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STATISTICAL APPROACH TO PREDICTING CHRONIC TOXICITY OF CHEMICALS TO FISHES FROM ACUTE TOXICITY TEST DATA

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PREFACE

The Office of Research and Development initiated a multi-laboratory Ecological Risk Assessment Research Program in 1986 to develop scientifically defensible methods for use by the Office of Pesticides and Toxic Substances (OPTS) in assessing ecological risks. The Ecological Risk Assessment Research Program provides the technical basis to improve Agency risk assessments for chemicals in view of the Agency's interest in protecting ecological resources and the OPTS state of the practice in conducting ecological risk assessments. Many research needs remain, and the demands on OPTS to consider risks to ecological resources in chemical regulation will continue to grow.

The area of ecological risk assessment described in this report involves a major advancement in predictive toxicology. For the last 20 years, we have continued to use and refine various acute-chronic ratios and correlation analyses of acute (LC50s) and chronic data (maximum acceptable toxicant concentrations) to estimate chronic toxicity from acute data. Until this research was conducted, no accurate method for truly predicting, and not estimating, chronic toxicity existed.

A technically defensible concept and methodology are described wherein simultaneous consideration is given to exposure, degree of response, and time course of effect, all of which are usually included in the results of an acute test, but seldom used. The predictive technique may reduce chronic testing requirements and will be highly beneficial in initial chronic assessments of chemicals and effluents and in predicting chronic toxicity for species difficult to culture, including those that are rare and endangered.

ABSTRACT

A comprehensive approach to predicting chronic toxicity from acute toxicity data was developed in which simultaneous consideration is given to concentration, degree of response, and time course of effect. A consistent endpoint (lethality) and degree of response (0%) were used to compare acute and chronic tests. Predicted no-effect concentrations were highly accurate 92% of the time (within a factor of 2.0 of the limits of the maximum acceptable toxicant concentrations for lethality) and did not vary by more than a factor of 4.8 when the technique was applied to a data base of 18 chemicals and 7 fish species. Growth effects can be predicted from chronic lethality, but reproductive effects should not be.

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INTRODUCTION

Using acute lethality data to estimate chronic toxicity to fishes customarily involves deriving an application factor (Mount and Stephan 1967) or an acute-chronic ratio (Kenaga 1982), both of which require acute-and chronic toxicity testing. Kenaga (1979) reviewed the principal measurements of the acute LC50, the maximum acceptable toxicant concentration (MATC), and the application factor (AF) used in determining chronic no-effect concentrations for many chemicals. The application factor is derived by dividing the MATC for a compound, as determined in a chronic toxicity test with a given fish species, by the acute flow-through LC50 for the same compound tested with the same species. The acute-chronic ratio (ACR) is the inverse of AF. The AF or ACR is then used to estimate chronic no-effect concentrations for other species for which only acute toxicity data exist (Buikema et al. 1982). Both approaches have limitations in using these ratios to estimate chronic toxicity.

One limitation is that the biological endpoints and degrees of response are often not comparable between acute and chronic toxicity data. When one uses either the AF or ACR, the acute median lethal concentration (LC50) is compared with the MATC, often derived from an endpoint other than lethality. Even though the mode of action for lethality is often assumed to be the same under acute and chronic exposures, the mode of action may not be the same for different endpoints (e.g., growth or reproduction compared with lethality). Although different degrees of response (acute 50%

versus chronic no-effect or 0%) could be used when response slopes are similar, the slopes may be different. Additionally, the use of the AF or ACR method does not take into consideration the progression of lethality through time that is observed in acute toxicity tests. The concentration-time-response interaction has been addressed by Shirazi and Lowrie (1988), but they directed their efforts toward better defining the LC50. The acute toxicity value represents only one point in time (96-h LC50), and the progression of degree of response with duration of exposure should be essential when one predicts chronic toxicity from acute toxicity data.

A more comprehensive, alternative approach is proposed here in which simultaneous consideration is given to concentration, degree of response, and time course of effect, all of which are usually included in the results of an acute test, but seldom used. A consistent endpoint (lethality) and degree of response (0%) are used to predict chronic lethality from acute toxicity tests. Two assumptions may be required: (1) concentration-response is a continuum in time, and (2) the mode of action for lethality is similar under acute and chronic exposures.

METHODS

Simple linear regression (Y = a+bX) was used to derive lethal concentrations of no effect (LC0 = 0.01%) for each observation time in an acute toxicity test and to predict the chronic no-effect concentration for lethality from those LC0's.

Degree of Response

In chronic toxicity tests, we are most often interested in the no-effect concentration (e.g., that concentration causing 0% effect), whereas in acute tests, the degree of response usually used is 50%. Although a probit value (Finney 1971) does not exist for 0% or 100%, an approximate value can be derived. In the use of probit analysis of acute toxicity data (Finney 1971, Litchfield and Wilcoxon 1949), the probit value used for 100% mortality is actually the probit value for 99.99%. An approximate value for LC0 can thus be derived by subtracting the probit value for 99.99% (8.7190) from 10 to provide a probit value of 1.2810 for 0.01% mortality.

Time Course of Effect

Predicting chronic toxicity from acute toxicity data requires a means of estimating the LC0 for an indefinite period of time (chronic) from an acute toxicity test conducted over a finite period of time (96-h LC50). Green (1965) and Sprague (1969) provided approaches to the problem of estimating tolerance over an indefinite time period, although it was with the LD50 or LC50. They noted that as the time of exposure becomes sufficiently long, the LD50 or LC50 approaches an asymptotic value. Green (1965)

suggested using a hyperbola to describe the relationship. A hyperbola can be expressed as a straight line by using the reciprocal of time (t) as the independent variable. The equation becomes LD50 = a+b(1/t). Since 1/t approaches zero as t approaches infinity, the intercept (a) represents the LD50 over an indefinite time of exposure. The proposed method for estimating LC0 makes use of Green's approach to predict chronic toxicity from acute toxicity data.

Technique

The acute toxicity test must be conducted with strict adherence to standard test methods (Committee on Methods for Toxicity and Tests with Aquatic Organisms 1975, American Society for Testing and Materials 1980) to obtain estimates of LC0 over time. The times of 24, 48, 72, and 96 hours were selected because observations in standard acute toxicity tests are usually made at these time periods. Less than 24-h observations were used when available. Inclusion of these observations is very important when most toxicity occurs during the early part of a 96-h test. The greatest concentration that causes no mortality and the least concentration that causes complete mortality were the concentrations used for 0% and 100% responses. All concentrations causing mortality ($0\% \leq x \leq 100\%$) were also included in our calculations. When regression analysis could not be conducted (less than 3 observations), the highest nonlethal concentration was used as the estimate of LC0 for that observation time. Having a range of mortalities for all time periods is best; although observation times with only 0 and 100% mortalities are acceptable

if a concentration-response is evident in time.

Linear regression analysis (Snedecor and Cochran 1980) was used to calculate the estimated LC0 at all observation times from acute flow-through tests (Fig. 1) as probit % mortality = a+b(log concentration). The LC0's at each time period were then regressed against the reciprocal of time (Fig. 2) where LC0 = a+b(1/t). The intercept (a) of this regression is the predicted no-effect concentration for chronic lethality. Log transformations, log LC0 = a+b (1/t) or log LC0 = a+b log (1/t), were required for ten tests because of negative intercepts and/or curvilinear nature of the data.

When test data permits, response-surface models (multiple regression) for analyzing all data from an acute toxicity test simultaneously (Fig. 3) are preferable to the two-step simple linear regression approach described above. We therefore developed a probit surface methodology and a user-friendly software program (simple linear and multiple regression models) to predict chronic toxicity based on acute time-exposure-effect data (Appendix A). This method is called Multifactor Probit Analysis (MPA) and uses the iterative reweighed least squares method to estimate the parameters of the probit surface. The independent variables consist of time of exposure and concentration of the toxicant. The dependent variable is the probit of the proportion responding to an exposure concentration. MPA allows the user to predict the concentration of a toxicant at any time and percent mortality as well as calculate a measure of variability (95% confidence limits).

The calculation of LC0s is dependent upon slope and time

course of effect, both of which are influenced by sample size (number of fish per concentration) and dose separation (dilution factor among concentrations). In this study, sample sizes ranged from 10 to 30 organisms and dilution factors ranged from 50 to 75%. The minimum acceptable sample size and maximum dilution factors were not determined in this study, but could be from the data set (Appendix B).

Data Base

The acute and chronic tests (Appendix B) selected for analyses were taken from those conducted at the Columbia National Fisheries Contaminant Research Center (U.S. Fish and Wildlife Service, Columbia, MO) and the Gulf Breeze Environmental Research Laboratory (U.S. Environmental Protection Agency, Gulf Breeze, FL) on seven fish species: rainbow trout, Oncorhynchus mykiss; cutthroat trout, O. clarki; brook trout, Salvelinus fontinalis; lake trout, S. namaycush; fathead minnow, Pimephales promelas; channel catfish, Ictalurus punctatus; and sheepshead minnow, Cyprinodon variegatus. With the exception of a few static acute tests used, acute and chronic tests were conducted in flow-through diluter systems modeled after that described by Mount and Brungs (1967). Each diluter delivered four to seven concentrations of toxicant and a control. Water temperature was maintained within ± 1° C of the desired temperature, and day length was regulated by the methods of Drummond and Dawson (1970). Acute and chronic tests were conducted in accordance with standard procedures (Committee on Methods for Toxicity Tests with Aquatic Organisms 1975, American Society for Testing and Materials 1980, Clesceri et al. 1989). The specific

methods and materials, experimental design, and measurements are included among the published articles cited as footnotes at the end Table 1. Concentrations of all chemicals were measured.

The concentration-response data in our historical data base for chlordane, endrin, EPN, heptachlor, methoxychlor, and toxaphene with sheepshead minnows were inadequate for observations prior to the 96 h point in time; for that reason, several acute tests with sheepshead minnows were repeated. Acute and chronic flow-through tests were also conducted with carbon tetrachloride and sheepshead minnows as another test of the LC0 method, because carbon tetrachloride is considered to have different modes of action between acute and chronic exposures with mammals (Haley 1987, Hardin 1954, Recknagel et al. 1989).

Two additional types of data sets having available and acceptable acute toxicity tests were analyzed -- a pond study with bluegills, Lepomis macrochirus, and fluorene, a component of petroleum (Boyle et al. 1985, Finger et al. 1985), and the other was with coturnix quail, Coturnix japonica, and mercuric or methyl mercuric chloride (Hill and Soares 1984). Fourteen 0.08 ha ponds were treated with various concentrations of fluorene (July 26, 1982). The ponds were drained approximately 70 days after exposure (early October, 1982), and the fish were counted, measured, and weighed to determine survival, growth, and production of recruits. Fluorene exposures in the ponds were based on average measured concentrations following treatment on days 1, 3, and 7. Acute toxicity tests with bluegills were conducted in the laboratory under static conditions to simulate pond exposures. Five-d acute

dietary tests were conducted with coturnix quail by presenting the chemicals at various concentrations in turkey starter mash for 5 days. Daily observations for evidence of toxicity were made until clinical signs were no longer detectable (10 days). Chronic toxicity was determined by feeding the mercurials at various concentrations in ad libitum diets from hatching to adulthood (9 weeks).

RESULTS AND DISCUSSION

When the predicted values were compared with the observed values of chronic tests (early life-stage and partial and full life cycle toxicity tests), they proved highly accurate for a variety of chemicals and fish species (Table 1; Appendix C, model 5). The predicted no-effect concentrations (PNEC) were very close to or within the limits (highest concentration without effect on survival and the next higher concentration with a significant effect) of the maximum acceptable toxicant concentrations (MATC) for lethality and varied by less than a factor of two from an MATC 92% of the time. The other 18% of the predictions (two observations) consisted of factors of 2.5 and 4.8 of the observed concentrations.

