			0.129	0.92504	5	6	0.83333
	0.05	0.37008	0.043	0.43191	0	6	0.00000
			0.086	0.86250	5	6	0.83333
			0.129	0.98661	5	6	0.83333
DIMETHYLPHTHALATE	0.01	0.38029	0.087	0.20429	0	4	0.00000
			0.173	0.63077	0	6	0.00000
			0.260	0.90840	0	5	0.00000
	0.05	0.34895	0.087	0.45707	0	4	0.00000
			0.173	0.86050	0	6	0.00000
			0.260	0.98242	0	5	0.00000
HEXACHLOROBENZENE	0.01	0.24046	0.036	0.18654	1	6	0.16667
			0.072	0.64141	6	6	1.00000
			0.108	0.93161	5	6	0.83333
	0.05	0.22135	0.036	0.43053	3	6	0.50000
			0.072	0.86633	6	6	1.00000
			0.108	0.98821	6	6	1.00000
ANTHRACENE	0.01	0.29502	0.024	0.17538	2	6	0.33333
			0.047	0.60952	6	6	1.00000
			0.071	0.92546	6	6	1.00000
	0.05	0.26955	0.024	0.41353	5	6	0.83333
			0.047	0.84682	6	6	1.00000
			0.071	0.98672	6	6	1.00000
PHENANTHRENE	0.01	0.31813	0.019	0.16978	2	6	0.33333
			0.038	0.62110	2	6	0.33333
			0.058	0.93888	5	6	0.83333
	0.05	0.29616	0.019	0.40482	2	6	0.33333
			0.038	0.85405	2	6	0.33333
			0.058	0.98991	6	6	1.00000
FLUORANTHENE	0.01	0.34962	0.030	0.17590	0	6	0.00000
			0.060	0.62110	5	6	0.83333
			0.090	0.92428	6	6	1.00000
	0.05	0.31741	0.030	0.41435	0	6	0.00000
			0.060	0.85405	6	6	1.00000
			0.090	0.98642	6	6	1.00000

TABLE 4-27. COMPARISON OF PHASE I AND PHASE II DETECTION RATE ESTIMATES, BY ANALYTE, FOR r=3

ANALYTE	p	Yp	X*	ESTIMATE OF 1-q	NO. DETECTED	NO. CASES	PROPORTN DETECTED
2-CHLORONAPHTHALENE	0.01	0.38604	0.043	0.26389	0	4	0.00000
			0.086	0.79380	2	4	0.50000
			0.129	0.98250	3	4	0.75000
	0.05	0.35959	0.043	0.53612	0	4	0.00000
			0.086	0.94276	4	4	1.00000
			0.129	0.99814	4	4	1.00000
DIMETHYLPHTHALATE	0.01	0.36531	0.087	0.28586	0	3	0.00000
			0.173	0.78703	0	4	0.00000
			0.260	0.97535	0	3	0.00000
	0.05	0.33862	0.087	0.56345	0	3	0.00000
			0.173	0.94028	0	4	0.00000
			0.260	0.99710	0	3	0.00000
HEXACHLOROBENZENE	0.01	0.23113	0.036	0.26265	2	4	0.50000
			0.072	0.79964	3	4	0.75000
			0.108	0.98483	4	4	1.00000
	0.05	0.21491	0.036	0.53457	2	4	0.50000
			0.072	0.94515	4	4	1.00000
			0.108	0.99846	4	4	1.00000
ANTHRACENE	0.01	0.28260	0.024	0.24652	2	4	0.50000
			0.047	0.77052	4	4	1.00000
			0.071	0.98264	4	4	1.00000
	0.05	0.26099	0.024	0.51406	4	4	1.00000
			0.047	0.93283	4	4	1.00000
			0.071	0.99816	4	4	1.00000
PHENANTHRENE	0.01	0.30741	0.019	0.23853	1	4	0.25000
			0.038	0.78133	1	4	0.25000
			0.058	0.98723	4	4	1.00000
	0.05	0.28876	0.019	0.50361	1	4	0.25000
			0.038	0.93752	1	4	0.25000
			0.058	0.99877	4	4	1.00000
FLUORANTHENE	0.01	0.33390	0.030	0.24739	0	4	0.00000
			0.060	0.78140	4	4	1.00000
			0.090	0.98225	4	4	1.00000
	0.05	0.30656	0.030	0.51518	2	4	0.50000
			0.060	0.93755	4	4	1.00000
			0.090	0.99811	4	4	1.00000

estimated detection rates at each Phase II concentration level are shown below:

r	p	Phase II Concentration Level Low	Middle	High
1	0.01	0.10	0.35	0.68
	0.05	0.28	0.64	0.89
2	0.01	0.18	0.63	0.93
	0.05	0.42	0.86	0.99
3	0.01	0.25	0.79	0.98
	0.05	0.52	0.94	0.99+

This design property allowed us to pool the Phase II results over analytes in a useful fashion -- namely, by aggregating the number of detections over analytes. Through such an aggregation we were able to achieve a larger effective sample size and thereby reduce the overall variation in the empirically derived estimates of detection rates. The results of this aggregation over five analytes (dimethylphthalate was excluded) are given in Table 4-28.

The results of this table indicate a close agreement between the Phase I predictions of detection rates and the observed rates of detection derived from Phase II. If the 60 cases for r=1, for instance, are treated as 60 independent binomial trials (though this is not strictly true), then binomial confidence limits for the true proportion detected can be constructed. Similar interval estimates for r=2 (30 trials) and r=3 (20 trials) can be developed. In all of the eighteen cases listed in Table 4-28, the point estimate for the detection rate from Phase I falls within the corresponding 95% confidence interval for the Phase II proportion, indicating no detectable departure of predicted from observed rates.

An alternative way of comparing the Phase I and Phase II results is to compare K_I and K_{II}, where

K_I is the expected number of detections derived from Phase I predictions, and

K_{II} is the observed number of detections in Phase II.

Values of K_I and K_{II} are shown in the table below:

Table 4-28. Comparison of Phase I and Phase II Detection Rate Estimates, Based on Aggregating the Phase II Results Over Five Analytes

r	p	Phase II Concentration	Approximate Phase I Detection Rate Est.	Phase II Results: Number of Detections	Phase II Results: Number of Cases	Phase II Results: Proportion Detected	Approximate Deviation in Phase I and Phase II Rates
1	0.01	low	0.10	4	60	0.0667	-0.03
		medium	0.35	15	60	0.2500	-0.10
		high	0.68	46	60	0.7667	0.08
1	0.05	low	0.28	17	60	0.2833	0.00
		medium	0.64	41	60	0.6833	0.04
		high	0.89	55	60	0.9167	0.03
2	0.01	low	0.18	5	30	0.1667	-0.01
		medium	0.63	20	30	0.6667	0.04
		high	0.93	27	30	0.9000	-0.03
2	0.05	low	0.42	10	30	0.3333	-0.09
		medium	0.86	25	30	0.8333	-0.02
		high	0.99	29	30	0.9667	-0.02
3	0.01	low	0.25	5	20	0.2500	0.00
		medium	0.79	14	20	0.7000	-0.09
		high	0.98	19	20	0.9500	-0.03
3	0.05	low	0.52	9	20	0.4500	-0.07
		medium	0.94	17	20	0.8500	-0.09
		high	0.99+	20	20	1.0000	0.00

			Phase II Concentration Level					
			low		medium		high	
r	p	No. Cases	K_I	K_{II}	K_I	K_{II}	K_I	K_{II}
1	0.01	60	6.0	4	21.0	15	40.8	46
1	0.05	60	16.8	17	38.4	41	53.4	55
2	0.01	30	5.4	5	18.9	20	27.9	27
2	0.05	30	12.6	10	25.8	25	29.7	29
3	0.01	20	5.0	5	15.8	14	19.6	19
3	0.05	20	10.4	9	18.8	17	19.9+	20

Among the six combinations of r and p shown above, the worst agreement occurs in the case r=1, p=0.01. Even for this case, however, the magnitudes of the differences between K_I and K_{II} are within the range that would be expected to occur simply by chance.

5. CONCLUSIONS AND RECOMMENDATIONS

The current approach utilized by EPA for defining/estimating a "Method Detection Limit" tends to emphasize achieving protection against reaching false positive conclusions -- i.e., erroneously claiming presence of an analyte in an environmental or biological medium. At least in certain situations, the consequences of reaching a false negative conclusion -- i.e., erroneously concluding that the analyte is absent when it is in fact present -- can be equally important. Yet the EPA approach makes no attempt to furnish adequate protection against this second type of error.

The methods described in Chapter 3 and Appendix A, on the other hand, provide a conceptually and statistically rigorous technique for estimating detection limits with specified assurance probabilities against both types of errors. The methods, which utilize standard calibration techniques and involve assumptions common to many calibration situations, are founded on the classical Neyman-Pearson criterion for performing tests of hypotheses. For a given detection rule involving a specified false-positive rate p, this method defines a detection limit $x_\ell(p,q)$ that provides protection against false negatives with probability q. Because of the power properties of the associated hypothesis test, this method (under normality assumptions) is guaranteed to produce the lowest such limit that satisfies the specified rates p and q.

The Phase I experiment described in Chapter 4 illustrates an application of the method using chemical methods similar to those employed in many EPA studies. The second phase of the experiment provides an empirical demonstration that the method does, in fact, perform in accordance with theory in the EPA laboratory environment.

In order to produce and report reliable estimates of detection limits therefore, EPA should consider the formulation and methods described in Chapter 3/Appendix A as a basis for defining and estimating such limits. Characteristics of the detection protocol (the calibration design, p, q, and r), should not be fixed arbitrarily, but should be adapted to meet the needs of specific studies and programs.

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APPENDIX A

Detectability and Detection Limits

[This appendix is a paper prepared by Peter M. Burrows and submitted to Technometrics]

DETECTABILITY AND DETECTION LIMITS

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Detectability of an analyte is considered when detection is based on a normally distributed measure calibrated against analyte concentrations. Detection rules are formulated as t-tests. Estimation of detection rates, at specified concentrations, requires estimation of the corresponding power functions (noncentral t-distribution functions). Estimation of detection limits with specified assurance probability requires estimation of the corresponding noncentrality parameters. Formulations for situations involving statutory limits, with assured alert rates, are developed in a similar manner.

KEY WORDS: calibration; inverse calibration; detection; noncentral t-distribution.

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1. INTRODUCTION

A substance A, usually referred to as the analyte or determinand, is present in unknown concentration X that varies from one sample preparation to another, including the possibility X = zero. In practice A may be an element, a compound or a macromolecule (including biologically functional particles). There exists a well defined measurement method that produces observations Y informative about X. In most cases Y is obtained from some convenient indirect measure of X, often not expressed in the same units as X, and is subject to random observational error resulting from sequential accumulation of uncontrollable errors at each step of the preparation and measurement methods. Measurement of physical, chemical or biological properties of A itself, or of consequences of the presence or activity of A, are typical sources of observations Y.

Theories of calibration and reverse calibration address the general problem of estimating X for a particular sample given one or more determinations of Y on that sample. But when A is present at low concentrations, in the neighborhood of X = zero, interest shifts to whether or not it is reasonable to declare that A is detectable given that observations Y are subject to error as just described. Instead of providing point and interval estimates for X, the problem is to provide a reliable rubric for asserting that X > zero. Assertions 'X > zero' are logically equivalent to declarations 'A is detected', regardless of their veracity or of the actual value of X when X > zero.