The technique worked very well in predicting chronic lethality of carbon tetrachloride to sheepshead minnows (PNEC = 10.4 mg/L, observed = $4.5 \leq x \leq 11$). Although this was only one study and one fish species, it may indicate that one assumption (i.e., the mode of action for lethality is similar under acute and chronic exposures) is not required for the technique. It may also be that carbon tetrachloride does not have different modes of action between acute and chronic exposures in fishes as has been observed for mammals (Haley 1987, Hardin 1954, Recknagel et al. 1989). Mount (personal communication) concluded that a relationship can be consistent even if acute and chronic modes of action are different. He further stated that the acute and chronic mode of action must be the same across species. Although the proposed approach works very well for both freshwater and marine fishes, its applicability to invertebrates needs to be determined.

The predictive technique was also highly accurate among various single chemicals and mixtures; it seemed representative of a wide range of octanol-water partition coefficients (log K_{ow}). Results of acute static tests might be used when flow-through tests results are not available and the log K_{ow} is low (e.g., fluridone). Chemicals that are highly water soluble will not adsorb to the test container or be taken up by the test organisms as much as with chemicals of low water solubility, and exposure will more closely resemble that for flow-through tests. However, additional research is needed to determine the log K_{ow} below which static test data can be used to predict chronic toxicity.

Although the other studies (pond and quail) analyzed represent a very small data set, it is notable that the PNECs were accurate. The ponds were dosed in a static acute manner (MATC for lethality = $0.0 \leq x \leq 67$ µg/L) and therefore, static acute toxicity test data were used to determine the PNEC (24 µg/L). With coturnix quail, the observed no-effect dietary concentrations for lethality were >32 ug/g for mercuric chloride and $2.0 \leq x \leq 8.0$ µg/g for methyl mercuric chloride with PNECs of 226 and 1.3 µg/g, respectively.

With Multifactor Probit Analysis (MPA), 64% of the PNECs were within a factor of two of MATCs for lethality, 24% ranged from 2.2 to 17, and 12% could not be determined due to the data (Appendix C, models 2,3,6 and 7). PNECs falling outside a factor of two included the butoxyethanol ester of 2,4-D (2.3, 4.7), endrin (17), fluridone (4.1), heptachlor (2.2), and methoxychlor (12). The deviation of four of the PNECs from respective MATCs are of little concern, but those for endrin and methoxychlor varied by more than

an order of magnitude. The cause is being investigated, and when determined, the MPA program will be modified to default to model 5 under those conditions. PNECs can be derived with model 5 when the other models do not work, but confidence limits are not provided.

The technique for deriving PNECs uses some aspects of concepts developed previously. Sprague (1969) recommended that acute tests be conducted until the toxicity curve becomes parallel to the time axis, indicating a threshold concentration. An incipient LC50 is then estimated by selecting an exposure time from the asymptotic part of the toxicity curve. The reciprocal of mean survival times within concentrations was used as early as 1917 by Powers (Jones 1964). Regressing the reciprocal of mean survival time on concentration to derive theoretical thresholds of toxicity was further developed by Abram (1964, 1967) and Alderdice and Brett (1957). Although observing survival times in acute tests has merits, it is laborious and is only infrequently done.

The approach of incorporating all data in an acute test (concentration, degree of response, and time course of effect) to predict chronic lethality has a technical basis. During the last 20 years, fish chronic toxicity tests have been shortened from full life cycle tests to 30-90 d early life stage or partial life cycle tests (Macek and Sleight 1977; McKim 1977, 1985) and then to 7-d subchronic tests (Norberg and Mount 1985). Reviews of subchronic, early life stage, partial life cycle, and full life cycle toxicity tests with several fish species demonstrated that the shorter tests are good estimators of chronic toxicity and MATCs observed in the longer life cycle tests (Macek and Sleight 1977; McKim 1977, 1985;

Norberg-King 1989; Woltering 1984). Although the success of developing briefer tests to estimate chronic toxicity is empirically based, it does support the toxicological concept of time course of effect in using acute data to predict chronic lethality.

Another use of acute toxicity data to estimate chronic toxicity is the toxicity threshold value or LC1 (Birge et al. 1985, Birge et al. 1989), which is calculated for 1.0% mortality and at one point in time. This application of acute tests should work well for those chemicals, effluents, and so on that differ little in toxicity between acute and chronic effects or where the LC1 is derived at a duration approaching or within chronic exposure conditions. However, the LC1 does not take into account time course of effect, and its use for predictive purposes is limited for a wide range of chemicals; particularly those that bioconcentrate or have cumulative effects.

Relation to Other Endpoints

Chronic toxicity tests commonly include the measurement of long-term effects of a contaminant on the survival, growth, and reproduction of a test organism. Assessments of sensitivity in relation to chronic endpoints in fishes have been conducted (Woltering 1984, Mayer et al. 1986, Suter et al. 1987, Ward and Parrish 1980). Survival and growth are often equally sensitive, and growth may not be of critical importance in establishing no-effect concentrations in most tests. In tests for which growth is the single most sensitive endpoint, survival could be used to estimate the no-effect concentration within a factor of 3.

Mayer et al. (1986) found growth-related endpoints to be highly predictable from survival effects with freshwater fishes (Table 2). Length was less variable than weight, and although all of the coefficients of determination (r^2) exceeded 0.9, they were slightly higher for length (0.941 to 0.949) than for weight (0.901 to 0.931). Also, no alteration was noted in the intercepts (a) for length versus survival between 30 and 90 days of exposure; the intercepts of weight versus survival varied, without trends, over time. Using these equations (Table 2), estimated no-effect concentrations for growth may be derived from the predicted values for chronic lethality.

No-effect concentrations were always less for reproduction endpoints than for survival (Mayer et al. 1986, Suter et al. 1987). Attempts to relate acute lethality to chronic reproductive effects by regression analysis have not been successful (Suter et al. 1987). Because of the likelihood of different modes of action between lethal and reproductive effects, we do not recommend that reproductive effects be predicted using the proposed method. However, the proposed technique is highly beneficial in the preliminary assessment of chronic toxicity of effluents and other chemicals and in predicting chronic no-effect concentrations for survival and growth with fish species that are difficult to culture under chronic testing conditions.

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Woodward, D.F. and F.L. Mayer. 1978. Toxicity of three herbicides (butyl, isooctyl, and propylene glycol butyl ether esters of 2,4-D) to cutthroat trout and lake trout. Technical Paper No. 97. U.S. Fish and Wildlife Service, Washington, DC.

Table 1. Comparison of observed maximum acceptable toxicant concentrations (MATC) and predicted no-effect concentrations (PNEC) for lethality based on flow-through acute tests.

Chemical and Species	Log Kow	MATC[a] (µg/L)	PNEC (µg/L)
Butyl benzyl phthalate	4.44		
Fathead minnows		>360	635[b]
Carbon tetrachloride	2.64		
Sheepshead minnows		4,500≤x≤11,200	10,427
Chlordane	5.80		
Sheepshead minnows		7.1≤x≤17	14[b]
Complex effluent			
Fathead minnows		2.0≤x≤3.5%	5.2%
2,4-D Butyl ester	2.81		
Cutthroat trout		24≤x≤44	112
Lake trout		33≤x≤60	67[b]
2,4-D PGBEE	4.88		
Cutthroat trout		31≤x≤60	59
Lake trout		52≤x≤100	74
Endosulfan	4.90-6.00[c]		
Sheepshead minnows		1.1≤x≤2.5,0.92≤x≤2.1	1.4
Endrin	4.56-5.30		
Sheepshead minnows		0.12≤x≤0.31	0.12
EPN	4.80		
Sheepshead minnows		4.1≤x≤7.9	3.9[b]
Fluridone	1.87		
Channel catfish		1,000≤x≤2,000	1,182[d]
Heptachlor	5.44		
Sheepshead minnows		1.9≤x≤2.8,2.2≤x≤3.5	2.6
Kepone	6.08		
Fathead minnows		1.2≤x≤3.1	2.9
Methoxychlor	4.20		
Rainbow trout		1.1≤x≤3.1	0.94[b,e]
Sheepshead minnows		12≤x≤23,23≤x≤48	12,12,17[b]

Pentachlorophenol	5.01		
Fathead minnows		>142	240
Phorate	3.50		
Sheepshead minnows		0.24≤x≤0.41	0.15[b]
Pydraul 50E	4.62-6.08[f]		
Fathead minnows		317≤x≤752	592
TFM			
Brook trout		4,000≤x≤8,800	4,311
Toxaphene	4.83		
Brook trout		0.068≤x≤0.14	0.041
Brook trout (adult)		0.14 ≤x≤0.29	1.4[b]
Fathead minnows		0.62 ≤x≤1.3	1.7[b]
Channel catfish		0.07 ≤x≤0.13	0.057
Sheepshead minnows		1.1 ≤x≤2.5	0.77[b]

---

[a]Source of MATC: butyl benzyl phthalate-unpublished; carbon tetrachloride-this study; chlordane-Parrish et al. [1976]; complex effluent-unpublished; 2,4-D butyl ester and PGBEE-Woodward and Mayer [1978]; endosulfan-Hansen and Cripe [1984] endrin-Hansen et al. [1977]; EPN-Cripe et al. [1984]; fluridone-Hamelink et al. [1986]; heptachlor-Goodman et al. [1976], Hansen and Parrish [1977]; Kepone-Buckler et al. [1981]; methoxychlor-unpublished (rainbow trout), Hansen and Parrish [1977] (sheepshead minnows); pentachlorophenol-Cleveland et al. [1982]; phorate-U.S. EPA [1981]; Pydraul 50E-Mayer et al. [1981]; TFM-Dwyer et al. [1978]; toxaphene-Mayer et al. [1975] (brook trout), Mayer et al. [1977] (fathead minnows, channel catfish), Goodman et al. [1976] (sheepshead minnows).

[b]Log transformation of LC0's required.

[c]Endosulfan I = 4.90, Endosulfan II = 6.0.

[d]Based on static test.

[e]Acute toxicity test for rainbow trout was not available and PNEC was based on brook trout test because of similarity in response to toxicants (Mayer et al. 1987).

[f]Pydraul 50E is a hydraulic fluid consisting of three components: triphenyl phosphate = 4.62, nonylphenyl diphenyl phosphate = 5.93, cumylphenyl diphenyl phosphate = 6.08.

Table 2. Regression correlations[a] of survival and growth[b] no observed effect concentrations among all fish species and chemicals at various time periods.

Analysis and days of exposure	n	Intercept (a)	Slope[c] (b)	Coefficient of Determination (r^2)	$\bar{y}\pm$95% C.I.
Weight vs. survival					
30	6	0.395	0.920	0.931	4.35±0.41
60	10	0.682	0.901	0.901	4.63±0.31
90	15	0.194	0.993	0.916	4.33±0.22
Length vs. survival					
30	16	0.284	0.968	0.945	4.64±0.18
60	17	0.263	0.965	0.949	4.60±0.15
90	18	0.275	0.971	0.941	4.41±0.17

[a]Log y = a+b(log x), where y is no-effect concentration for survival and x is no-effect concentration (ng/L) for growth (length or weight).

[b]Mayer et al. (1986).

[c]All slopes were significantly different from 0 ($p\leq0.01$).

Fig. 1. Dose-response curves used to derive the LC0 (0.01%) for various observation times in acute toxicity tests (1.281 = a+bX). Probit % mortality: 1.281 = 0.01%, 5.000 = 50%, and 8.719 = 99.99%.