A detection protocol is defined by specifying the measurement method, performing at least a minimal calibration of Y against X, and adopting a

detection rule. With the assumption that the calibration function (standard curve) is monotonic, a detection rule with known properties in repeated applications is provided by the Neyman-Pearson apparatus for constructing tests of the hypothesis X = zero against alternatives X > zero, with specified protection against frequent false positives (erroneous assertions that X > zero). The resulting rule always takes the form of referring Y to a specified action value $\hat{y}$, also known as the detection threshold: if Y is expected to increase with increasing X, then assert X > zero whenever $Y > \hat{y}$, but if Y is expected to decrease with increasing X (as happens when Y results from some property of a substance in competition with $\underset{\sim}{A}$), then assert X > zero whenever $Y < \hat{y}$. If several determinations of Y are obtained for a single source then their average $\bar{Y}$ is substituted for Y in these rules and threshold $\hat{y}$ is modified accordingly.

Detection rates in repeated applications of the defined detection protocol are given by the power function of this detection rule. There are other criteria that lead to rules of this same general type (see Voller, Bidwell and Bartlett (1977) for examples), but superior power properties of the Neyman-Pearson criterion ensure that among all such rules it attains the highest detection rates at all concentrations X > zero, and therefore the lowest detection limits as defined below.

Previous discussions of detectability, especially among analytical chemists, have concentrated on detection limits (see Liteanu and Rîcă (1980) for a review and bibliography). Definition of detection limits with specified assurance probability, and estimation of detection rates and detection limits, are all

applications of noncentral t-distributions as shown in §3. For this purpose the following notations are adopted:

Z : a normally distributed random variable with zero mean and unit variance,

S_v : independent of Z, vS_v^2 is distributed as chi-square on v degrees of freedom,

$T_v(\delta)$: follows the noncentral t-distribution with noncentrality parameter δ and v degrees of freedom; $T_v(\delta) = (Z+\delta)/S_v$,

$t_{v,p}$: the upper 100p percentage point of Student's t-distribution with v degrees of freedom; $\Pr[T_v(0) > t_{v,p}] = p$.

When there is no risk of confusion, v is suppressed in the notations S, $T(\delta)$ and t_p.

A comprehensive review of properties of $T(\delta)$ is given by Owen (1968). Availability of arithmetic routines that produce $\Pr[T(\delta)<t]$ for specified v, δ and t, and also δ such that $\Pr[T(\delta)<t] = q$ for specified v, t and q, is assumed throughout. With modern computing facilities it is a simple matter to prepare such routines; those employed here are based on quadrature methods described previously (Burrows, 1985).

Results for a general formulation of the standard curve relating Y to X are developed in §2 (calibration against known standards) and §3 (detectability); detailed results for linear (straight line) calibration are elaborated in §4 which also contains criticisms of previous formulations of detectability. An example of detectability estimation, under linear calibration, is given in §5. Situations involving statutory limiting concentrations of A are considered in §6. Then detection translates into assertions of noncompliance which constitute 'alerts'; estimates of rates of false alerts are derived using the same methods.

Some general comments on the usefulness of detection limits, and conventional specification of false positive rates and false negative rates, are included in the discussion in §7.

2. CALIBRATION

A model of observations Y is necessary to formulate the detection protocol and its behavior in repeated applications. Here it is assumed that when X = x, known or unknown,

$$(2.1) \qquad Y = \alpha + f(x,\underline{\beta}) + e \ , \quad \underline{\beta}' = (\beta_1, \beta_2, \ldots, \beta_m)$$

where e is an additive observational error distributed normally with mean zero and variance σ^2 constant for all x in the range of X of interest. Errors in r repeated determinations of Y, obtained for a single source with X = x, are assumed independent so that their average $\bar{Y} = [\alpha + f(x,\underline{\beta}) + Z\sigma/\sqrt{r}]$.

Two features of the function $f(x,\underline{\beta})$ are assumed throughout. First, $f(o,\underline{\beta})$ = zero, so that parameter α is the expected background value of Y, attributable to such phenomena as leakage and nonspecific reactions. In the case of a laboratory instrument, α is the expected reading for a 'blank' sample. Second, $f(x,\underline{\beta})$ is assumed to be a monotonic function of x over all x > zero, which imposes conditions (usually inequalities) on its parameters $\underline{\beta}$. Monotonicity is necessary to avoid possible ambiguities in inverse calibration. Here $f(x,\underline{\beta})$ is assumed to be monotonic increasing in x so that detection rules of the type 'assert X > zero whenever $\bar{Y} > \hat{y}$' are appropriate. Modifications required when $f(x,\underline{\beta})$ is monotonic decreasing in x are obvious throughout.

Calibration is assumed to be based on standard preparations of $\underset{\sim}{A}$ with known concentrations $x_1, x_2, \ldots, x_n$ including at least (m+1) distinct standards. The model for observation Y_i, obtained at standard x_i, is that at (2.1) with pairwise independence of corresponding observational errors e_i. Let $\hat{\alpha}$ and $\hat{\underline{\beta}}$ denote the resulting least squares estimates of parameters α and $\underline{\beta}$, and let $\hat{\sigma}^2$ denote the estimate of σ^2. Then the following distributional properties are

assumed throughout:

(2.2) $[\hat{\alpha},\hat{\underline{\beta}}]$ follows a multivariate normal distribution with mean $[\alpha,\underline{\beta}]$ and variance-covariance matrix $\sigma^2 V$,

(2.3) $\hat{\sigma} = \sigma S_v$,

(2.4) $\bar{Y}$ and $\hat{\sigma}$ are independent of each other and of $[\hat{\alpha},\hat{\underline{\beta}}]$.

In many cases degrees of freedom $v = (n-m-1)$, but v is left general here to allow for other possibilities including incorporation of experimental design features in the calibration exercise, pooling of information from several independent calibrations, and use of variation among the r replicated observations about their average $\bar{Y}$. Matrix V is $(m+1) \times (m+1)$ with known elements that are functions of the n standard concentrations and of the form assumed for $f(x,\underline{\beta})$.

If $f(x,\underline{\beta})$ is linear in its parameters $\beta_1,\beta_2,\ldots,\beta_m$, which includes cases of polynomial standard curves treated by Scheffé (1973), then properties (2.2), (2.3) and (2.4) follow from the observational error structure assumed for Y and Y_i. If $f(x,\underline{\beta})$ is nonlinear in its parameters $\underline{\beta}$, so that nonlinear least squares estimation is necessary, then these distributional properties (and those below) are only large sample approximations. Two immediate consequences of (2.2) and (2.4) are stated now for use later. Given any x,

(2.5) $$f(x,\hat{\underline{\beta}}) = f(x,\underline{\beta}) + Z\sigma u_x \ , \quad u_x^2 = \mathrm{var}[f(x,\hat{\underline{\beta}})/\sigma] \ ,$$

and when $X = x$ for the source of $\bar{Y}$,

(2.6) $$(\bar{Y}-\hat{\alpha}) = f(x,\underline{\beta}) + Z\sigma w \ , \quad w^2 = \mathrm{var}(\hat{\alpha}/\sigma) + 1/r \ .$$

Both u_x and w are readily obtained from V.

Let $\delta_x = f(x,\underline{\beta})/\sigma u_x$. A point estimate for δ_x is $\hat{\delta}_x = f(x,\hat{\underline{\beta}})/\hat{\sigma} u_x$, but when $f(x,\underline{\beta})$ is linear in its parameters $\underline{\beta}$ the minimum variance unbiassed estimate is usually $\hat{\delta}_x/M_v$ where $E(1/\hat{\sigma}) = M_v/\sigma$; values of M_v are given in the second column of Table 1. An interval estimate for δ_x is obtained by combining (2.5) and (2.3) to yield $\hat{\delta}_x = (Z+\delta_x)/S = T(\delta_x)$. Let γ^- (>0) and γ^+ (>0) be such that $(\gamma^-+\gamma^+) = \gamma < 1$, and locate d_x^- $(<\hat{\delta}_x)$ and d_x^+ $(>\hat{\delta}_x)$ such that

$$\Pr[T(d_x^-) > \hat{\delta}_x] = \gamma^- \quad , \quad \Pr[T(d_x^+) < \hat{\delta}_x] = \gamma^+ \ . \tag{2.7}$$

Then the interval (d_x^-, d_x^+) covers δ_x with probability $(1-\gamma)$. The choice $\gamma^- = \gamma^+ = \gamma/2$ is obviously convenient, but to obtain an interval that is symmetric about $\hat{\delta}_x$, or the interval that is narrowest given $\hat{\delta}_x$ and γ, requires manipulation of γ^- and γ^+ subject to $(\gamma^-+\gamma^+) = \gamma$.

3. DETECTION RULES, RATES AND LIMITS

Let $\hat{y}_p = (\hat{\alpha}+w\hat{\sigma}t_p)$ and $\Delta_x = f(x,\underline{\beta})/\sigma w$. Then at concentrations $X = x$, rates of detection when using the rule 'assert X > zero whenever $\bar{Y} > \hat{y}_p$' are given by

(3.1) $$1-q(x) = \Pr[(\bar{Y}-\hat{\alpha}) > w\hat{\sigma}t_p \mid X=x]$$

$$= \Pr[(Z+\Delta_x) > St_p] = \Pr[T(\Delta_x) > t_p]$$

because $(\bar{Y}-\hat{\alpha}) = w\sigma(Z+\Delta_x)$ from (2.6) and $\hat{\sigma} = \sigma S$ at (2.3). Threshold $\hat{y}_p$ has been chosen so that rates of false positives under the rule '$\bar{Y} > \hat{y}_p$' are fixed at p: Δ_o = zero and

(3.2) $$1-q(0) = \Pr[T(0) > t_p] = p \ .$$

Rates of false negatives at concentrations $X = x$ are given by $q(x)$.

The definition of detection limits adopted here is based on the concept favored by Currie (1968) who reviewed several inconsistent alternatives: the detection limit should be an analyte concentration that is almost assured of detection. Such a limit is inseparable from the adopted detection rule, so that a formal definition is stated as follows:

> if $x_\ell = x_\ell(p,q)$ is the lowest analyte concentration for which the rate of detection is at least (1-q) when using the rule 'declare $\underline{A}$ detected whenever $\bar{Y} > \hat{y}_p$', then x_ℓ is <u>the detection limit with assurance probability (1-q)</u> for that rule; alternatively, the rate of false negatives is at most q for all analyte concentrations exceeding x_ℓ.

Thus x_ℓ corresponds to just one point on the curve of false negative rates $q(x)$ in relation to analyte concentrations x, namely, that point selected by specification of q. Let $\Delta = \Delta(v,p,q)$ satisfy $\Pr[T(\Delta)<t_p] = q$. Then parameter x_ℓ is a solution x to the equation

$$(3.3) \qquad \Delta = \Delta_x \quad \text{or} \quad f(x,\underline{\beta}) = \sigma w \Delta$$

obtained by setting $q(x) = q$ in (3.1). In practice both p and q are specified as small as possible. Some values of Δ for the special cases $q = p = 0.05$, 0.01 and 0.001 are given in columns 6, 7 and 8 of Table 1; corresponding values of t_p required for threshold $\hat{y}_p$ are given in columns 3,4 and 5 of that table.

Detection rates, $[1-q(x)]$, and detection limits, x_ℓ, are parameters. Characterization of detectability for a particular detection protocol, or construction of a detection protocol (including calibration design and choice of r) to match a specified detectability criterion (if it is not so stringent as to be unattainable), requires estimates of these parameters. Point estimates of $q(x)$ for any x, and of x_ℓ, can be obtained by substitution of $\hat{\underline{\beta}}$ and $\hat{\sigma}$ for $\underline{\beta}$ and σ in Δ_x:

$$(3.4) \qquad \hat{\Delta}_x = u_x\hat{\delta}_x/w \text{ or } u_x\hat{\delta}_x/wM_v \ , \quad \hat{q}(x) = \Pr[T(\hat{\Delta}_x) < t_p]$$

though little can be said about their properties, and the required solution $\hat{x}_\ell$ to $\hat{\Delta}_x = \Delta$ may not be readily tractable depending on the form of $f(x,\underline{\beta})$. But interval estimates are available from the interval (d_x^-,d_x^+) developed for δ_x at (2.7). First, and for each x of interest, convert (d_x^-,d_x^+) to an interval estimate (D_x^-,D_x^+) for Δ_x:

$$(3.5) \qquad (D_x^-,D_x^+) \equiv (u_x d_x^-/w, u_x d_x^+/w) \quad .$$

Then, since $Pr[T(\delta) < t_p]$ is a monotonic decreasing function of δ, the interval

$$\{q^-(x), q^+(x)\} \equiv \{Pr[T(D_x^+) < t_p], Pr[T(D_x^-) < t_p]\} \tag{3.6}$$

covers $q(x)$ with probability $(1-\gamma)$. An interval estimate for x_ℓ is then obtained by inverse interpolations from the specified q through curves $q^-(x)$ and $q^+(x)$ to (x_ℓ^-, x_ℓ^+) as illustrated in Figure 1: $q^-(x_\ell^-) = q = q^+(x_\ell^+)$. This last construction ignores the possibility, and its probability, that although $f(x,\underline{\beta})$ is assumed to be monotonic increasing in x, the standard curve fitted by least squares may not obey this condition. The consequences of this possibility are discussed in more detail in the next section.

4. LINEAR CALIBRATION

Considerable simplification results when a straight line relationship, $f(x,\underline{\beta}) = \beta x$, is adopted for the standard curve. In particular, interval estimates for x_ℓ are obtained directly without recourse to inverse interpolation as in Figure 1. For this special case with $m = 1$, the elements of V are given by

$$\mathrm{var}(\hat{\alpha}/\sigma) = (n^{-1}+\bar{x}^2/Q) \ , \quad \mathrm{cov}(\hat{\alpha}/\sigma,\hat{\beta}/\sigma) = -\bar{x}/Q \ , \quad \mathrm{var}(\hat{\beta}/\sigma) = 1/Q$$

where $n\bar{x} = \sum_i x_i$ and $Q = \sum_i (x_i-\bar{x})^2$. Then, for (2.5) and (2.6),

$$u_x = x/Q^{\frac{1}{2}} \quad \text{and} \quad w = (r^{-1}+n^{-1}+\bar{x}^2/Q)^{\frac{1}{2}}$$

so that $\delta_x = Q^{\frac{1}{2}}\beta/\sigma$ is not a function of x and its subscript x can be omitted throughout this section: $\delta = Q^{\frac{1}{2}}\beta/\sigma$ and $\hat{\delta} = Q^{\frac{1}{2}}\hat{\beta}/\hat{\sigma}$. Note that (β/σ) is the sensitivity ratio referred to as Gaddum's constant. Threshold $\hat{y}_p$ and noncentrality parameter Δ_x define detection rates for the rule 'assert x > zero whenever $\bar{Y} > \hat{y}_p$' as at (3.1):

(4.1) $$1-q(x) = \Pr[T(\Delta_x) > t_p] \ , \quad \Delta_x = \beta x/\sigma w \ .$$

Hubaux and Vos (1970) offer a curious development of x_ℓ, endorsed by Currie, Filliben and DeVoe (1972) and by Liteanu and Rîcă (1980), in terms of inverse interpolations in the space of Y and x, rather than via a graph of q(x) against x. With the present terminology and notation, their graphical formulation can be stated algebraically as follows:

Step (i). The rule 'assert X > zero whenever $\bar{Y} > \hat{y}_p$', with $\hat{y}_p = (\hat{\alpha}+w\hat{\sigma}t_p)$, achieves level-p protection against false positives as at (3.2).

Step (ii). Let $w_x = [r^{-1}+n^{-1}+(x-\bar{x})^2/Q]^{\frac{1}{2}}$ and consider any point $(x,\hat{y})$, x not a random variable, on the curve $\hat{y} = (\hat{\alpha}+\hat{\beta}x-w_x\hat{\sigma}t_q)$. Then $x_\ell(\cdot,q) = x$ for the

rule 'assert X > zero whenever $\bar{Y} > \hat{y}$', because $(\bar{Y}-\hat{\alpha}-\hat{\beta}x) = Z\sigma w_x$ when $X = x$ and the detection rate when $X = x$ is

$$\Pr[(\bar{Y}-\hat{\alpha}-\hat{\beta}x) > -w_x\hat{\sigma}t_q \mid X = x] = \Pr[T(o) > -t_q] = (1-q) .$$

The qualification that x not be a random variable is required because no accounting of any stochastic variation in x has been made when formulating this detection rate. Since $x = x_\ell(.,q)$ for this rule, any concentration x can be a detection limit with assurance probability (1-q). To each different x there corresponds a different rate of false positives determined by threshold $\hat{y}$.

Step (iii). Let $\hat{x}$ be the solution x to $\hat{y} = \hat{y}_p$:

$$\hat{\beta}\hat{x} = \hat{\sigma}(wt_p + w_{\hat{x}}t_q) . \tag{4.2}$$

Then $x_\ell(p,q) = \hat{x}$ because the rule in step (ii), with detection rate (1-q) when $x = \hat{x}$, has been made to coincide with the rule in step (i) with false positive rate p.

Hubaux and Vos (1970) do not provide an explicit solution to (4.2) which is quadratic in $\hat{x}$. The typographical inconvenience is reduced by the following sequence of notations. Let $\hat{x}_p = (\hat{y}_p-\hat{\alpha})/\hat{\beta}$; $\hat{x}_p$ is the analyte concentration obtained by inverse interpolation from $\hat{y}_p$ through the fitted calibration line. Though it has no useful meaning, a popular convention is to regard $\hat{x}_p$ as a detection limit, often in the mistaken belief that concentrations $X = \hat{x}_p$ are detectable at rate (1-p), as in the following quotation from Maggio (1980):

> "If one makes the generally reasonable assumption that the system noise, the overall variability in the measurement of enzyme activity, may be described by a Gaussian distribution, then the minimum detectable concentration may be conveniently expressed in terms of the standard deviation. For example, we may define the minimum detectable concentration as the interpolated analyte concentration which lies two standard deviations above the zero value on the dose-response curve. This concentration may be distinguished from zero with greater than 95% confidence."

Let $(1+R_q) = (\hat{\delta}/t_q)^2$; in practice R_q is a positive quantity because otherwise the hypothesis β = zero is not rejected by the level-q t-test which refers $\hat{\delta}$ to $t_{v,q}$. Indeed, R_q > zero is the condition for existence of sensible yet nontrivial interval estimates, with coverage probability $(1-2q)$, for inverse calibration of X given $\bar{Y}$ using the formulation of Bowker and Lieberman (1959) which is a special case of Fieller's method (1954); a general (non-Bayesian) remedy that produces useable intervals for X given $\bar{Y}$, even when $\hat{\delta} < t_q$, is given by Scheffé (1970).

Let $\hat{x}_* = \hat{x}_p + (\hat{x}_p - \bar{x})/R_q$; then roots of (4.2) are given by

$$\hat{x} = \hat{x}_* \pm [\hat{x}_*^2 - \hat{x}_p^2(1-R_p/R_q)]^{\frac{1}{2}}$$

and when q = p, $\hat{x} = 2\hat{x}_*$ is the required solution.

This formulation, $x_\ell = \hat{x}$, is seductive but obviously fallacious because if valid this method would always be available to circumvent noncentral t-distributions when considering power of t-tests. Hubaux and Vos (1970) regard $\hat{x}$ first as a definition of x_ℓ and later, after noting that $\hat{x}$ is a random variable, as an estimate. The fallacy arises because $x = \hat{x}$ violates the restriction that x not be a random variable in step (ii). The rule in

step (ii) is of some interest however, and is used in §6. Also as $n \to \infty$, $\hat{\beta} \to \beta$, $\hat{\sigma} \to \sigma$ and $t_p \to z_p$, the upper 100p percentage point of Z. Thus in the limit, (4.2) reduces to

$$\beta\hat{x} = (z_p + z_q)\sigma/\sqrt{r}$$

and $\hat{x}$ does coincide with the definition of $x_\ell(p,q)$ for the case where α, β and σ are known perfectly.

In contrast with $\hat{x}_p$ and $\hat{x}$, the exact formulation of parameter $x_\ell(p,q)$ is obtained by specifying $q(x_\ell) = q$ in (4.1):

(4.3) $$x_\ell = w\Delta\sigma/\beta$$

where $\Delta = \Delta(v,p,q)$ as at (3.3); $\Delta \to (z_p + z_q)$ as $v \to \infty$.

Estimation of detectability parameters follows from the general recipe in §3. Point estimate $\hat{q}(x)$, for any x, is given by (3.4) with $\hat{\Delta}_x = \hat{\beta}x/\hat{\sigma}w$ and interval $[q^-(x), q^+(x)]$ is given by (3.6). For this last purpose, note that d^- and d^+ (defined as at (2.7) but with subscript x ommitted again because these are not functions of x) provide an interval estimate for $\delta = Q^{\frac{1}{2}}(\beta/\sigma)$, and that

$$(D_x^-, D_x^+) \equiv (xd^-/wQ^{\frac{1}{2}}, xd^+/wQ^{\frac{1}{2}}) \ .$$

Then the interval estimate for x_ℓ, defined by $q^-(x_\ell^-) = q = q^+(x_\ell^+)$ at the end of §3, is obtained directly as

(4.4) $$x_\ell^- = w\Delta(Q^{\frac{1}{2}}/d^+) \quad , \quad x_\ell^+ = w\Delta(Q^{\frac{1}{2}}/d^-) \ .$$

Thus in comparison with $x_\ell = w\Delta(\sigma/\beta)$, the interval (x_ℓ^-, x_ℓ^+) is constructed as if $(Q^{\frac{1}{2}}/d^+, Q^{\frac{1}{2}}/d^-)$ is an interval estimate for (σ/β) with coverage probability $(1-\gamma)$, whereas $(d^-/Q^{\frac{1}{2}}, d^+/Q^{\frac{1}{2}})$ is defined to cover (β/σ) with probability $(1-\gamma)$. This situation is similar to that discussed by Koopmans, Owen and Rosenblatt (1964). An interval estimate for δ is available. An interval estimate for

$\lambda = 1/\delta$ is required. Is it satisfactory to invert the endpoints of the interval for δ?