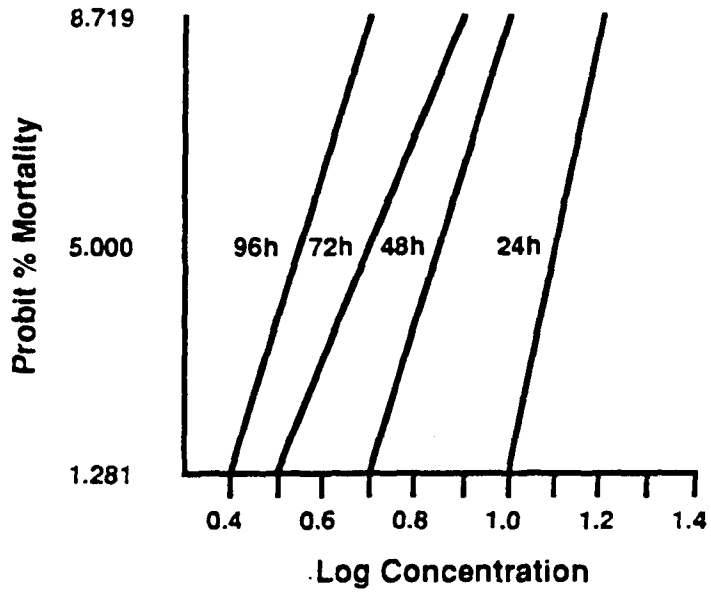

8.719
5.000
1.281
Probit % Mortality
96h
72h
48h
24h
0.4
0.6
0.8
1.0
1.2
1.4
Log Concentration

Fig. 2. Prediction of the chronic no-effect value for lethality from acute toxicity test data with Kepone and fathead minnows (LC0 = a+b [1/t]). The intercept (a) represents the LC0 (2.9 µg/L) over an indefinite exposure time, and the maximum acceptable toxicant concentration (MATC) for chronic lethality was between 1.2 and 3.1 µg/L.

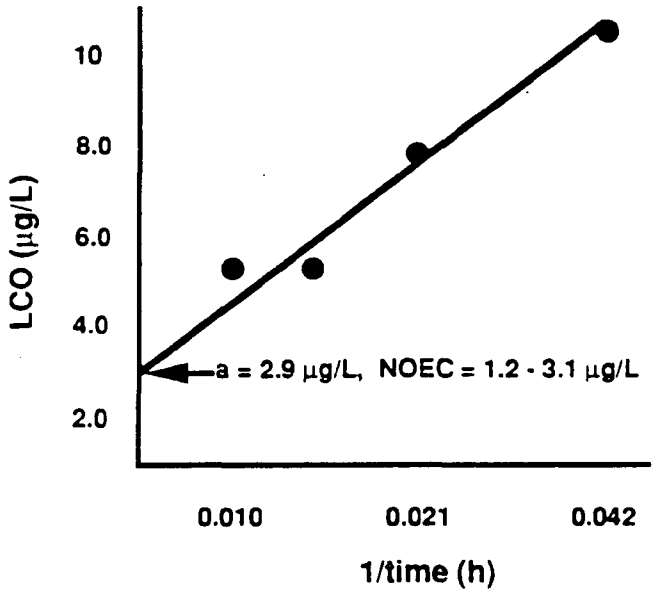
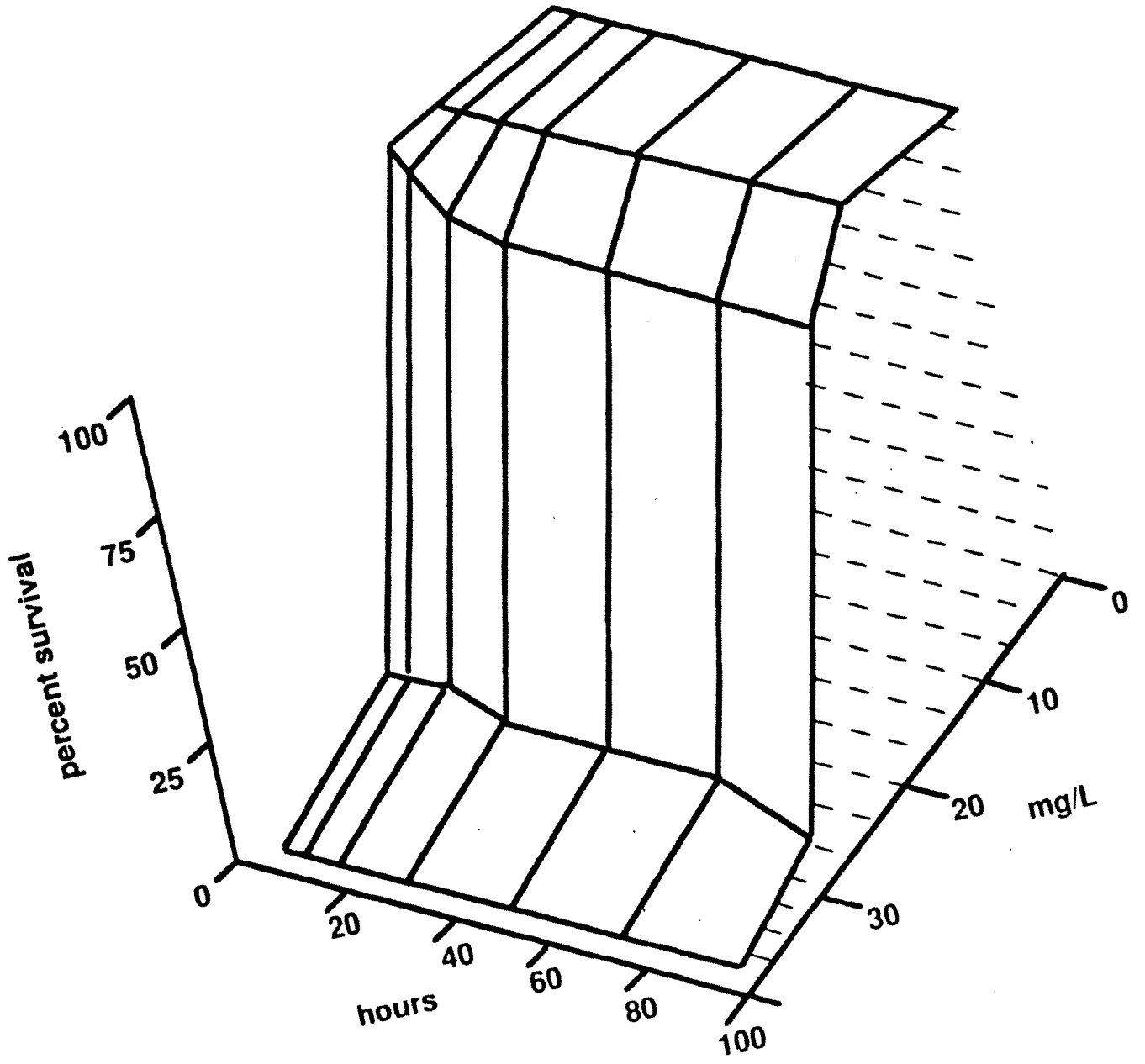

10
8.0
6.0
4.0
2.0
LCO (µg/L)
a = 2.9 µg/L, NOEC = 1.2 - 3.1 µg/L
0.010
0.021
0.042
1/time (h)

Fig. 3. Acute flow-through toxicity test results with carbon tetrachloride and sheepshead minnows demonstrating dose-response data in time (96-h LC50 = 19 mg/L).


100
75
50
25
0
percent survival
0
20
40
60
80
100
hours
0
10
20
30
mg/L

APPENDIX A

Multifactor Probit Analysis Program

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MULTIFACTOR PROBIT ANALYSIS

by

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DISCLAIMER

The research described in this article has been subjected to Agency review for internal Agency distribution. Mention of trade names does not constitute endorsement or recommendation for use.

TABLE OF CONTENTS

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ABSTRACT

Environmental toxicologists are interested in the long-time-exposure effect of a low concentration of a toxic substance. Long-time-exposure toxicity testing is time consuming and expensive; consequently, accurate methods for estimating long-time-exposure effects which eliminate this time and expense, are desirable. In the past long-time-exposure toxicity was determined by calculating an application factor or an acute-chronic ratio for a limited number of species and then applying these multiplicative factors to other species. This method may not give accurate estimates and does not give any measure of the sampling variance of the estimate.

A methodology has been developed that will predict long-time-exposure effect toxicity based on acute data. This method is called Multifactor Probit Analysis (MPA) and uses the iterative reweighed least squares method to estimate the parameters of the probit surface. The independent variables are time of exposure and concentration of the toxicant. The dependent variable is the probit of the proportion responding to a concentration. MPA allows the user to predict the concentration of a toxicant at any time and percent mortality, $LC_{t,p}$. The Multifactor Probit Analysis calculates a point estimate and a measure of dispersion (95% approximate confidence limits).

The Multifactor Probit Analysis software is versatile and the user can choose from seven different probit models and seven different transformation combinations of the independent variables. This software is entirely menu driven.

MPA predicts long-time-exposure mortality from acute data. This prediction represents the amount of a toxic substance that can exist in a laboratory environment for an extended exposure time that will produce 0.01 percent mortality.

SECTION 1
INTRODUCTION

Environmental toxicologists are interested in determining low concentration mortality of a chemical to an organism when exposed for extended periods of time. More specifically: What concentration of a chemical can exist in a laboratory environment with only small effect on biological life? In the past, estimates for these chronic no-effect concentrations have been estimated using a combination of chronic and acute data for a particular species.

A methodology and a computer program have been developed cooperatively by the Environmental Protection Agency and the University of Missouri-Columbia that predicts the long-time-exposure lethality of chemicals from acute toxicity test data. The software is called Multifactor Probit Analysis (MPA). This software calculates the lethal concentration of a chemical for expected effect, P (probability of response), for extended periods of exposure time.

SECTION 2
CONCLUSIONS

Statistical models are programmed that utilize information from several acute bioassay data sets to quantify the relationship between exposure time, and dose of a chemical and mortality. This user friendly software provides maximum likelihood estimates of relevant parameters. Output from this software includes a predicted concentration LC_p, that has expected effect P, (P is the proportion of subjects responding which may be very small), under extended exposure time. Approximate confidence limits are provided on true LC_p.

Computer software, called MULTIFACTOR PROBIT ANALYSIS, may be used to: a) assist selection of a model to relate exposure time and concentration to probit mortality, b) estimate the functional relationship among parameters in the best model and exposure time to predict long-time-exposure LC_p. P may be very small. Comparison of the predicted LC_p to long-time-exposure LC_p estimates from long-time-exposure trials may be done. Evaluation of the appropriateness of this scheme would be dependent upon this comparison.

SECTION 3
RECOMMENDATIONS AND IMPORTANT CONSIDERATIONS

1. Short term exposure tests should be independent. The methodology used assumes this condition. If observed mortality at time t and a concentration is cumulative, then bias may result.

2. Choice of model depends on mode of action of the toxicant. Parallel concentrations-mortality lines assumes mode of action is constant as time varies. An interaction between concentration and time allows for changes in mode of action. Several models may be evaluated using the same data. This permits a scheme, based on heterogeneity chi-square to select the best model of those tried.

3. If control mortality is observed at zero dose (control), Abbott's adjustment can be used to adjust non-zero dose mortality. However, if control mortality is not constant over the entire range of exposure time, Abbott's adjustment is no longer valid. In this situation analysis can be done by ignoring control mortality. A rule of thumb is, control mortality should not be greater than 10%. (If control mortality is greater than 10% the entire test should be redone).

4. If exposure tests are done at different times, a separate probit analysis at each time will give an indication of parallel or non parallel slopes. If the slopes are similar, a multifactor probit model using parallel lines should be used. If the slopes are different, a model with non-parallel slopes should be used.