The inituitive point estimate for λ is $\hat{\lambda} = 1/\hat{\delta}$. Its mean square error is infinite for the same reason that this is true of inverse calibrated estimates $\hat{X} = (\bar{Y}-\hat{\alpha})/\hat{\beta}$, as noted by Williams (1969). Estimate $\hat{\lambda}$ is distributed as $W(\lambda) = 1/T(\delta)$. It is assumed now that the possibility of negative estimates $\hat{\beta}$ has negligible probability (so that the fitted line, $\hat{\alpha}+\hat{\beta}x$, is monotonic increasing in $x >$ zero as is assumed for the line $\alpha+\beta x$; actually, the occurrence of $\hat{\beta} <$ zero would almost certainly lead to a discarding of those particular calibration results and probably to a questioning of the measurement method also). Then $\hat{\lambda} >$ zero and, analogous to (2.7), an interval estimate (ℓ^-,ℓ^+) for λ is constructed by locating ℓ^- $(< \hat{\lambda})$ and ℓ^+ $(> \hat{\lambda})$ such that

$$\gamma_*^- = \Pr[W(\ell^-) > \hat{\lambda}] \equiv \Pr[T(1/\ell^-) < \hat{\delta}] - \Pr[T(1/\ell^-) < \text{zero}] ,$$

$$\gamma_*^- = \Pr[W(\ell^+) < \hat{\lambda}] \equiv \Pr[T(1/\ell^+) > \hat{\delta}] + \Pr[T(1/\ell^+) < \text{zero}] ,$$

where $(\gamma_*^- + \gamma_*^+) = \gamma < 1$. Unless the ratio β/σ, together with the calibration design features Q and v, are illsuited for sensitive detection, ℓ^+ is so small that $\Pr[T(1/\ell^+) < \text{zero}] \equiv \Pr[Z < -1/\ell^+]$ is negligible; these are precisely the circumstances under which $\Pr[\hat{\beta} < \text{zero}]$ is negligible as assumed above. Then, in comparison with (2.7), $\ell^- = 1/d^+$ with $\gamma_*^- = \gamma^+$ and $\ell^+ = 1/d^-$ with $\gamma_*^+ = \gamma^-$, which justifies the use of $(Q^{\frac{1}{2}}/d^+, Q^{\frac{1}{2}}/d^-)$ as an interval estimate for (σ/β) when $\Pr[\hat{\beta} < \text{zero}]$ is negligible.

5. AN EXAMPLE OF DETECTABILITY

Liteanu and Rîcă (1980;Table 7.1) present an example of calibration results in which analyte $\underset{\sim}{A}$ is tungsten and concentrations X refer to tungsten content in steel samples (scaled as ppm here). The measure Y was obtained by emission spectrometry with results recorded as coded digits over the range 110 to 280. A total of n = 84 observations Y_i are listed, twelve each at seven standards x = zero,10,60,120,480,770 and 1050 ppm. A linear calibration function, $f(x,\underline{\beta}) = \beta x$, with constant variance σ^2 is a satisfactory representation over this range of concentrations. The following summary statistics are obtained:

$$n = 84, \quad \bar{x} = 355.714, \quad Q^{\frac{1}{2}} = 3563.433,$$

$$\hat{\alpha} = 113.022, \quad \hat{\beta} = 0.153888, \quad \hat{\sigma} = 2.39472 \text{ on 82 degrees of freedom.}$$

Thus $\hat{\delta} = (Q^{\frac{1}{2}}\hat{\beta}/\hat{\sigma}) = 228.992$ and the interval (d^-,d^+) obtained as at (2.7), with $\gamma^- = \gamma^+ = 0.025$, is (193.927,263.990). A false positive rate of p = 0.01 is specified and, with v = 82, $t_p = 2.37269$ is required for threshold $\hat{y}_p$.

For a detection limit $x_\ell(p,q)$ with assurance probability (1-q) = 0.99, the required $\Delta = \Delta(82,0.01,0.01) = 4.73164$ (linear interpolation in Table 1 yields 4.73171). The following listing contains w, x_ℓ, $\hat{x}_\ell = (w\Delta\hat{\sigma}/\hat{\beta})$, and interval estimate (x_ℓ^-,x_ℓ^+) obtained from (d^-,d^+) with $(1-\gamma) = 0.95$, for r = 1,2 or 3 observations yielding $\bar{Y}$ in the rule 'declare $\underset{\sim}{A}$ detected if $\bar{Y} > \hat{y}_p$' :

	r=1	r=2	r=3
w :	1.010876	0.722405	0.505989
x_ℓ (ppm) :	4.7831(σ/β)	3.4182(σ/β)	2.8200(σ/β)
$\hat{x}_\ell$ (ppm) :	74.4	53.2	43.9
interval (ppm) :	(64.6,87.9)	(46.1,62.8)	(38.1,51.8)

A more complete characterization of detectability, under the same detection rule, is given in Figure 2 which contains curves of detection rates for r = 1, 2 or 3, constructed from point estimates $[1-\hat{q}(x)]$ at (3.4) with $\hat{\Delta}_x = (0.06426)x/w$. An indication of the reliability of these estimated curves is given in Figure 3 which contains interval estimates from (3.6), with $\gamma = 0.05$, $D_x^- = (0.05384)x$ and $D_x^+ = (0.07324)x$, for the case r = 1 only.

6. STATUTORY LIMITS

Another form of limiting concentration is that imposed by statute or contractual obligation. Let x_s denote that analyte concentration specified as the statutory limit. Of the two possibilities that in which $X \leq x_s$ corresponds to compliance, and $X > x_s$ to noncompliance, will be considered here. Numerous examples can be found in the Acts and Guidelines governing clean air, clean water and potentially hazardous compounds in foodstuffs. Appropriate modifications for the reverse situation, compliance if $X \geq x_s$, are obvious throughout. The term detection now refers to assertions of noncompliance which constitute 'alerts'.

Implicit in the notion of a statutory limit, if not explicitly stated in the statute or contract, is the requirement that noncompliance be assured of detection for all $X > x_s$. Unless $x_\ell(p,q) << x_s$, a detection rule meeting this requirement with assurance (1-q) is necessary. Analogous to u_x,w and δ_x of §2, and Δ_x of §3, let

$$u^2_{s,x} = \mathrm{var}\{[f(x_s,\hat{\underline{\beta}})-f(x,\hat{\underline{\beta}})]/\sigma\} \quad , \quad w^2_s = \mathrm{var}\{[\bar{Y}-\hat{\alpha}-f(x_s,\hat{\underline{\beta}})]/\sigma\} \ ,$$

$$\delta_{s,x} = [f(x_s,\underline{\beta})-f(x,\underline{\beta})]/\sigma u_{s,x} \quad , \quad \Delta_{s,x} = u_{s,x}\delta_{s,x}/w_s \ ,$$

and let $\hat{y}_q = [\hat{\alpha}+f(x_s,\hat{\underline{\beta}})-w_s\hat{\sigma}t_q]$. Then, with the distributional assumptions in §2, the rule 'assert noncompliance if $\bar{Y} > \hat{y}_q$' yields alert rates p(x) at concentrations X = x given by

$$\begin{aligned} (6.1) \qquad p(x) &= \Pr\{[\bar{Y}-\hat{\alpha}-f(x_s,\hat{\underline{\beta}})] > -w_s\hat{\sigma}t_q \mid X = x\} \\ &= \Pr[(Z-\Delta_{s,x}) > -St_q] = \Pr[T(\Delta_{s,x})<t_q] \end{aligned}$$

because $[\bar{Y}-\hat{\alpha}-f(x_s,\hat{\underline{\beta}})] = [f(x,\underline{\beta})-f(x_s,\underline{\beta})] + Z\sigma w_s$ when $X = x$, and $Pr[T(-\delta) > -t] \equiv Pr[T(\delta) < t]$. Threshold $\hat{y}_q$ has been chosen so that the alert rate at the statutory limit is

$$p(x_s) = Pr[T(o) < t_q] = (1-q)$$

because $\Delta_{s,x}$ = zero when $x = x_s$. Moreover, since $f(x,\underline{\beta})$ is monotonic increasing in x, $\Delta_{s,x}$ < zero for all $x > x_s$ and alert rate p(x) exceeds (1-q) for all $x > x_s$. The price for this specified level of protection against failure to detect noncompliance is 'false alerts' at rate p(x) when $x < x_s$ given by (6.1). Frequent false alerts, a consequence of insensitivity of the detection protocol in relation to stringency of the statute and the specified level (1-q), may be intolerable.

Estimation in this context is limited to interest in p(x) and follows the pattern established in §2 and §3. Point estimates of p(x), for any x, can be obtained by substitution of $\hat{\underline{\beta}}$ and $\hat{\sigma}$ for $\underline{\beta}$ and σ in $\Delta_{s,x}$:

$$(6.2) \qquad \hat{\delta}_{s,x} = [f(x_s,\hat{\underline{\beta}})-f(x,\hat{\underline{\beta}})]/\hat{\sigma}u_{s,x} \quad , \quad \hat{\Delta}_{s,x} = u_{s,x}\hat{\delta}_{s,x}/w_s$$

and $\hat{p}(x) = Pr[T(\hat{\Delta}_{s,x}) < t_q]$. Interval estimates for p(x) are obtained by developing an interval estimate $(d^-_{s,x},d^+_{s,x})$ for $\delta_{s,x}$ analogous to that for δ_x at (2.7):

$$(6.3) \quad \hat{\delta}_{s,x} = T(\delta_{s,x}) \quad , \quad Pr[T(d^-_{s,x}) > \hat{\delta}_{s,x}] = \gamma^- \quad , \quad Pr[T(d^+_{s,x}) < \hat{\delta}_{s,x}] = \gamma^+ \ ,$$

and then converting $(d^-_{s,x},d^+_{s,x})$ to an interval for $\Delta_{s,x}$:

$$(6.4) \qquad (D^-_{s,x}, D^+_{s,x}) \equiv (u_{s,x}d^-_{s,x}/w_s, u_{s,x}d^+_{s,x}/w_s) \ .$$

Then the interval

$$(6.5) \qquad \{p^-(x), p^+(x)\} \equiv \{\Pr[T(D^+_{s,x}) < t_q], \Pr[T(D^-_{s,x}) < t_q]\}$$

covers $p(x)$ with probability $(1-\gamma)$.

Under linear calibration, as in §4, $u_{s,x} = (x_s - x)/Q^{\frac{1}{2}}$ and $w_s = [r^{-1} + n^{-1} + (x_s - \bar{x})^2/Q]^{\frac{1}{2}}$ so that $\delta_{s,x} = Q^{\frac{1}{2}}\beta/\sigma = \delta$, $\Delta_{s,x} = (x_s - x)\beta/\sigma w_s$, $\hat{\delta}_{s,x} = \hat{\delta}$, and interval (d^-, d^+) in §4 supplies $(d^-_{s,x}, d^+_{s,x})$ at (6.3) <u>for all x</u>. Then in (6.4),

$$[D^-_{s,x}, D^+_{s,x}] \equiv [(x_s - x)d^-/w_s Q^{\frac{1}{2}}, (x_s - x)d^+/w_s Q^{\frac{1}{2}}]$$

ready for (6.5).

7. DISCUSSION

Detection limits are valuable to a researcher as an indication of quality of existing methods and of the need for refinement of those methods. They provide another criterion for comparisons among alternative methods. They are also a useful feature to report in communications to other researchers when describing measurement techniques and the meaning of measurements. In the context of statutory limits, characterization of detectability is an essential ingredient when evaluating methods: a method with detection limit exceeding the statutory limit is obviously inadequate. The general formulations in §3 and §5 provide definitions of parameters, and practical procedures for their estimation, that are required to characterize detectability. One option, not elaborated here but implicit throughout, is the possibility of evaluating the effects of different calibration designs on detectability using existing estimates $\hat{\alpha}$, $\hat{\underline{\beta}}$ and $\hat{\sigma}$. Then it is possible to match a detection protocol to a particular criterion of detectability.

There remains the important matter of choosing rates p and q when evaluating and reporting detectability. There is a long tradition of conservatism, indeed scepticism, inherited from the philosophy of scientific method, when reporting 'positive' results (especially in technical journals). It operates by emphasizing repeatability and protecting against false positives, in the general sense, by adopting Type I error levels set at $p = 0.05$ or 0.01. This mode of inference is more behavorial than inductive and these levels are quite arbitrary. Perhaps the best argument in their defense is the empirical justification offered by Bross (1971): they do appear to have controlled the

rate of misinformation in communications among researchers at a conventional low level. To Bross they are linguistic conventions in such communications, conventions that have evolved and survived because they are useful.

In the context of analyte detection p and q have natural frequency interpretations (rates) in repeated applications, so that part of the philosophical dispute is avoided. If rates of false positives in this context are to be set by convention also, then it is advisable to incorporate this precedent and adopt the same levels of protection in the same communications. Consequences of such a convention should not be overlooked however. Arbitrary designation of p implies that all benefits of improved sensitivity and calibration design, and of increases in sample sizes n and r, are then devoted to higher detection rates at all concentrations $x >$ zero, and therefore lower detection limits at specified assurance level $(1-q)$, all at the expense of an arbitrarily fixed rate of false positives, p. There will be combinations of protocol sensitivity, calibration designs and sample sizes, for which p can be set below these conventional levels without serious losses in detectability.

When describing detection limits to other researchers there is no such precedent or convention for choice of assurance probability $(1-q)$. A convenient convention for analyte detection would be $p = kq$ with k a preassigned constant (see Lehmann (1958) for constructions of this type in a more general setting). The choice $k = 1$ is assumed for values of Δ given in Table 1. A similar table can be prepared for other choices: $k = 5$, for example, yields $(1-q) = 0.998$ when $p = 0.01$ and $(1-q) = 0.99$ when $p = 0.05$. Another suggestion, also convenient, is to borrow a convention from toxicology and report estimates of the 'median detectable concentration' (MDC or X_{50}) along

with p : MDC = $x_\ell(p,0.5)$ of course. But convenience is not a dominant criterion and it is preferable to report estimates of detection limits for several choices of q, or of detection rates at several analyte concentrations (as in Figures 2 and 3) for each p. These suggestions are also appropriate in some other areas of technical communications where characterizations of detectability are valuable in demonstrating technical competence when selling services or competing for contracts that contain detection specifications. Such characterizations can be included in expert testimony, in legislative hearings and before Administrative Law Courts for example, whether defending or disputing the quality and suitability of a particular detection protocol.

The convention q = p, when reporting detection limit $x_\ell(p,q)$, corresponds to a reversal of the 'equal probability' test criterion (Arrow, 1960). Instead of a preassigned departure ($X = x_\ell$), from the null condition (X = zero), at which the probability of a Type II error (false negative) is to be equal to the probability of a Type I error (false positive) because at that departure the costs of these different errors are equal, this convention defines x_ℓ as that departure at which q = p with p preassigned. In communications among researchers there are no habitual formulations of costs of false positives or false negatives, which explains in part why conventional levels of protection are considered so useful: they provide a convenient refuge. But this is not the case in the context of statutory limits where costs can be characterized at least on a relative basis. A false alert, for example, incurs costs that are not functions of $x < x_s$ and can be estimated precisely. Appeal to traditional linguistic conventions is inappropriate in such a context. In

cases where detection protocol and assurance level (1-q) are to be stipulated in a contract, or in guidelines attendant to a statute, there will be interested parties with conflicting demands regarding q, especially if protocol sensitivity results in levels p(x) that promise to be costly in false alerts. In the arena of public policy, where social costs are invoked, this conflict occurs among increasingly antagonistic camps with their vehement public advocates; the likelihood of rational resolution of q, by negotiation and compromise, diminishes accordingly. A two point decision space (compliance versus noncompliance) is too simplistic for detection rules in these circumstances.

8. REFERENCES

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Table 1. Values M_v, t_p and Δ for specified v and p=q=0.05, 0.01 and 0.001 (see footnotes).

v	M_v	$t_{.05}$	$t_{.01}$	$t_{.001}$	$\Delta_{.05}$	$\Delta_{.01}$	$\Delta_{.001}$
5	1.18942	2.01505	3.36493	5.89343	3.86994	6.68320	12.60124
6	1.15124	1.94318	3.14267	5.20763	3.75160	6.21267	10.88815
7	1.12587	1.89458	2.99795	4.78529	3.67302	5.91456	9.86151
8	1.10778	1.85955	2.89646	4.50079	3.61713	5.71003	9.18600
9	1.09424	1.83311	2.82144	4.29681	3.57538	5.56152	8.71150
10	1.08372	1.81246	2.76377	4.14370	3.54304	5.44903	8.36169
11	1.07532	1.79588	2.71808	4.02470	3.51725	5.36100	8.09409
12	1.06844	1.78229	2.68100	3.92963	3.49622	5.29030	7.88328
13	1.06272	1.77093	2.65031	3.85198	3.47872	5.23231	7.71324
14	1.05788	1.76131	2.62449	3.78739	3.46396	5.18390	7.57337
15	1.05373	1.75305	2.60248	3.73283	3.45133	5.14291	7.45640
16	1.05014	1.74588	2.58349	3.68615	3.44041	5.10776	7.35722
17	1.04700	1.73961	2.56693	3.64577	3.43087	5.07727	7.27212
18	1.04423	1.73406	2.55238	3.61048	3.42245	5.05060	7.19830
19	1.04176	1.72913	2.53948	3.57940	3.41499	5.02706	7.13372
20	1.03956	1.72472	2.52798	3.55181	3.40833	5.00615	7.07674
21	1.03758	1.72074	2.51765	3.52715	3.40232	4.98744	7.02611
22	1.03579	1.71714	2.50832	3.50499	3.39690	4.97059	6.98084
23	1.03416	1.71387	2.49987	3.48496	3.39198	4.95537	6.94012
24	1.03267	1.71088	2.49216	3.46678	3.38749	4.94153	6.90332
25	1.03130	1.70814	2.48511	3.45019	3.38338	4.92890	6.86989
26	1.03005	1.70562	2.47863	3.43500	3.37960	4.91732	6.83939
27	1.02889	1.70329	2.47266	3.42103	3.37611	4.90667	6.81144
28	1.02782	1.70113	2.46714	3.40816	3.37288	4.89684	6.78577
29	1.02683	1.69913	2.46202	3.39624	3.36989	4.88774	6.76208
30	1.02590	1.69726	2.45726	3.38518	3.36710	4.87930	6.74017
32	1.02423	1.69389	2.44868	3.36531	3.36207	4.86411	6.70094
34	1.02276	1.69092	2.44115	3.34793	3.35765	4.85082	6.66681
36	1.02145	1.68830	2.43449	3.33262	3.35374	4.83910	6.63686
38	1.02029	1.68595	2.42857	3.31903	3.35025	4.82870	6.61039
40	1.01925	1.68385	2.42326	3.30688	3.34713	4.81939	6.58680
42	1.01831	1.68195	2.41847	3.29595	3.34431	4.81101	6.56566
44	1.01746	1.68023	2.41413	3.28607	3.34176	4.80343	6.54660
46	1.01668	1.67866	2.41019	3.27710	3.33944	4.79655	6.52934
48	1.01597	1.67722	2.40658	3.26891	3.33730	4.79027	6.51363
50	1.01532	1.67591	2.40327	3.26141	3.33536	4.78451	6.49927
52	1.01472	1.67469	2.40022	3.25451	3.33356	4.77921	6.48609
54	1.01416	1.67356	2.39741	3.24815	3.33189	4.77433	6.47396
56	1.01365	1.67252	2.39480	3.24226	3.33035	4.76981	6.46275
58	1.01317	1.67155	2.39238	3.23680	3.32892	4.76561	6.45237
60	1.01272	1.67065	2.39012	3.23171	3.32759	4.76170	6.44272
65	1.01173	1.66864	2.38510	3.22041	3.32462	4.75302	6.42134
70	1.01088	1.66691	2.38081	3.21079	3.32207	4.74562	6.40318
75	1.01014	1.66543	2.37710	3.20249	3.31989	4.73923	6.38757
80	1.00950	1.66412	2.37387	3.19526	3.31796	4.73367	6.37400
85	1.00893	1.66298	2.37102	3.18890	3.31628	4.72877	6.36210
90	1.00843	1.66196	2.36850	3.18327	3.31478	4.72444	6.35157
95	1.00798	1.66105	2.36624	3.17825	3.31344	4.72057	6.34221
100	1.00758	1.66023	2.36422	3.17374	3.31224	4.71711	6.33380
INF	1.00000	1.64485	2.32635	3.09023	3.28971	4.65270	6.18046

Column 1: v = degrees of freedom for estimate $\hat{\sigma}^2$.

Column 2: M_v such that expectation of $1/\hat{\sigma}$ is M_v/σ.

Columns 3,4,5: values t_p such that $Pr[T(0) > t_p] = p$ (Students's t).

Columns 6,7,8: values Δ such that $Pr[T(\Delta) < t_p] = p$ (noncentral t).

(Captions for Figures 1,2,3)

Figure 1. Construction of interval estimate (x_ℓ^-, x_ℓ^+), for the detection limit $x_\ell(p,q)$, using inverse interpolation.

Figure 2. Estimated detection rates for the tungsten content example with $r = 1,2,$ or 3 and $p = 0.01$.

Figure 3. Interval estimates ($\gamma = 0.05$) of detection rates for the tungsten content example with $r = 1$ and $p = 0.01$.