ESTIMATING NO OBSERVABLE EFFECT CONCENTRATIONS

We set the tolerable long-time-exposure effect at 0.01%. The concentration that causes this effect (probit value of 1.281) will be called the No Observable Effect Concentration (NOEC).

First Approach:

This approach is based on a simple probit analysis or a least square linear regression for each exposure time (Type 5). The procedure is as follows:

For given time t, NOEC will be estimated from the estimated simple probit line. Then one estimates the regression line between NOEC estimates and reciprocal of exposure using the

following model:

$$NOEC = \alpha + \beta/time.$$

If the model is correct, NOEC converges to α as time become large.

Note 1:
Several dose and time transformations should be evaluated for each different chemical and species. Estimated NOEC will be the one which has maximum R-square for the regression model.

NOTE 2:
Suppose there are not enough data points to estimate a probit regression line. In this situation, we recommend that the maximum no-mortality concentration for a specific toxicant be included for analysis. In this case, the experimenter should identify the NOEC for different exposure times and check the monotonicity assumption. For example, NOEC of 48 hours cannot be less than 72 hours but should be greater than 24 hours. Generally, we recommend the analysis include the maximum no-mortality concentration for a large number of exposure times in the experiment. This will give more information about NOEC at time infinity.

NOTE 3:
The use of the least-squares-method to estimate the probit regression line when responses are the same at different exposure times, may have a danger of over estimation, i.e. NOEC estimate may be higher than the true NOEC. The reason is, that data at 96 hours gives more information for time infinity predictions than earlier exposure times. This method treats them equally. If slopes between any two (NOEC,time) points changes after some period of time, for example mode of action changes, this method is very insensitive to change and provides a compromised slope which will be smaller.

Note 4:
MPA program uses MLE (Maximum Likelihood Estimation) of simple probit analysis and also calculates a simple LS (least square) analysis for each time. Theoretically, the ML estimator is usually superior to LS estimator. ML estimation is sensitive to changes in observed data. However, there is a greater possibility of an estimate against the monotonicity assumption. For example, ML estimate of NOEC increases as time increases or p% lethal concentration decreases as p increases. With MLE, we highly recommend the option of screen plotting (which is provided by MPA program) be chosen and checked for the monotonicity

assumption, i.e. 72 hours simple probit line estimate should lie above the 48 hours simple probit line estimate. If MLE provides an unreasonable estimate, LS should be selected. The results from LS has the serious overestimation problem and is quite sensitive to the choice of concentration in the experiment. LS, however, guarantees the monotonicity assumption as long as the observed data holds the assumption and always produces an estimate of NOEC when MLE may not.

Second Approach:

This approach is based on a multiple probit model, Dose-Time-Response surface. Unlike the first approach, which estimates the NOEC probit for each time, this approach solves the Dose-Time-Response equation simultaneously. The MPA program can compute four cases. The cases are as follows:

Case 1:
Specific long time exposure is specified and assumes equal slope for every time (Type 2).

Dose - Time - Response relationship is defined as

$$\text{Probit}(p) = \alpha + \beta*(\text{Dose}) + \gamma*(\text{Time}).$$

Let us denote specific long exposure time as T. NOEC at T hours can be found as follows:

$$NOEC_T = \frac{1.281-\alpha-\gamma*T}{\beta}$$

where, 1.281 is the probit value of 0.01%.

Case 2:
Specific long time exposure is unknown and equal slope is expected for every time (Type 6).

Dose - Time - Response relationship is defined as

$$\text{Probit}(p) = \alpha + \beta*(\text{Dose}) + \gamma/(\text{Time}).$$

NOEC at long exposure time can be found as follows:

$$NOEC = \frac{1.281-\alpha}{\beta}$$

where, 1.281 is the probit value of 0.01%.

Case 3:
Specific long time exposure is specified and one assumes slope changes with constant rate as time increases (Type 3).

Dose - Time - Response relationship is defined as

$$\text{Probit}(p) = \alpha + \beta*(\text{Dose}) + \gamma*(\text{Time}) + \delta*(\text{Dose})*(\text{Time}).$$

Let us denote specific long exposure time as T. NOEC at T hours can be found as follows:

$$NOEC_T = \frac{1.281-\alpha-\gamma*T}{\beta+\delta*T}$$

where, 1.281 is probit value of 0.01%.

Case 4:
Specific long time exposure is unknown and one assumes slope changes with constant rate as time increase (Type 7).

Dose - Time - Response relationship is defined as

$$\text{Probit}(p) = \alpha + \beta*(\text{Dose}) + \gamma/(\text{Time}) + \delta*(\text{Dose})/(\text{Time}).$$

NOEC at infinity hours can be found as follows:

$$NOEC = \frac{1.281-\alpha}{\beta}$$

where, 1.281 is probit value of 0.01%.

Note 5:
Type 6 and Type 7 can be utilized with specified long exposure time. NOEC at T hours can be found as:

$$NOEC_T = \frac{1.281-\alpha-\gamma/T}{\beta} \quad \textit{if Type6 is appiled}$$

$$NOEC_T = \frac{1.281-\alpha-\gamma/T}{\beta+\delta/T} \quad \textit{if Type7 is appiled.}$$

Note 6:
Several dose and time transformations should be evaluated for each different chemical and species. The best estimated NOEC will be the one which has the minimum computed heterogeneity factor. The heterogeneity factor equals the computed chi-square divided by degrees of freedom.

Note 7:
Since long exposure time is quite dependent on different species (or average life of species), in some cases, an experimenter may want to set a specific time (for example, 1440 hours). If there is knowledge of a life cycle of a species, estimation of NOEC should be based on the average life time to avoid underestimation. NOEC at time infinity is always less than NOEC at a specified time.

Note 8:
When a cross product term is used, i.e. δ*(Dose)*(Time) term in Type 3 and δ*(Dose)/(Time) term in Type 7, there is still a small chance to get an estimate against the monotonicity assumption. If this happens, both Type 3 and Type 7 of any dose time transformation should not be considered to estimate NOEC. In this situation, the assumption of monotonicity is not met even though it has a small computed chi-square.

Note 9:
If no candidate model has reasonable small computed chi-square, i.e. every candidate model has large chi-square which is greater than 10 times the degrees of freedom, care should be taken when estimating the NOEC. Multiple probit model is not appropriate with a large chi-square.

SECTION 4
APPROACH

MPA has two primary functions. The first function is for entering and editing datum not only for the MPA subroutine, but also for other software. Data files already prepared in ASCII format can be retrieved using this function. The data entry and editing function is described in detail in a later section.

The second function is statistical analysis. Once a data set has been entered, a selected MPA subroutine executes an analysis. The output which is produced depends on the analysis option chosen.

The analysis options are:

A:
Simple probit analysis using one independent variable, which is concentration. A single exposure time is assumed.

B:
Multifactor probit analysis which includes two independent variables, (concentration and exposure time). This option assumes parallel probit regression lines at each exposure time.

C:
This is also a multifactor probit analysis using concentration, time and interaction as independent variables. This option is different than B in that non-parallel probit lines over time are assumed.

D:
This option computes a multifactor probit analysis with three independent variables (concentration of toxicant 1, exposure time and a third variable which could be a second toxicant).

E.
This is a simple probit analysis using concentration as the independent variable and is computed at each level of exposure time.

F:
This option is the same as option B except that the independent variable representing exposure time is the reciprocal of time, (1/time).

G:
This option is the same as C except that the independent variable representing exposure time is 1/Time.

Since the MPA uses time as one of the independent variables, the mortality or other quantal response needs to be observed at different times. Mortality must be observed at least two times. However, when only two time tests are available relationships will be poorly determined resulting in wide confidence intervals. Therefore, it is preferable to observe mortality more than two times.

If the model chosen includes the independent variable 1/Time then the long time exposure small effect concentration is estimated conditional on 1/Time or 1/(log time) being 0 and a choice of mortality (perhaps .01 percent). The estimate is the y-intercept of the regression of an LC value on x (x being the time factor), the predicted concentration of a toxicant that will essentially produce small effect (perhaps .01 percent) under long-time-exposure.

SECTION 5
MULTIFACTOR PROBIT ANALYSIS SOFTWARE

The anticipated computer is an IBM PS/2 or an earlier PC. High resolution graphics are preferred. The Multifactor Probit Analysis (MPA) Software is initiated by placing the program disk into disk drive A and typing A:MPA. A logo should appear on the screen. This will remain until you press the <ENTER> key.

After pressing the <ENTER> key, the following MAIN PROGRAM MENU, (above the dashed line below) and Current Program Status, (below the dashed line below) will appear on the monitor.

```
          MULTIFACTOR PROBIT ANALYSIS
      MAIN PROGRAM MENU

1    CHOOSE TYPE OF PROBIT MODEL AND LOG TRANSFORMATION
2    CHOOSE EXPOSURE TIMES
3    ENTER NEW DATA
4    EDIT DATA IN MEMORY
5    GET DATA FROM DISK
6    SAVE DATA ON DISK
7    DEFINE A TITLE, CHANGE GRAPHICS MODE
8    STATISTICAL ANALYSIS
9    QUIT

CHOOSE 1-9 (enter a single number, you do not press <ENTER>)
-------------------------------------------------------------
                  CURRENT MODEL STATUS

CURRENT MODEL.....................................
..ONE INDEPENDENT VARIABLE (DOSE)
CURRENT EXPOSURE TIMES ......24 48 72 96
CURRENT TRANSFORMATION ......NATURAL LOG
LAST DISK FILE READ ........
LAST DISK FILE WRITTEN ON ..
TITLE .......................
```

Above the dashed line is a menu of the possible operations that the MPA has available. Below the dashed line is the Current Model Status showing the current statistical model, current exposure times, current transformation, last disk file read, last disk file written on, and title.

DESCRIPTION OF MULTIFACTOR PROBIT ANALYSIS MAIN MENU

MAIN PROGRAM MENU ITEM 1:

1) CHOOSE TYPE OF PROBIT MODEL AND LOG TRANSFORMATION.
Number 1 in the MAIN PROGRAM MENU serves three functions. The first function permits the choice of model. If number 1 is selected from the MAIN PROGRAM MENU a second menu appears on the monitor:

```
                PROBIT MODEL
      MENU OF STATISTICAL MODELS

1     ONE INDEPENDENT VARIABLE (DOSE)
2     TWO INDEPENDENT VARIABLES (DOSE AND TIME) WITH
      PARALLEL SLOPE
3     INTERACTION BETWEEN DOSE AND TIME WITH NON-PARALLEL SLOPE
4     THREE INDEPENDENT VARIABLES (DOSE, TIME AND A THIRD
      VARIABLE. eg. second dose)
5     ONE INDEPENDENT VARIABLE (DOSE) GROUPED BY TIME
6     TWO INDEPENDENT VARIABLES WITH PARALLEL SLOPE USING
      RECIPROCAL OF TIME
7     TWO INDEPENDENT VARIABLES WITH NON-PARALLEL SLOPE USING
      RECIPROCAL OF TIME
8     QUIT

CHOOSE 1-8 (enter a single number, you do not need to press
<ENTER>)
```

THE STATISTICAL MODELS:

NOTATION:
Suppose n subjects are tested at k different dose levels.

- r : the response frequency from n subjects given dose level z.
- x : the transformed value of z (natural log or log10 transformation).
- P : the proportion of subjects responding at dose level z, ($P = r/n$).
- t_x : the representation of exposure time for these values of r, n and z.
- f_t : the third transformed interval scale factor which may be a dose level of a second chemical.

The basic Probit function of P is:

$$Probit(p) = \Phi^{-1}(p) + 5 \qquad , where \quad \Phi(x) = \int_{-\infty}^{x} \frac{1}{\sqrt{2\pi}} e^{-\frac{1}{2}x^2} = p$$

Type 1 : Probit model with one independent variable.
Probit(p) = a+bx.
where a is the intercept and
b is the coefficient on the metric of
dose concentration x.