Fig. 1

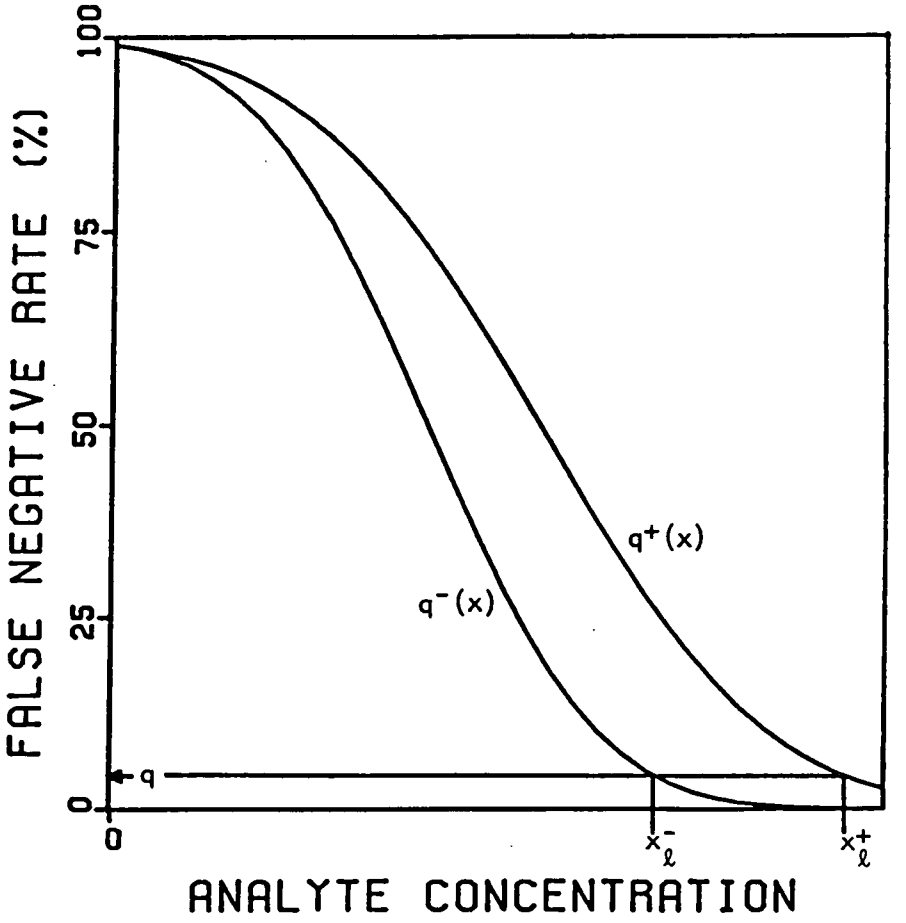

FALSE NEGATIVE RATE (%)
100
75
50
25
0
q
0
x_ℓ^-
x_ℓ^+
$q^+(x)$
$q^-(x)$
ANALYTE CONCENTRATION

Fig. 2

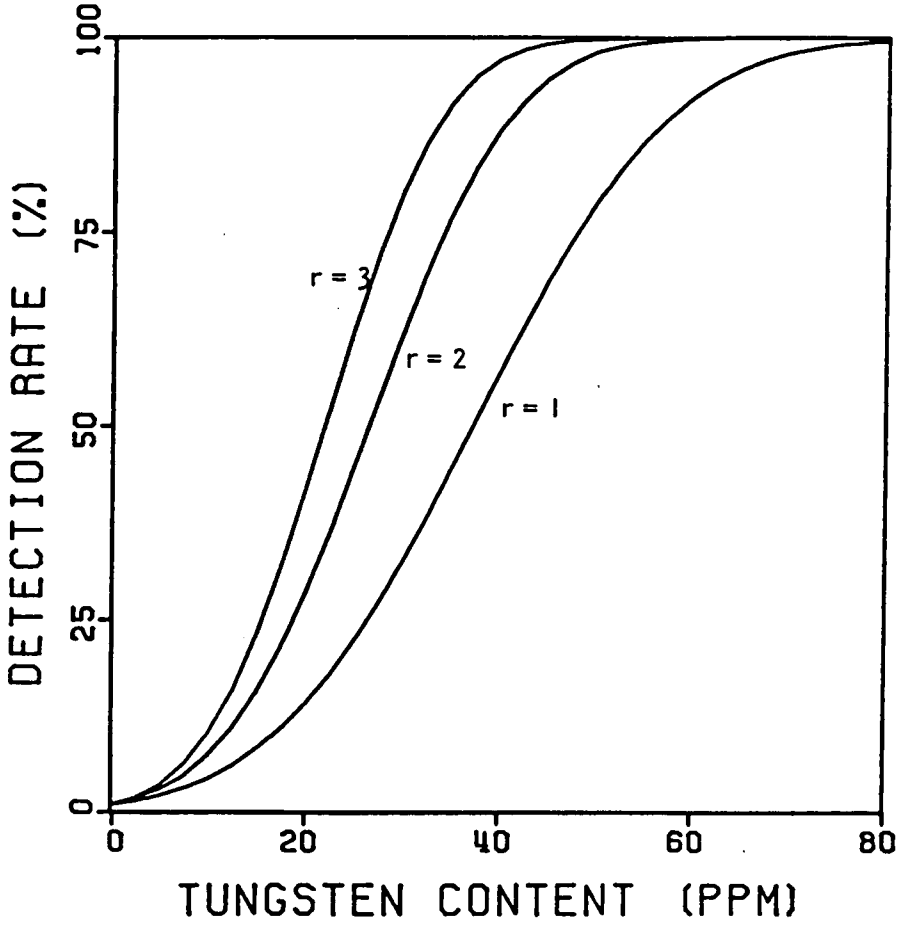
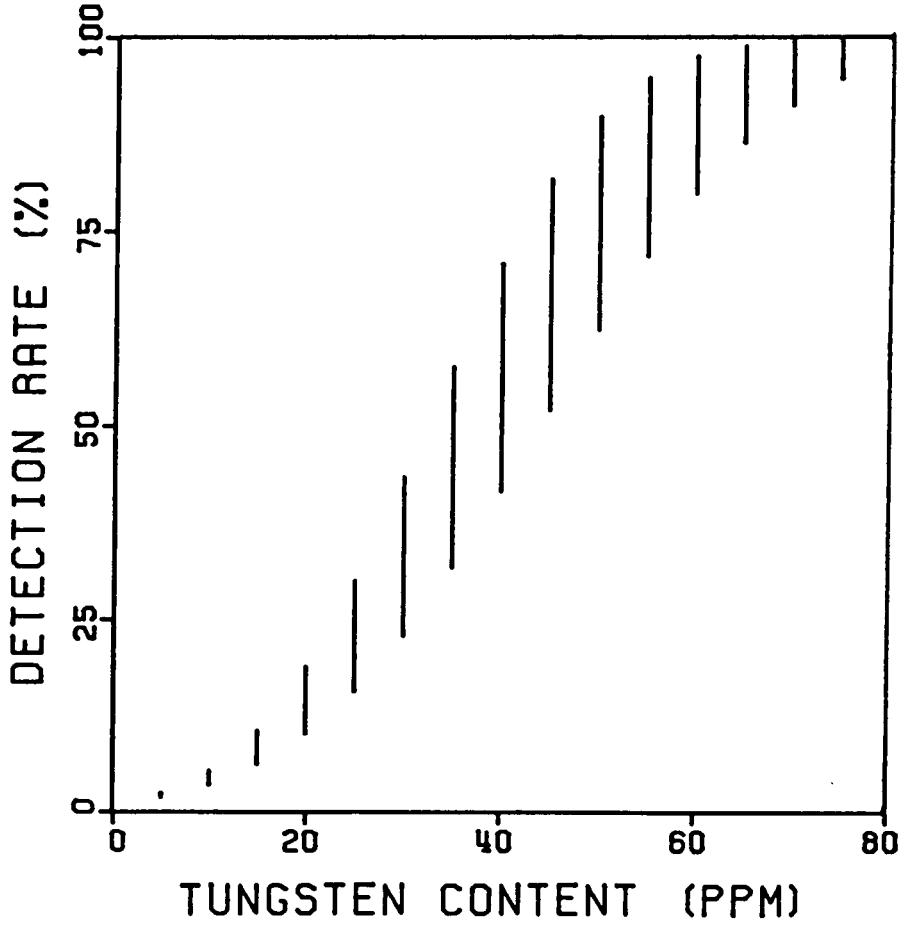

DETECTION RATE (%)
100
75
50
25
0
r = 3
r = 2
r = 1
0
20
40
60
80
TUNGSTEN CONTENT (PPM)

Fig. 3


DETECTION RATE (%)
0
25
50
75
100
0
20
40
60
80
TUNGSTEN CONTENT (PPM)

APPENDIX B

SAS Algorithms for Determining Parameters Associated With the Noncentral t-Distribution

[Author: Peter M. Burrows]

```
46          MACRO NCTP;
47          *                                                                  ;
48          * ASSUMES T(V,DELTA) FOLLOWS NON-CENTRAL T DISTRIBUTION            ;
49          * ON V DEGREES OF FREEDOM, NON-CENTRALITY PARAMETER DELTA:         ;
50          *                                                                  ;
51          * LOAD    : V IN PV, DELTA IN PD, T IN PT                          ;
52          *                                                                  ;
53          * RETURNS : Q(DELTA,T,V) = PR( T(V,DELTA) < T ) IN PQ              ;
54          *           DERIVATIVE Q'(DELTA,T,V) IN PQD                        ;
55          *           PR( T(V,ZERO) > T ) IN PP (STUDENT'S T)                ;
56          *                                                                  ;
57          * CONDITIONS : DELTA > -6 IF T > ZERO (OTHERWISE Q = 1)            ;
58          *              DELTA <  6 IF T < ZERO (OTHERWISE Q = 0)            ;
59          *                                                                  ;
60          * PETER M. BURROWS/EXST/CE .NCT1/MAY 4,1985                        ;
61          *                                                                  ;
62          PPI=0.398942280401432;
63          IF PT=0 THEN DO; PQ=PROBNORM(-PD); PQD=-PPI*EXP(-0.5*PD*PD);
64          PP=0.5; GO TO P1; END;
65          PP=1-PROBT(PT,PV);
66          PNI=1; IF PT < 0 THEN PNI=-1;
67          PT=PNI*PT; PD=PNI*PD;
68          PA=-6; IF PD < 6 THEN PA=-PD; PB=6; PC=PB-PA;
69          PCV=PV/(PT*PT);
70          PQ=0; PQD=0;
71          ARRAY PU (PI) PU1-PU8;
72          ARRAY PZ PZ1-PZ16;
73          ARRAY PW PW1-PW16;
74          PZ1 =0.0483076656877383; PW1 =0.0965400885147278;
75          PZ2 =0.1444719615827965; PW2 =0.0956387200792749;
76          PZ3 =0.2392873622521371; PW3 =0.0938443990808046;
77          PZ4 =0.3318686022821276; PW4 =0.0911738786957639;
78          PZ5 =0.4213512761306353; PW5 =0.0876520930044038;
79          PZ6 =0.5068999089322294; PW6 =0.0833119242269468;
80          PZ7 =0.5877157572407623; PW7 =0.0781938957870703;
81          PZ8 =0.6630442669302152; PW8 =0.0723457941088485;
82          PZ9 =0.7321821187402897; PW9 =0.0658222227763618;
83          PZ10=0.7944837959679424; PW10=0.0586840934785355;
84          PZ11=0.8493676137325699; PW11=0.0509980592623762;
85          PZ12=0.8963211557660521; PW12=0.0428358980222267;
86          PZ13=0.9349060759377397; PW13=0.0342738629130214;
87          PZ14=0.9647622555875064; PW14=0.0253920653092621;
88          PZ15=0.9856115115452683; PW15=0.0162743947309057;
89          PZ16=0.9972638618494816; PW16=0.0070186100094701;
90          DO OVER PZ;
91          PF=0; PFD=0;
92          PU1=(7*PA+PB-PC*PZ)/8; PU2=(7*PA+PB+PC*PZ)/8;
93          PU3=PU1+PC/4;          PU4=PU2+PC/4;
94          PU5=PU3+PC/4;          PU6=PU4+PC/4;
95          PU7=PU5+PC/4;          PU8=PU6+PC/4;
96          DO OVER PU; PCH=PCV*((PU+PD)**2);
97          PE=PPI*(EXP(-0.5*PU*PU))*(PROBCHI(PCH,PV));
98          PF=PF+PE; PFD=PFD+PU*PE; END;
99          PQ=PQ+PW*PF; PQD=PQD+PW*PFD; END; PQD=-PC*PQD/8;
100         IF PNI=1 THEN PQ=1-PC*PQ/8;
101         ELSE DO; PQ=PC*PQ/8; PD=PNI*PD; PT=PNI*PT; END;
```

```
102         *                                                                ;
103         * CLEAN UP (DROP ALL BUT PV PD PT PQ PQD PP)                      ;
104         *                                                                ;
105         DROP PA PB PC PCH PCV PE PF PFD PI PNI PU1-PU8 PW1-PW16 PZ1-PZ16;
106         P1: DROP PPI;
107         %
108         MACRO NCTD;
109         *                                                                ;
110         * ASSUMES T(V,DELTA) FOLLOWS NON-CENTRAL T DISTRIBUTION          ;
111         * ON V DEGREES OF FREEDOM, NON-CENTRALITY PARAMETER DELTA:       ;
112         *                                                                ;
113         * LOAD    :  V IN DV, T IN DT, Q IN DQ                           ;
114         *                                                                ;
115         * RETURNS :  DELTA IN DD SATISFYING PR( T(V,DELTA, < T ) = Q     ;
116         *                                                                ;
117         * USES MACRO NCTP                                                ;
118         *                                                                ;
119         * PETER M. BURROWS/EXST/CE .NCT2/MAY 4,1985                      ;
120         *                                                                ;
121         DD1=DT-PROBIT(DQ)*SQRT(1+DT*DT/(2*DV)); PV=DV; PT=DT;
122         D1: PD=DD1; NCTP;
123             DD=DD1+(DQ-PQ)/PQD;
124             IF ABS(DD-DD1) < 0.000001 THEN GO TO D2;
125             DD1=DD; GO TO D1;
126         D2: DROP DD1;
127         %
128         *                                                                ;
129         * 5/04/85                                                        ;
130         *                                                                ;
131         * EXAMPLE ONE:                                                   ;
132         *                                                                ;
133         * GET PR( T(DF,DELTA) < T ) & PR( T(DF,0) > T ) GIVEN DF, DELTA ,T ;
134         *                                                                ;
135         DATA ONE; TITLE 'EXAMPLE ONE'; INPUT DF DELTA T;
136         PV=DF; PD=DELTA; PT=T;
137         NCTP; PROBD=PQ; PROBO=PP;
200         CARDS;
```

NOTE: DATA SET WORK.ONE HAS 6 OBSERVATIONS AND 11 VARIABLES. 510 OBS/TRK.
NOTE: THE DATA STATEMENT USED 0.41 SECONDS AND 100K.

```
207         PROC SORT; BY DF PROBD;
```

NOTE: 4 CYLINDERS DYNAMICALLY ALLOCATED ON DISK FOR EACH OF 3 SORT WORK DATA SETS.
NOTE: DATA SET WORK.ONE HAS 6 OBSERVATIONS AND 11 VARIABLES. 510 OBS/TRK.
NOTE: THE PROCEDURE SORT USED 0.38 SECONDS AND 200K.

```
208         PROC PRINT; BY DF; ID DF; VAR DELTA T PROBD PROBO;
209         *                                                                ;
210         * EXAMPLE TWO                                                    ;
211         *                                                                ;
212         * GET DELTA SUCH THAT PR( T(DF,DELTA) < T ) = Q  GIVEN DF, T, Q  ;
213         *                                                                ;
```

NOTE: THE PROCEDURE PRINT USED 0.11 SECONDS AND 132K AND PRINTED PAGE 1.

```
214        DATA TWO; TITLE 'EXAMPLE TWO'; INPUT DF T Q;
215        DV=DF; DT=T; DQ=Q;
216        NCTD; DELTA=DD;
299        CARDS;

NOTE: DATA SET WORK.TWO HAS 18 OBSERVATIONS AND 14 VARIABLES. 404 OBS/TRK.
NOTE: THE DATA STATEMENT USED 1.52 SECONDS AND 108K.

318        PROC SORT; BY DF Q;

NOTE: DATA SET WORK.TWO HAS 18 OBSERVATIONS AND 14 VARIABLES. 404 OBS/TRK.
NOTE: THE PROCEDURE SORT USED 0.25 SECONDS AND 200K.

319        PROC PRINT; BY DF; ID DF; VAR T Q DELTA;

NOTE: THE PROCEDURE PRINT USED 0.11 SECONDS AND 132K AND PRINTED PAGE 2.
NOTE: SAS USED 200K MEMORY.

NOTE: SAS INSTITUTE INC.
      SAS CIRCLE
      PO BOX 8000
      CARY, N.C.  27511-8000
```

EXAMPLE ONE

DF	DELTA	T	PROBD	PROBO
8	9.18600	4.50079	0.0010000	0.0010000
	5.71003	2.89646	0.0100000	0.0100000
	3.61713	1.85955	0.0499999	0.0499998
48	6.51363	3.26891	0.0010000	0.0010000
	4.79027	2.40658	0.0100000	0.0100000
	3.33730	1.67722	0.0500004	0.0500004

EXAMPLE TWO

DF	T	Q	DELTA
8	4.50079	0.0010000	9.18600
	2.89646	0.0100000	5.71003
	1.85955	0.0500000	3.61713
16	3.68615	0.0010000	7.35722
	2.58349	0.0100000	5.10776
	1.74588	0.0500000	3.44041
24	3.46678	0.0010000	6.90332
	2.49216	0.0100000	4.94153
	1.71088	0.0500000	3.38749
32	3.36531	0.0010000	6.70094
	2.44868	0.0100000	4.86411
	1.69389	0.0500000	3.36207
40	3.30688	0.0010000	6.58680
	2.42326	0.0100000	4.81939
	1.68385	0.0500000	3.34713
48	3.26891	0.0010000	6.51363
	2.40658	0.0100000	4.79027
	1.67722	0.0500000	3.33730

APPENDIX C

Definition and Procedure for the Determination of the Method Detection Limit [EPA-Revision 1.11]

Definition

The method detection limit (MDL) is defined as the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing analyte.

Scope and Application

This procedure is designed for applicability to a wide variety of sample types ranging from reagent (blank) water containing analyte to wastewater containing analyte. The MDL for an analytical procedure may vary as a function of sample type. The procedure requires a complete, specific and well defined analytical method. It is essential that all sample processing steps of the analytical method be included in the determination of the method detection limit.

The MDL obtained by this procedure is used to judge the significance of a single measurement of a future sample.

The MDL procedure was designed for applicablity to a broad variety of physical and chemical methods. To accomplish this, the procedure was made device- or instrument-independent.

Procedure

1. Make an estimate of the detection limit using one of the following:

(a) The concentration value that corresponds to an instrument signal/noise in the range of 2.5 to 5.

(b) The concentration equivalent of three times the standard deviation of replicate instrumental measurements of the analyte in reagent water.

(c) That region of the standard curve where there is a significant change in sensitivity, i.e., a break in the slope of the standard curve.

(d) Instrumental limitations.

It is recognized that the experience of the analyst is important to this process. However, the analyst must include the above considerations in the initial estimate of the detection limit.

2. Prepare reagent (blank) water that is as free of analyte as possible. Reagent or interference free water is defined as a water sample in which analyte and interferent concentrations are not detected at the method detection limit of each analyte of interest. Interferences are defined as systematic errors in the measured analytical signal of an established procedure caused by the presence of interfering species (interferent). The interferent concentration is presupposed to be normally distributed in representative samples of a given matrix.

3. (a) If the MDL is to be determined in reagent (blank) water, prepare a laboratory standard (analyte in reagent water) at a concentration which is at least equal to or in the same concentration range as the estimated method detection limit. (Recommend between 1 and 5 times the estimated method detection limit.) Proceed to Step 4.

(b) If the MDL is to be determined in another sample matrix, analyze the sample. If the measured level of the analyte is in the recommended range of one to five times the estimated detection limit, proceed to Step 4.

If the measured level of analyte is less than the estimated detection limit, add a known amount of analyte to bring the level of analyte between one and five times the estimated detection limit.

If the measured level of analyte is greater than five times the estimated detection limit, there are two options.

(1) Obtain another sample with a lower level of analyte in the same matrix if possible.

(2) The sample may be used as is for determining the method detection limit if the analyte level does not exceed 10

times the MDL of the analyte in reagent water. The variance of the analytical method changes as the analyte concentration increases from the MDL, hence the MDL determined under these circumstances may not truly reflect method variance at lower analyte concentrations.

4. (a) Take a minimum of seven aliquots of the sample to be used to calculate the method detection limit and process each through the entire analytical method. Make all computations according to the defined method with final results in the method reporting units. If a blank measurement is required to calculate the measured level of analyte, obtain a separate blank measurement for each sample aliquot analyzed. The average blank measurement is subtracted from the respective sample measurements.

(b) It may be economically and technically desirable to evaluate the estimated method detection limit before proceeding with 4a. This will: (1) prevent repeating this entire procedure when the costs of analyses are high and (2) insure that the procedure is being conducted at the correct concentration. It is quite possible that an inflated MDL will be calculated from data obtained at many times the real MDL even though the level of analyte is less than five times the calculated method detection limit. To insure that the estimate of the method detection limit is a good estimate, it is necessary to determine that a lower concentration of analyte will not result in a

significantly lower method detection limit. Take two aliquots of the sample to be used to calculate the method detection limit and process each through the entire method, including blank measurements as described above in 4a. Evaluate these data:

(1) If these measurements indicate the sample is in desirable range for determination of the MDL, take five additional aliquots and proceed. Use all seven measurements for calculation of the MDL.

(2) If these measurements indicate the sample is not in correct range, reestimate the MDL, obtain new sample as in 3 and repeat either 4a or 4b.

5. Calculate the variance (S^2) and standard deviation (S) of the replicate measurements, as follows:

$$S^2 = \frac{1}{n-1}\left[\sum_{i=1}^{n} X_i^2 - \left(\sum_{i=1}^{n} X_i\right)^2 \Big/ n\right]$$

$$S = (S^2)^{1/2}$$

where:

X_i; i=1 to n, = are the analytical results in the final method reporting units obtained from the n sample aliquots and Σ refers to the sum of the X values from i=1 to n.

where: LCL and UCL are the lower and upper 95% confidence limits respectively based on seven aliquots.

7. Optional iterative procedure to verify the reasonableness of the estimate of the MDL and subsequent MDL determinations.

a.) If this is the initial attempt to compute MDL based on the estimate of MDL formulated in Step 1, take the MDL as calculated in Step 6, spike in the matrix at the calculated MDL and proceed through the procedure starting with Step 4.

b.) If this is the second or later iteration of the MDL calculation, use S^2 from the current MDL calculation and S^2 from the previous MDL calculation to compute the F-ratio. The F-ratio is calculated by substituting the larger S^2 into the numerator S_2^A and the others into the denominator S_B^2. The computed F-ratio is then compared with the F-ratio found in the table which is 3.05 as follows:

$$\text{if} \quad \frac{S_A^2}{S_B^2} < 3.05,$$

then compute the pooled standard deviation by the following equation:

$$S_{pooled} = \left[\frac{6S_A^2 + 6S_B^2}{12}\right]^{1/2}$$

if $\frac{S_A^2}{S_B^2} > 3.05$, respike at the most recent calculated MDL and process the samples through the procedure starting with Step 4. If the most recent calculated MDL does not permit qualitative identification when samples are spiked at that level, report the MDL as a concentration between the current and previous MDL which permits qualitative identification.

c.) Use the S_{pooled} as calculated in 7b to compute the final MDL according to the following equation:

$$MDL = 2.681 \quad (S_{pooled})$$

where 2.681 is equal to $t_{(12,\ 1-\alpha\ =\ .99)}$.

d.) The 95% confidence limits for MDL derived in 7c are computed according to the following equations derived from precentiles of the chi squared over degrees of freedom distribution.

$$LCL = 0.72\ MDL$$

$$UCL = 1.65\ MDL$$

where LCL and UCL are the lower and upper 95% confidence limits respectively based on 14 aliquots.

Table of Students' t Values at the 99 Percent Confidence Level

Number of Replicates	Degrees of Freedom (n-1)	$t_{(n-1,.99)}$
7	6	3.143
8	7	2.998
9	8	2.896
10	9	2.821
11	10	2.764
16	10	2.602
21	20	2.528
26	25	2.485
31	30	2.457
61	60	2.390
∞	∞	2.326

Reporting

The analytical method used must be specifically identified by number or title and the MDL for each analyte expressed in the appropriate method reporting units. If the analytical method permits options which affect the method detection limit, these conditions must be specified with the MDL value. The sample matrix used to determine the MDL must also be identified with MDL value. Report the mean analyte level with the MDL and indicate if the MDL procedure was iterated. If a laboratory standard or a sample that contained a known amount analyte was used for this determination, also report the mean recovery.