Type 2 : Probit model with two independent variables.
Probit(p) = $a+bx+ct_x$.
where a is the intercept,
b is the coefficient on the metric of
dose concentration x and
c is the coefficient on the metric of
time (hours).

NOTE:
In Type 2, parallel probit lines are assumed for each time.

Type 3 : Probit model with two independent variables and
interaction between dose and time.
Probit(p) = $a+bx+ct_x+dxt_x$.
where a is the intercept,
b is the coefficient on the metric of
dose concentration x,
c is the coefficient on the metric of
time (hours) and
d is the coefficient on cross product term.

NOTE:
In Type 3, the slopes are changing at rate d so the slope will be $b+dt_x$. For example, if d equals -0.2, then the slope will decrease -0.2 as time increases 1 unit.

Type 4 : Probit model with three independent variables for
exposure time i.
Probit(p) = $a+bx+ct_x+df_t$.
where a is the intercept,
b is the coefficient on the metric of
dose concentration x,
c is the coefficient on the metric of
time (hours) and
d is the coefficient on the metric of
third interval scale factor f_t.

Type 5 : Probit model with one independent variable.
Probit(p_i) = a_i+b_ix

NOTE:
May be used for a regression at each level of time.

Type 6 : Probit model with two independent variables one of which is the inverse of t_x.
Probit(p) = $a+bx+c/t_x$.

Type 7 : Probit model with two independent variables and interaction which uses inverse of t_x.
Probit(p) = $a+bx+c/t_x+dx/t_x$.

NOTE:
Type 6 and Type 7, assume parallel probit lines and non-parallel probit lines, respectively. Type 2 and Type 3 has time as one of the independent variables. This differs from Type 6 and Type 7 which involves the reciprocal of time ($1/t_x$) as one of the independent variables.

2)<u>CHOOSE TYPE OF TRANSFORMATION</u>. After a probit model has been chosen by selecting one of the seven models, a Data Transformation Menu will appear. The transformation selected applies to independent variables. This is the second function of MAIN PROGRAM MENU Item 1.

```
            DATA TRANSFORMATION MENU

1     NATURAL LOG OF DOSE AND NATURAL LOG OF TIME
2     LOG 10 OF DOSE AND LOG 10 OF TIME
3     INPUT VALUE OF DOSE AND INPUT VALUE OF TIME
4     NATURAL LOG OF DOSE AND LOG 10 OF TIME
5     NATURAL LOG OF DOSE AND INPUT VALUE OF TIME
6     LOG 10 OF DOSE AND NATURAL LOG TIME
7     LOG 10 OF DOSE AND INPUT VALUE OF TIME
8     QUIT

CHOOSE 1-8 (enter a single number, you do not need to press
<ENTER>)
```

When a transformation has been selected by entering a number, the MAIN PROGRAM MENU will appear.

3) The third function identifies the order of variables in the input record for the model selected. This is a data requirement. You will not see this until you utilize MAIN PROGRAM MENU Item 3.

Three variable orders are possible.

The Type 1 model requires the order be z, n and r.

The Type 2, 3, 5, 6 and 7 models requires the order be z, t, n and r.

The Type 4 model requires the ordering z, t, f_t, n and r.

MAIN PROGRAM MENU ITEM 2:

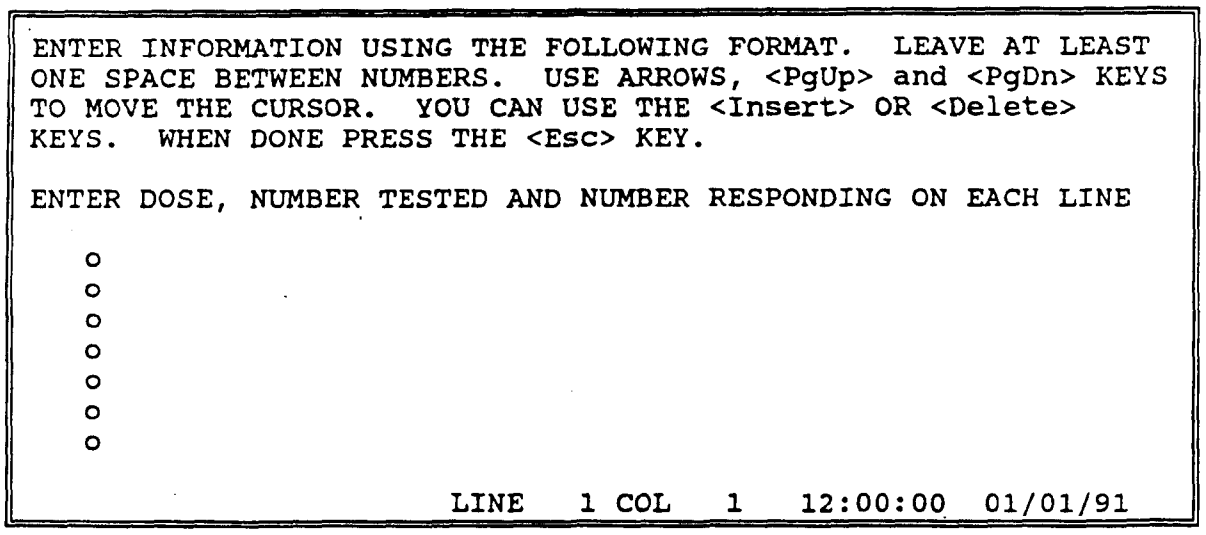
CHOOSE EXPOSURE TIMES. These only influence output. The default times are 24, 48, 72 and 96 for model types 2, 3 and 4. For model types 6 and 7, the default times are 24, 48, 72, 96 and time infinity. If MAIN PROGRAM MENU Item 2 is used, times other than the default times can be chosen. These times (in hours) dictate the times for which LC values will be calculated and presented in PRINTED OUTPUT. If times other than default times are needed they can be entered in response to the cue from MAIN PROGRAM MENU Item 2. Ex:

```
ENTER EXPOSURE TIMES SEPARATED BY BLANKS
(Example: 24 48 72 96)
?
```

Model types 1 and 5 calculate simple probit analysis, so exposure times are not present. Exposure times should be entered within one line separated by blanks.

MAIN PROGRAM MENU ITEM 3:

ENTER NEW DATA. If data has not been entered previously, it can now be entered by selecting item 3. The following instructions and data input prompt will now appear on the monitor, (this is an illustration assuming a Type 1 model was chosen, see third function description of MAIN PROGRAM MENU Item 1).



The cursor should be at the first line and column as indicated at the bottom of the monitor. The order of entry will come from a prompt. At least one space is needed between values for successive variables.

There are a number of commands that are useful for data entry:

<Home>,<End>
<Home> Key or the <End> Key is depressed to send the cursor to the top of a file (Home) or bottom of a file (End).

<PgUp>,<PgDn>
If the <PgUp> or <PgDn> keys are depressed it moves the file up or down one editor screen.

<Ins>
The <INS> key puts the user in and out of insert mode. If one is in the insert mode it is indicated in the first window. This key is only used for inserting data or characters within a line.

<Del>
The <DEL> key deletes data or characters within a line.

<Tab>
The <TAB> key allows data to be typed in specific columns. (Default is every 5 spaces.)

<Arrows>
The <ARROWS> can be used to position the cursor while in the

work window.

<Alt> <I>
This allows the user to insert a line previous to the current line.

<Alt> <D>Deletes the current line.

To exit MAIN PROGRAM MENU Item 3, press <ESC>.

MAIN PROGRAM MENU ITEM 4:

EDIT DATA IN MEMORY. The same commands used to enter new data also work for editing existing data sets. Once the data are in memory, use number 4 for editing. The same screen presented under the discussion on MAIN PROGRAM MENU Item 3 will appear. If data are in memory and one utilizes MAIN PROGRAM MENU Item 3, the data in memory and on the screen will be erased, waiting for new data to be entered. The data must be in memory for the program to run. To exit MAIN PROGRAM MENU Item 4, press <ESC>.

MAIN PROGRAM MENU ITEM 5:

GET DATA FROM DISK. If a data set already resides on a disk, choose MAIN PROGRAM MENU Item 5. The screen will now present the statement.

```
ENTER THE ASCII FILE NAME  (Example. B:PROBIT.DAT)
?
```

If one enters the ASCII data set name and presses enter, this data set will be in memory and can be edited by MAIN PROGRAM MENU Item 4.

MAIN PROGRAM MENU ITEM 6:

SAVE DATA ON DISK. After data has been entered and/or edited it should be saved. Enter 6 and the following message will appear on the screen.

```
ENTER A FILE NAME  (Example. B:PROBIT.DAT)
?
```

After entering the ASCII data set name and pressing the <ENTER> key, the following WARNING will appear on the screen.

```
THIS PROCESS WRITES OVER AN OLD DATA SET WITH THE SAME
NAME IF ONE EXISTS.  ARE YOU SURE YOU WANT TO DO THAT  ?
(Y/N)   ?
```

If Y is entered the data set will be saved or replaced. If N is entered the data will not be saved under the name specified, however, it still resides in memory. After the Y or N is entered the MAIN PROGRAM MENU will appear. One may enter MAIN PROGRAM MENU Item 6 again and save the ASCII file under a different name.

MAIN PROGRAM MENU ITEM 7:

1)**DEFINE A TITLE, CHANGE GRAPHICS MODE**. Item 7 in the MAIN PROGRAM MENU serves two main functions. The first is to define a title. If a number of data sets are to be analyzed it is important to title each output. If MAIN PROGRAM MENU Item 7 is selected a Miscellaneous Menu will appear:

```
MISCELLANEOUS MENU

1DEFINE A TITLE
2CHANGE THE GRAPHICS MODE
3QUIT

CHOOSE 1-3 (enter a single number, you do not need to
press <ENTER>)
```

If 1 is selected from the Miscellaneous Menu, the response will be:

```
ENTER A TITLE:
```

After a title has been entered the program will return to the MAIN PROGRAM MENU.

2)**GRAPHICS HARDWARE CONTROL**. The second function of MAIN PROGRAM MENU Item 7 serves as graphic hardware control. When selected the Miscellaneous Menu will appear. If 2 is selected from Miscellaneous Menu (CHANGE THE GRAPHICS MODE), the following response will appear:

```
ENTER THE GRAPHICS MODE (eg. EGAMONO, HIRES, EGAHIRES, VGA)

?
```

Four responses are possible and are defined as follows:

EGAMONO:
Monochrome graphics with 640 pixels horizontally by 350 pixels vertically.

HIRES:
CGA High Resolution Graphics.

EGAHIRES:
EGA High Resolution Graphics. This can be used if the computer is equipped with an EGA card. This is default.

VGA:
VGA High Resolution Graphics.

After entering one of the 4 options the program will return to the MAIN PROGRAM MENU.

If 3 is entered from the Miscellaneous Menu, the MAIN PROGRAM MENU will appear.

MAIN PROGRAM MENU ITEM 8:

1)**STATISTICAL ANALYSIS**. After the data has been typed or retrieved from disk, a statistical model chosen and a title (optional) entered, the data will be analyzed when 8 is selected. If this is done, the following response will appear if models chosen were 2, 3, 4, 5, 6 or 7. The plotting option is not available if model 1 was chosen.

DO YOU WANT TO DO SCREEN PLOTTING? (Y/N)?

If Y or N is entered (which is the command for Y (yes) or N (no) screen plotting of probit line), another menu will appear if mortality occurs at any 0 dose level. If no mortality exists at dose level 0, screen plotting will begin. If N was entered (no plotting), all screen graphics are suppressed and the program proceeds to the output control menu after control mortality is checked.

2) CONTROL MORTALITY OPTIONS.

```
     NON-ZERO RESPONSE IS PRESENT AT DOSE LEVEL 0.

1    STOP PROCESSING.
2    IGNORE RESPONSE AT DOSE LEVEL 0.
3    ADJUST RESPONSE USING ABBOTT'S FORMULA.

CHOOSE 1-3 (enter a single number, you do not need
to press <ENTER>)
```

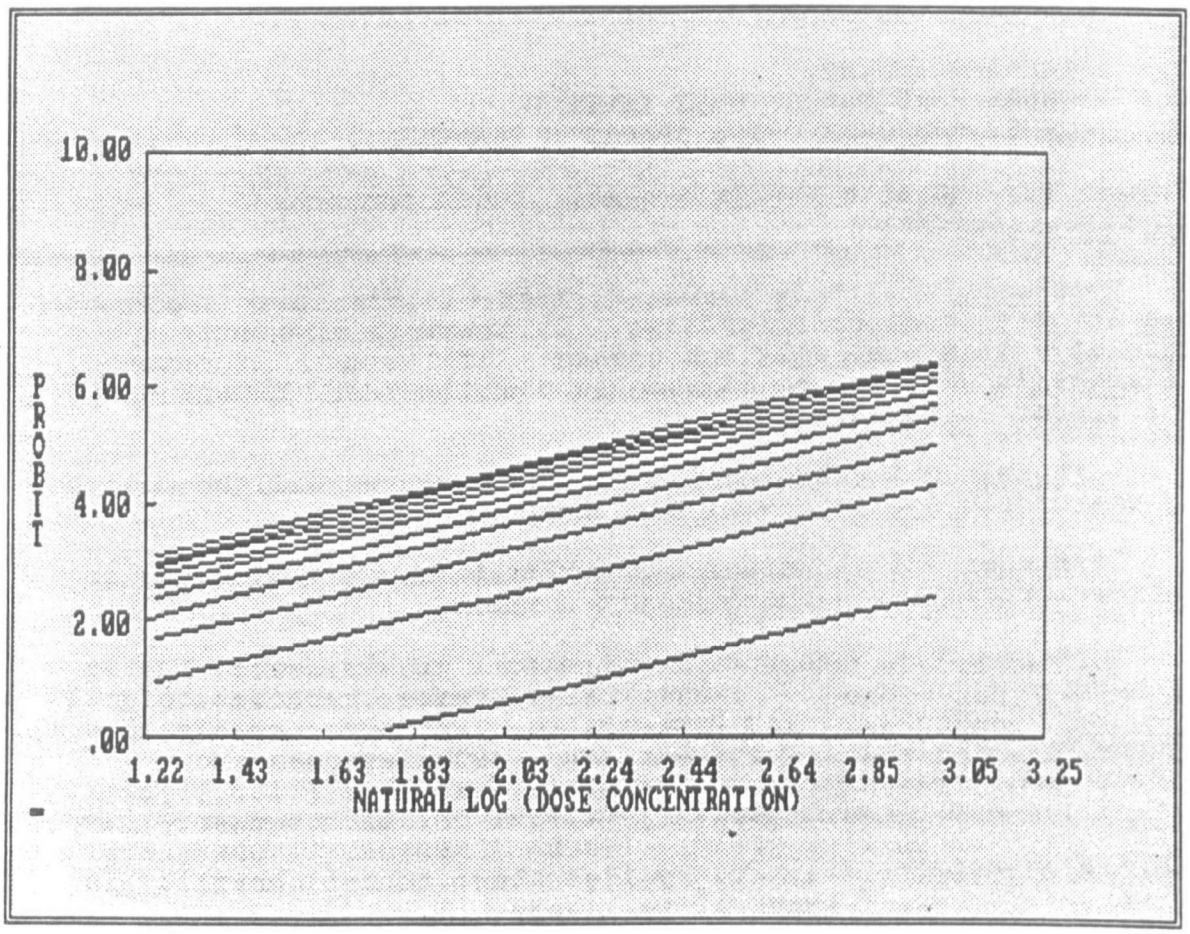
This menu will only appear if there is non-zero response at dose level 0 (control mortality). If there is no control mortality, this menu will not appear. If number 1 is entered, the analysis will not be calculated and the MAIN PROGRAM MENU will appear on the screen.

If number 2 is entered, all 0 dose records will be deleted and the probit analysis will be computed.

If number 3 is entered, all mortalities are adjusted for control mortalities using Abbott's formula.

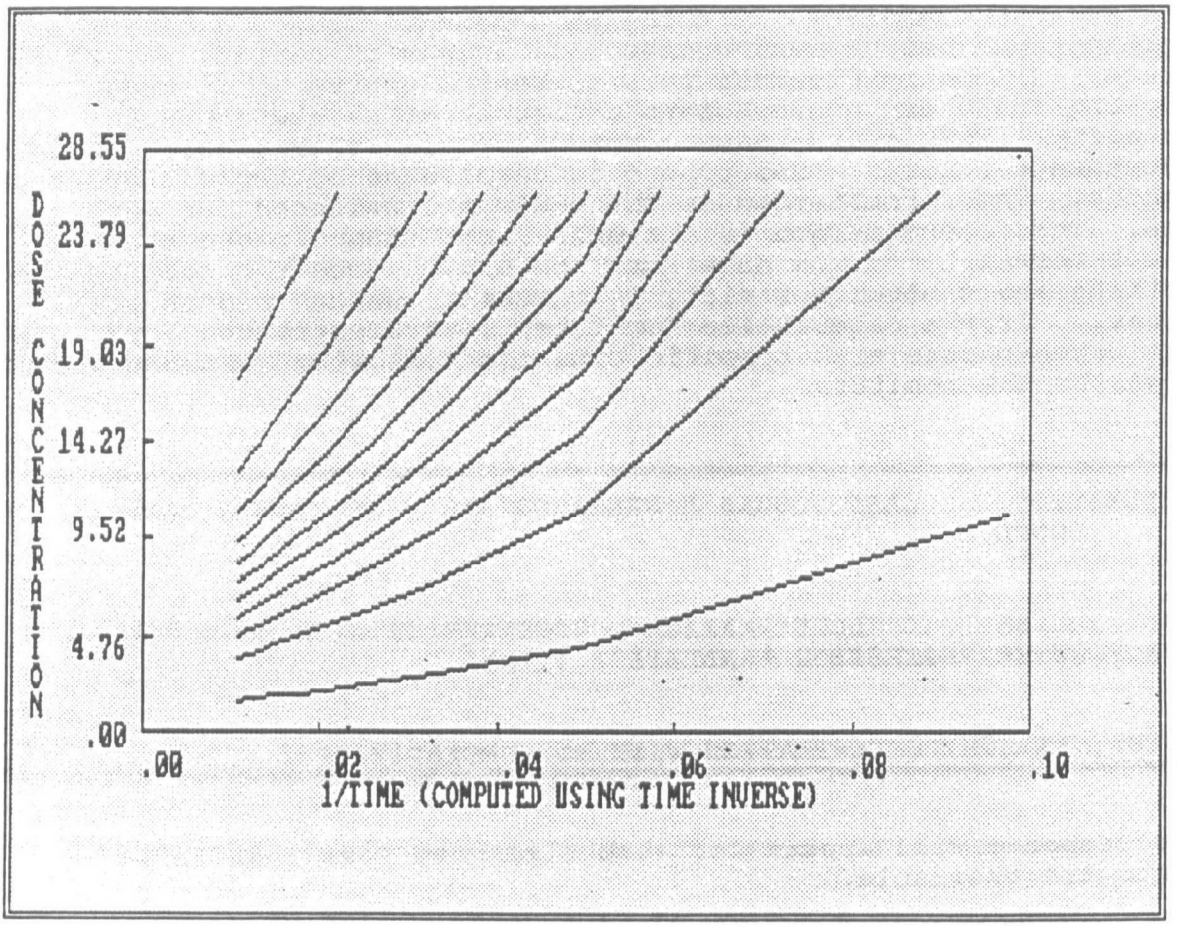
To facilitate computation the data file is sorted by time and dose, then stored. Further the software truncates off all records, except that for highest dose, with zero response and all records, except that for lowest dose, with response = n.
If you have more than one independent variable, (i.e., Type 2, 3, 4, 6, 7), and control mortality varies for each exposure time, we recommend one choose option 2, (IGNORE RESPONSE AT DOSE LEVEL 0). Abbott's adjustment is only applied when control mortality is constant for every exposure time assay.

Once control mortality method (solution 2 or 3) has been selected, Y (yes for plotting on the monitor) entered, and if the type of Model chosen was 2, 3, 4, 6 or 7 a plot will appear on the monitor.



The plot (for an example, use the figure above) has Dose Concentration on the X axis and the Probit on the Y axis. Each line represents time starting at 10 hours exposure with 10 hour increments ending at 100 hours. The lines are in order with time from bottom to top if mortality increases with time, if decreasing they are in order from top to bottom.

To proceed to the next plot, depress the <ENTER> key, the following plot will appear. This graph will NOT appear if Probit Model Type 5 was chosen.



This plot (figure above), has either time or 1/Time as the X axis (this determined by the model chosen) and dose concentration as the Y axis. The lines represent LC_P values for P: .01%, 5%, 15%, 25%, 35%, 45%, 55%, 65%, 75%, 85% and 95%, graphed over time from bottom to top.

If the probit model chosen is Type 5, which is a separate probit line for each exposure time, a different plot and an additional option are available. As with other probit models the option after entering Item 8 from the MAIN PROGRAM MENU (Statistical Analysis), is the plot command. Once a decision on plotting is made a second prompt will appear if control mortality occurs. If control mortality is present a prompt will appear allowing the user to use Abbott's formula or delete control mortality. This is the same command for all other probit models discussed earlier. Model type 5 is calculated by two different methods. The first method is the maximum likelihood for each time. The second method is a simple least square regression of probit mortality on log dose for each time. Once the decision on plotting and control mortality is checked, another prompt will appear. If the calculation for least square estimates have less than three points at a specific time the following response will appear on the monitor.

```
ESTIMATION OF LEAST SQUARE REGRESSION HAD LESS THAN 3 OBS. AT
      HOURS.

DO YOU WISH TO INCLUDE MAXIMUM CONCENTRATION WITH NO MORTALITY
FOR FURTHER REGRESSION ANALYSIS ? (Y/N)
?

NOTE: MAXIMUM CONCENTRATION WITH NO MORTALITY IS
```

This response will appear for each time when less than three points are available.