If the level of analyte in the sample was below the determined MDL or does not exceed 10 times the MDL of the analyte in reagent water, do not report a value for the MDL.

APPENDIX D

Extraction and Analysis of Selected Organics in Sediments by Ultrasonication

EXTRACTION AND ANALYSIS OF SELECTED ORGANICS IN SEDIMENTS BY ULTRASONICATION

1. Scope and Application
   1.1 This method was originally designed for the determination of extractable priority pollutants in sediments by GC/MS (source: W. Loy, Environmental Research Laboratory, USEPA, Athens, GA) with a detection limit of 5 ppm.
   1.2 For purposes of this experiment, the method was implemented as modified for capillary GC/FID in the evaluation of extraction methods under EPA Contract No. 68-03-3119 (Evaluation of Analytical Test Procedures for the Measurement of Organic Pollutants in Sediment)[1].
   1.3 No cleanup procedure was used in the application of the method to this experiment; however, if required, the procedures given in EPA Test Method 625-S[2] are appropriate.
2. Summary of the Method
   2.1 A 30g portion of sediment is mixed with anhydrous sodium sulfate and immediately extracted with methylene chloride/acetone (1:1) using a 300W ultrasonic probe. The extract is filtered, and concentrated by K-D and nitrogen blowdown. The extract is analyzed for selected organics by the internal standard, capillary GC/FID procedure.
3. Apparatus and Materials
   3.1 Disposable pipets, 1 mL
   3.2 Sonic cell disruptor, Heat Systems - Ultrasonics, Inc. Model 375C or equivalent (375 watt with pulsing capability and a 3/4" high-gain probe).
   3.3 Screwcap ointment jars, 16 oz
   3.4 Buchner funnels
   3.5 Filter paper (Whatman No. 41)
   3.6 Kuderna-Danish (K-D) apparatus
      3.6.1 Concentrator tube - 10 mL, graduated (Kontes K-570040-1029 or equivalent).
      3.6.2 Evaporative flask - 500 mL (Kontes K570001-0500 or equivalent).
      3.6.3 Snyder column - three-ball macro (Kontes K-503000-0121 or equivalent).
      3.6.4 Modified Snyder column - (Kontes K-569251 or equivalent).
   3.7 Boiling chips - extracted, approximately 10/40 mesh
   3.8 Nitrogen blowdown apparatus
   3.9 Steam bath or equivalent
   3.10 Gas chromatograph - analytical capillary GC/FID meeting the requirements outlined in EPA Test Method 625-S (Section 5.2)[2]
   3.11 Capillary GC column - 15 m X 0.25 mm ID wall coated open tubular capillary column coated with SE-30 or equivalent with a 0.25 um film thickness. The column should provide at least 25,000 effective theoretical plates, measured at C_{13}.

4. Reagents
   4.1 Sodium sulfate - Baker anhydrous powder.
   4.2 Methylene chloride - pesticide quality, distilled in glass.
   4.3 Acetone - pesticide quality, distilled in glass.
   4.4 Standard compounds
      4.4.1 Dissolve 0.060 g of individual reference materials for each analyte in pesticide quality acetone and dilute to volume in a 10 mL glass stoppered volumetric flask. Individual aliquots are combined and diluted to the appropriate volume for use. Store all solutions in a refrigerator and protect from light.
      4.4.2 Check frequently for signs of degradation or solvent evaporation (e.g., color or volume changes, precipitation).
5. Calibration
   5.1 For this experiment, no separate instrument calibration was used. Where required, calibration is performed according to EPA Test Method 625-S (Section 7.0)[2] using triplicate standards at three concentrations corresponding to 20, 50, and 100% of the sediment fortification level.
6. Quality Control
   6.1 In this experiment, quality control was provided by statistical procedures. Separate analytical quality control procedures as outlined in EPA Test Method 625-S[2] were not applied. Where required, appropriate quality control procedures are outlined in EPA Test Method 625-S (Section 8.0)[2].
7. Sample Collection, Preservation, and Handling
   7.1 Grab samples must be collected in glass containers. Conventional sampling practices should be followed except that the bottle must not be pre-rinsed with sample before collection. Composite samples should be collected in accordance with the requirements of the program. Automatic sampling equipment must be free of potential sources of contamination.
   7.2 The samples must be maintained at 4°C in the dark from the time of collection until extraction.
8. Sample Extraction
   8.1 Decant and discard the water layer over the sediment. Mix samples thoroughly and discard foreign objects such as sticks, leaves, and rocks using a 1/4" screen.
   8.2 Weigh 30 g of sample into a 16 oz jar and add 65 g of anhydrous sodium sulfate. Mix well and add more sodium sulfate as necessary to achieve a sandy texture. Immediately add surrogates (if used) and the extraction solvent (see step 8.3).
   8.3 Add 100 mL of 1:1 methylene chloride/acetone to the sediment/sodium sulfate mixture.
   8.4 Place the sonic probe ca 1 cm below the surface of the solvent but above the sediment layer.
   8.5 Sonicate for 3 min at full power with a 50% pulse.
   8.6 Decant the solvent into a Buchner funnel and suction filter. Repeat 8.3-8.6 twice more.
   8.7 Pour the entire sample into a Buchner funnel and rinse with 1:1

methylene chloride/acetone.

8.8 Concentrate the extract to 5-7 mL using the K-D apparatus and subsequently to ca 1 mL by N_2 blowdown for the final concentration.

NOTE: Discontinue evaporation if the concentrated extract becomes excessively viscous or if precipitation occurs. Redilute with the appropriate solvent, if necessary, and record final volume.

9. Sample Extract Analysis

9.1 Capillary GC/MS analysis of sample extracts is outlined in EPA Test Method 625-S (Section 12.1.1)[2].

9.2 Capillary GC/FID analysis of sample extracts is described below.

9.2.1 Instrumentation: Varian Model 3700 GC equiped with FID detector and Grob-type splitless/split capillary injector.

9.2.2 Capillary Column: DB-5 (SE-54) WCOT, fused silica column (15m X .25 mm ID), .25 um film thickness.

9.2.3 Conditions: Injector temperature, 240°C
Detector temperature, 260°C
Column temperature, 70°C for 3 min
70-220°C at 5°/min

Splitless time, 60 sec
Injection volume, 1.5 uL

Carrier gas, helium at 0.6 mL/min (column)
Septum purge, 2.2 mL/min
Injector split, 76.5 mL/min
Detector gases, air at 287 mL/min
hydrogen at 47 mL/min
Makeup gas (detector), helium at 35 mL/min

9.2.4 A representative capillary GC/FID chromatogram of the analytes included in this study is shown in Figure 1.

10. References

1. Hines, J. W. Evaluation of Analytical Test Procedures for the Measurement of Organic Pollutants in Sediment. EPA Contract No. 68-03-3119, Draft Final Report, U.S. Environmental Protection Agency, Cincinnati, Ohio, June, 1985.

2. Test Method 625-S: Protocol for the Analysis of Extractable Organic Priority Pollutants in Industrial and Municipal Wastewater Treatment Sludge. Environmental Monitoring and Support Laboratory. U.S. EPA, Cincinnati, Ohio, December 10, 1982.

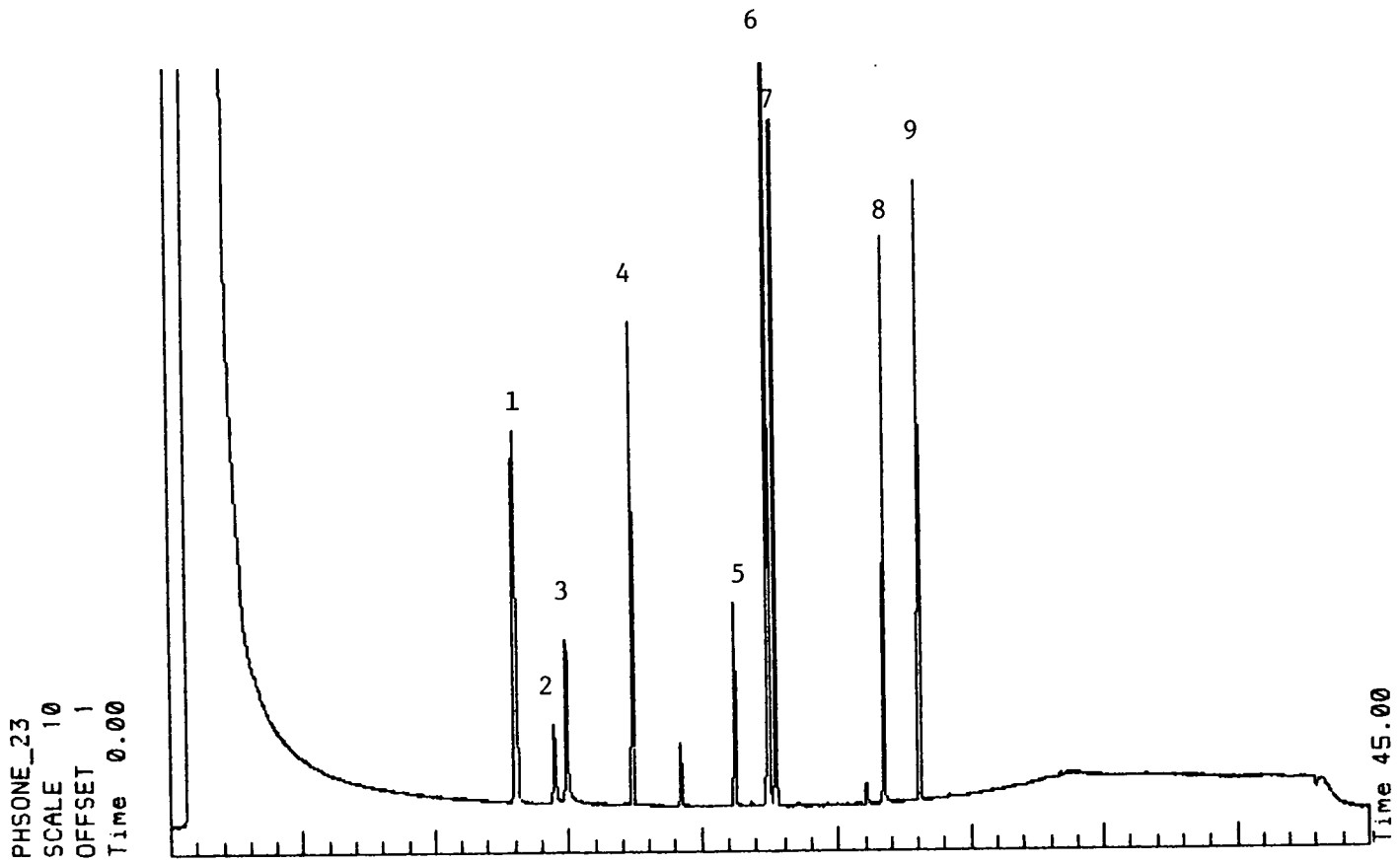


Figure 1 Representative capillary GC/FID chromotogram of ≈ 0.8 ppm standard.
Peak identity: 1 = 2-chloronaphthalene; 2 = 2,6-dinitrotoluene;
3 = dimethylphthalate; 4 = pentadedane;
5 = hexachlorobenzene; 6 = anthracene;
7 = phenanthrene; 8 = aldrin;
9 = fluoranthene.