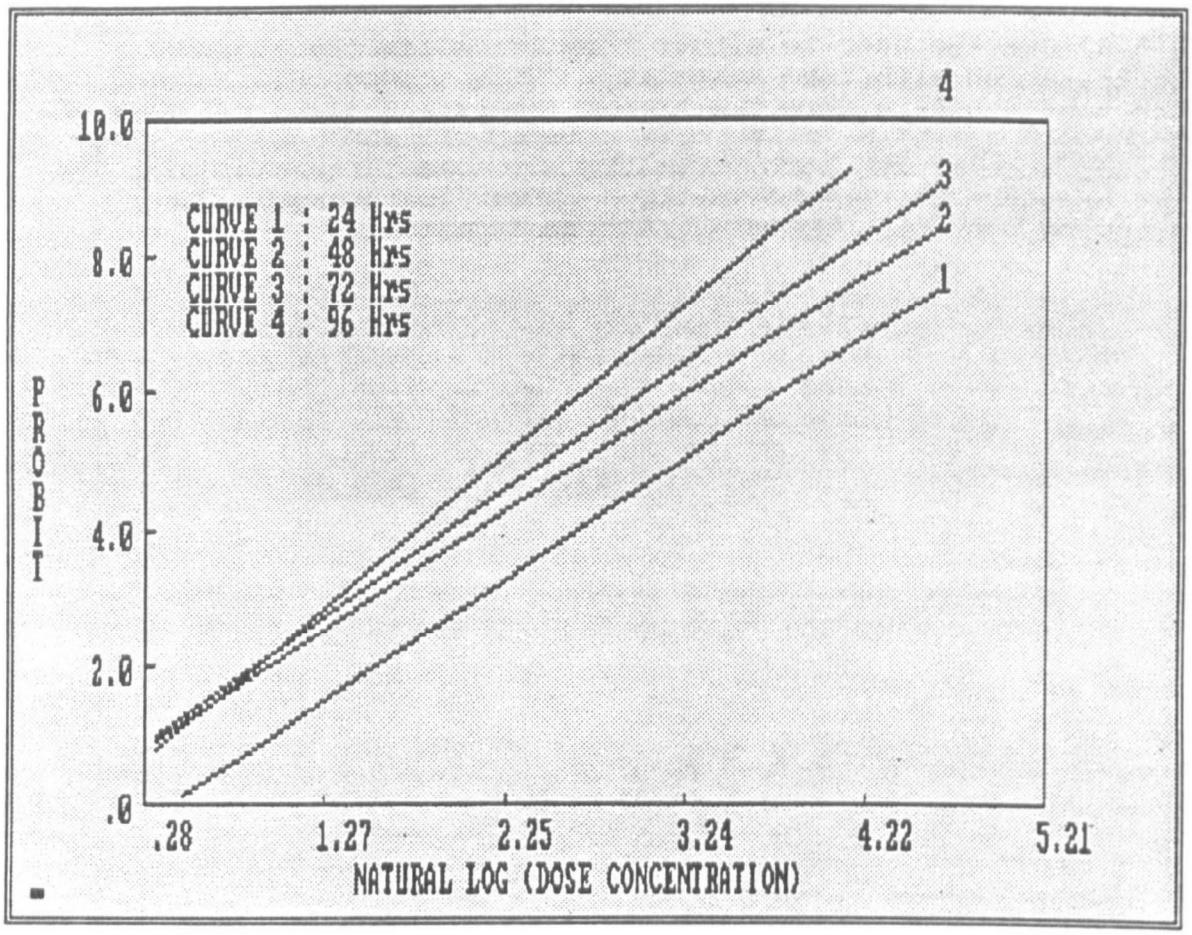
After the least square regression method has been checked for each time, a plot will be produced similar to the plot on page 20, if plotting was requested. The maximum likelihood prompt will appear if probit analysis cannot be computed at a specific time. This situation will occur if the responses at a specific time are all zeros, there are no partial mortalities or convergence is not obtained. The response will be,

```
ESTIMATION OF PROBIT REGRESSION HAS FAILED AT      HOURS.

DO YOU WISH TO INCLUDE MAXIMUM CONCENTRATION WITH NO MORTALITY
FOR FURTHER REGRESSION ANALYSIS ? (Y/N)
?

NOTE: MAXIMUM CONCENTRATION WITH NO MORTALITY IS
```

This allows the user to either keep or delete the highest concentration with zero mortality. This prompt will appear for each time-exposure were the probit analysis fails. If none of the probit analysis fails, this prompt will NOT appear. Those concentrations with zero mortality are used in predicting long time exposure at .01% mortality. After this prompt, the following plot will appear if it was requested.



The plot (for example, use the figure above) has Dose Concentration on the X axis and the Probit on the Y axis. In this example, probit lines are estimated for each 24, 48, 72 and 96 hours.
The equations for the lines at each time are shown in the output.

```
24 hours : Y = -0.799385 + 1.881835 X
48 hours : Y = 0.367254 + 1.831574 X
72 hours : Y = 0.446714 + 1.973819 X
96 hours : Y = 0.087199 + 2.354164 X
```

This plot will remain until the user presses the <ENTER> key.

OUTPUT CONTROL

After the second plot appears, press <ENTER>, the monitor will present the following menu. If one previously entered N for plotting, this menu would have appeared without showing the graphs.

```
        OUTPUT MENU

   1ON THE SCREEN
   2ON A PRINTER
   3ON A DISK
   4QUIT

   CHOOSE 1-4 (enter a single number, you do not need to
   press <ENTER>)
```

If number 4, is selected, the MAIN PROGRAM MENU will appear. If 3 is selected, a prompt will appear asking for disk drive identification and a data set name to identify the output that will be stored. Number 1 and 2 direct the output to the monitor or printer, respectively.

DESCRIPTION OF OUTPUT

The analysis procedures use the iterative reweighed least squares method to estimate the parameters of a probit plane. During the process, MPA uses the convergence criteria of 10^{-5} or 100 maximum iterations to determine the completion of analysis. The 10^{-5} criteria is based on the regression equation intercept and regression coefficients. If the difference from one iteration to the next is less than 10^{-5} for the intercept and regression coefficient or partial regression coefficients, depending on the model chosen, the convergence criteria is met. If 10^{-5} is not met for any of the parameters in the model, a further iteration is performed. If the 10^{-5} criteria is not met after 100 iterations the analysis is terminated. After one of these criteria has been met, a goodness-of-fit chi-square statistic is computed. The output includes:

A.
The data and the data points that have been deleted as a result of multiple 0% lethality or 100% lethality.

B.
Title if one is specified using MAIN PROGRAM MENU Item 7 and the description of the probit model and transformation.

C.
A listing of the values of the estimator is given for each iteration. Each iteration produces an estimate of the intercept and regression coefficient or partial regression coefficients of concentration and time.

D.
Chi-square value is given for the goodness-of-fit test.

E.
The chi-square critical value $\alpha = .05$, and the DF for chi-square.

F.
The variance covariance matrix of estimators are given.

G.
Statement of applying a heterogeneity factor if the calculated chi-square is greater than or equal to the critical value of chi-square. The heterogeneity factor is computed by dividing the goodness of fit chi-square statistic by the degrees of freedom. This application of heterogeneity factor is discussed by Finny (1971).

H.
The adjusted variance covariance matrix and heterogeneity factor are printed. (Output items G and H are not printed if the calculated chi-square is less than tabulated chi-square).

I.
All analysis (except type 4) includes a listing of mortality, lower and upper approximate confidence limits (95%) and the point estimate of LC_p.

If type 1 was chosen, a probit analysis is computed for a single exposure time.

If type 5 was chosen, three different analysis are performed.

The regression equation, (Dose = Intercept + Slope/Time), is calculated along with the ANOVA table for percent probit probabilities of .01, .1, 1, 5, 10, 50 and 90 percent. The transformation of dose and time found in the regression equation is controlled by the Data Transformation Menu described earlier. This additional output is described below.

J.
The description of the regression equation is printed.

K.
Probit probability, the intercept and slope for the equation present in step J.

L.
The estimate of the lethal dose concentration at time infinity is given. This estimate is calculated according to the regression equation described in step J.

M.
Analysis of variance table and R-square value for the probit analysis are printed for each probit.

If one of type 2, 3, 6 or 7 was chosen, the statistical analysis is based on a multiple regression equation in which the default times are 24, 48, 72 and 96 hour.

If type 6 or 7 was chosen, an additional time of infinity is present.

REFERENCES

Literature

1. Finney, D. J. (1971). Probit analysis. Cambridge University Press, London.

2. Finney, D. J. (1978). Statistical method in biological assay. Griffin, London.

3. Hastings, C. (1959). Approximations for digital computers. Princeton Univ. Press, Princeton, NJ.

4. Mayer, F. L. (1990). Predicting chronic lethality to fishes from acute toxicity data. Proc. Soc. Environ. Toxicol. Chem. 11:93.

5. Mayer, F. L. (1990). Predicting chronic lethality of chemicals to fishes from acute toxicity test data. U.S. Environmental Protection Agency, Report No. EPA/600/X-90/147, Gulf Breeze, FL. 15p.

Software

6. True BASIC Reference Manual. (1985). Addison-Wesley, Reading, MA.

APPENDIX B

Acute and Chronic Toxicity Data Base

FRESHWATER FISH TESTS

ACUTE TOXICITY

Chemical: **Butyl benzyl phthalate**
Species: Fathead minnows
Number/Concentration: 30
Age (days): Mean=

Length (mm): Mean= 47
Weight (g): Mean= 0.35
Temperature (C): Mean= 21
pH: Mean= 8.1
Hardness (mg/L): Mean= 297

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	24	48	72	96			
0	0	0	0	0			
740	0	0	0	0			
1060	0	1	1	1			
2100	2	7	8	8			
2770	22	27	28	28			
3230	28	29	29	29			

CHRONIC TOXICITY

Test type: ELS Test duration (days): 30
Observed no-effect concentrations (ug/L):
Lethality: >360 Growth: 140-360 Reproduction:

Reference:

Unpublished data, Monsanto Company.

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MARINE FISH TESTS

ACUTE TOXICITY

Chemical: **Carbon tetrachloride**
Species: Sheepshead minnows
Number/Concentration: 20
Age (days): Mean=

Length (mm): Mean= 16
Weight (g): Mean= 0.11
Temperature (C): Mean= 26
Salinity (o/oo): Mean= 21

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	3	6	12	24	48	72	96
0	0	0	0	0	0	0	0
11700	0	0	0	0	0	0	0
16100	0	1	3	4	4	4	4
25400	17	17	17	18	18	18	19
38500	20	20	20	20	20	20	20

CHRONIC TOXICITY

Test type: ELS Test duration (days): 28
Observed no-effect concentrations (ug/L):
Lethality: 4500-11200 Growth: Reproduction:

Reference:

This study

MARINE FISH TESTS

ACUTE TOXICITY

Chemical: **Chlordane**
Species: Sheepshead minnows
Number/Concentration: 20
Age (days): Mean= 27

Length (mm): Mean= 9
Weight (g): Mean= 0.02
Temperature (C): Mean= 25
Salinity (o/oo): Mean= 22

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	24	48	72	96			
0	0	0	0	0			
5.1	0	0	0	0			
9.3	0	0	3	6			
14	0	0	0	0			
19	0	4	10	15			
31	0	11	19	20			

Reference:

This study

CHRONIC TOXICITY

Test type: ELS Test duration (days): 28
Observed no-effect concentrations (ug/L):
Lethality: 7.1-17 Growth: Reproduction:

Reference:

Parrish, P.R., S.C. Schimmel, D.J. Hansen, J.M. Patrick, and J. Forester. 1976. Chlordane: Effects on several estuarine organisms. J. Toxicol. Environ. Hlth. 1:485-494.

FRESHWATER FISH TESTS

ACUTE TOXICITY

Chemical: **Complex effluent**
Species: Fathead minnows
Number/Concentration: 40
Age (days): Mean=

Length (mm): Mean=
Weight (g): Mean=
Temperature (C): Mean=
pH: Mean= 7.3
Hardness (mg/L): Mean=

Measured [conc] %	Number dead at selected observation time (hours)						
	24	48	72	96			
0	0	0	0	0			
5.6	0	0	0	0			
10	2	8	10	13			
18	4	15	39	40			
32	40	40	40	40			
56	40	40	40	40			

CHRONIC TOXICITY

Test type: ELS Test duration (days): 14
Observed no-effect concentrations (ug/L):
Lethality: 2.0-3.5% Growth: 1.1-2.0% Reproduction: 0.6-1.1%

Reference:

Unpublished data, Monsanto Company.

FRESHWATER FISH TESTS

ACUTE TOXICITY

Chemical: **2,4-D BE**
Species: Cutthrout trout
Number/Concentration: 30
Age days): Mean= 210

Length (mm): Mean= 79
Weight (g): Mean= 4.2
Temperature (C): Mean=10
pH: Mean= 7.4
Hardness (mg/L): Mean=162

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	24	48	72	96			
0	0	0	0	0			
48	0	0	0	0			
100	0	0	0	0			
191	0	0	0	0			
386	0	1	6	10			
785	23	30	30	30			

CHRONIC TOXICITY

Test type: ELS Test duration (days): 60
Observed no-effect concentrations (ug/L):
Lethality: 24-44 Growth: Reproduction:

Reference:

Woodward, D.F. and F.L. Mayer. 1978. Toxicity of three herbicides (butyl, isooctyl, and propylene glycol butyl ether esters of 2,4-D) to cutthrout trout and lake trout. Technical Paper No. 97. U.S. Fish and Wildlife Service, Washington, DC.

FRESHWATER FISH TESTS

ACUTE TOXICITY

Chemical: **2,4-D BE**
Species: Lake trout
Number/Concentration: 30
Age (days): Mean= 120

Length (mm): Mean= 60
Weight (g): Mean= 1.5
Temperature (C): Mean= 10
pH: Mean= 7.4
Hardness (mg/L): Mean= 162

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	24	48	72	96			
0	0	0	0	0			
48	0	0	0	0			
100	0	0	0	0			
191	0	1	1	1			
386	0	1	4	14			
785	29	30	30	30			

CHRONIC TOXICITY

Test type: ELS Test duration (days): 60
Observed no-effect concentrations (ug/L):
Lethality: 33-60 Growth: 15-33 Reproduction:

Reference:

Woodward, D.F. and F.L. Mayer. 1978. Toxicity of three herbicides (butyl, isooctyl, and propylene glycol butyl ether esters of 2,4-D) to cutthrout trout and lake trout. Technical Paper No. 97. U.S. Fish and Wildlife Service, Washington, DC.

FRESHWATER FISH TESTS

ACUTE TOXICITY

Chemical: **2,4-D PGBEE**
Species: Cutthroat trout
Number/Concentration: 30
Age (days): Mean=

Length (mm): Mean= 79
Weight (g): Mean= 4.2
Temperature (C): Mean= 10
pH: Mean= 7.4
Hardness (mg/L): Mean= 162

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	24	48	72	96			
0	0	0	0	0			
40	0	0	0	0			
75	0	0	0	0			
153	0	0	0	0			
308	0	23	26	29			
617	30	30	30	30			

CHRONIC TOXICITY

Test type: ELS Test duration (days): 60
Observed no-effect concentrations (ug/L):
Lethality: 31-60 Growth: Reproduction:

Reference:

Woodward, D.F. and F.L. Mayer. 1978. Toxicity of three herbicides (butyl, isooctyl, and propylene glycol butyl ether esters of 2,4-D) to cutthrout trout and lake trout. Technical Paper No. 97. U.S. Fish and Wildlife Service, Washington, DC.

FRESHWATER FISH TESTS

ACUTE TOXICITY

Chemical: **2,4-D PGBEE**
Species: Lake trout
Number/Concentration: 30
Age (days): Mean=

Length (mm): Mean= 60
Weight (g): Mean= 1.5
Temperature (C): Mean= 10
pH: Mean= 7.4
Hardness (mg/L): Mean= 162

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	24	48	72	96			
0	0	0	0	0			
40	0	0	0	0			
75	0	0	0	0			
153	0	0	0	0			
308	0	5	19	23			
617	30	30	30	30			

CHRONIC TOXICITY

Test type: ELS Test duration (days): 60
Observed no-effect concentrations (ug/L):
Lethality: 52-100 Growth: 52-100 Reproduction:

Reference:

Woodward, D.F. and F.L. Mayer. 1978. Toxicity of three herbicides (butyl, isooctyl, and propylene glycol butyl ether esters of 2,4-D) to cutthrout trout and lake trout. Technical Paper No. 97. U.S. Fish and Wildlife Service, Washington, DC.

MARINE FISH TESTS

ACUTE TOXICITY

Chemical: **Endosulfan**
Species: Sheepshead minnows
Number/Concentration: 20
Age (days): Mean=

Length (mm): Mean =
Weight (g): Mean=
Temperature (C): Mean= 30
Salinity (o/oo): Mean=

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	24	48	72	96			
0	0	0	0	0			
0.12	0	0	0	0			
0.36	0	0	0	0			
0.54	0	0	0	0			
0.90	0	0	0	0			
1.4	0	0	0	0			
4.1	1	6	18	20			
6.5	9	19	20	20			
11	19	20	20	20			

CHRONIC TOXICITY

Test type: ELS Test duration (days): 28
Observed no-effect concentrations (ug/L):
Lethality: 1.1-2.5 Growth: 0.31-0.54 Reproduction:
0.92-2.1 0.50-0.92

Reference:

Hansen, D.J. and G.M. Cripe. 1984. Interlaboratory comparison of the early life-stage toxicity test using the sheepshead minnow (Cyprinodon variegatus). EPA-600/X-84-081. U.S. Environmental Protection Agency, Gulf Breeze, Fl.

MARINE FISH TESTS

ACUTE TOXICITY

Chemical: Endrin
Species: Sheepshead minnows
Number/Concentration: 20
Age (days): Mean= 23

Length (mm): Mean = 9
Weight (g): Mean=
Temperature (C): Mean= 26
Salinity (o/oo): Mean= 18

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	24	48	72	96			
0	0	0	0	0			
0.16	0	0	0	0			
0.26	0	0	11	15			
0.52	3	17	20	20			
0.76	16	20	20	20			
1.4	20	20	20	20			

Reference:

This study

CHRONIC TOXICITY

Test type: LC Test duration (days): 140
Observed no-effect concentrations (ug/L):
Lethality: 0.12-0.31 Growth: 0.12-0.31 Reproduction: 0.12-0.31

Reference:

Hansen, D.J., S.C. Schimmel, and J. Forester. 1977. Endrin: Effects on the entire life cycle of a saltwater fish, Cyprinodon variegatus. J. Toxicol. Environ. Hlth. 3:721-733.

MARINE FISH TESTS

ACUTE TOXICITY

Chemical: **EPN**
Species: Sheepshead minnows
Number/Concentration: 20
Age (days): Mean= 22

Length (mm): Mean = 12
Weight (g): Mean=
Temperature (C): Mean= 25
Salinity (o/oo): Mean= 20

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	24	48	72	96			
0	0	0	0	1			
46	0	0	1	2			
63	1	2	4	5			
150	6	9	13	19			
213	14	19	19	20			
381	20	20	20	20			

Reference:

This study

CHRONIC TOXICITY

Test type: PLC Test duration (days): 229
Observed no-effect concentrations (ug/L):
Lethality: 4.1-7.9 Growth: 4.1-7.9 Reproduction: >7.9

Reference:

Cripe, G.M., L.R. Goodman, and D.J. Hansen. 1984. Effect of chronic exposure to EPN and to Guthion on the critical swimming speed and brain acetylcholinesterase activity of Cyprinodon variegatus. Aquatic Toxicol. 5:255-266.

FRESHWATER FISH TESTS

ACUTE TOXICITY (Static)

Chemical: **Fluorene**
Species: Bluegills
Number/Concentration: 10
Age (days): Mean=

Length (mm): Mean=
Weight (g): Mean= 0.8
Temperature (C): Mean= 22
pH: Mean= 7.5
Hardness (mg/L): Mean= 280

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	24	48	72	96			
0	0	0	0	0			
133	0	0	0	0			
237	0	0	0	0			
414	0	0	0	0			
740	0	5	10	10			
1332	0	8	10	10			
2368	1	10	10	10			
4144	4	10	10	10			
7400	9	10	10	10			

Reference:

Finger, S.E., E.F. Little, M.G. Henry, J.F. Fairchild, and T.P. Boyle. 1985. Comparison of laboratory and field assessment of fluorene- Part I: Effects of fluorene on the survival, growth, reproduction, and behavior of aquatic organisms in laboratory tests. Pages 120-133 in T.P. Boyle, ed. Validation and Predictability of Laboratory Methods for Assessing the Fate and Effects of Contaminants in Aquatic Ecosystems. American Society for Testing and Materials STP 865, Philadelphia, PA.

CHRONIC TOXICITY

Test type: Pond study Test duration (days): 70
Observed no-effect concentrations (ug/L)*:
Lethality: 0-67 Growth: >433 Reproduction: 0-67

* Measured concentration based on average of day 1,3, and 7 analyses.

Reference:

Boyle, T.P., S.E. Finger, R.L. Paulson, and C.F. Rabeni. 1985.Comparison of laboratory and field assessment of fluorene- Part II: Effects on the ecological structure and function of experimental pond ecosystems. Pages 134-151 in T.P. Boyle, ed. Validation and Predictability of Laboratory Methods for Assessing the Fate and Effects of Contaminants in Aquatic Ecosystems. American Society for Testing and Materials STP 865, Philadelphia, PA.

FRESHWATER FISH TESTS

ACUTE TOXICITY (Static)

Chemical: **Fluridone**
Species: Channel catfish
Number/Concentration: 10
Age (days): Mean=

Length (mm): Mean=
Weight (g): Mean= 0.70
Temperature (C): Mean= 22
pH: Mean= 7.1
Hardness (mg/L): Mean= 40

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	24	48	72	96			
0	0	0	0	0			
1800	0	0	0	0			
3200	0	0	0	0			
5600	0	0	0	1			
10000	0	2	6	9			
18000	1	1	5	10			
32000	10	10	10	10			
56000	10	10	10	10			
100000	10	10	10	10			

CHRONIC TOXICITY

Test type: ELS Test duration (days): 60
Observed no-effect concentrations (ug/L):
Lethality: 1000-2000 Growth: 1000-2000 Reproduction:

Reference:

Hamelink, J.L., D.R. Buckler, F.L. Mayer, D.U. Palawski, and H.O. Sanders. 1986. Toxicity of fluridone to aquatic invertebrates and fish. Environ. Toxicol. Chem. 5:87-94.

MARINE FISH TESTS

ACUTE TOXICITY

Chemical: **Heptachlor**
Species: Sheepshead minnows
Number/Concentration: 20
Age (days): Mean= 37

Length (mm): Mean = 10
Weight (g): Mean= 0.02
Temperature (C): Mean= 25
Salinity (o/oo): Mean= 22

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	24	48	72	96			
0	0	0	0	1			
4.2	0	0	0	0			
6.8	0	0	0	1			
11	0	6	9	14			
15	2	14	18	20			
31	20	20	20	20			

Reference:

This study

CHRONIC TOXICITY

Test type: PLC, ELS Test duration (days): 96,28
Observed no-effect concentrations (ug/L):
Lethality: 1.9-2.8 Growth: ---- Reproduction: 0.97-1.9
2.2-3.5 2.2-3.5

Reference:

Goodman, L.R., D.J. Hansen, J.A. Couch, and J. Forester. 1976. Effects of heptachlor and toxaphene on laboratory-reared embryos and fry of the sheepshead minnow. Southeast Assoc. Game and Fish Comm. 30:192-202. Hansen, D.J. and P.R. Parrish. 1977. Suitability of sheepshead minnows (Cyprinodon variegatus) for lifecycle toxicity tests. Pages 117-126 in F.L. Mayer and J.L. Hamelink, eds. Aquatic Toxicology and Hazard Evaluation. American Society for Testing and Materials STP 634, Philadelphia, PA.

FRESHWATER FISH TESTS

ACUTE TOXICITY

Chemical: **Kepone**
Species: Fathead minnows
Number/Concentration: 20
Age (days): Mean= 30

Length (mm): Mean= 15
Weight (g): Mean= 0.03
Temperature (C): Mean= 25
pH: Mean= 7.8
Hardness (mg/L): Mean= 290

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	24	48	72	96			
0	0	0	0	0			
10	0	1	5	5			
16	3	7	12	12			
22	5	12	13	13			
27	8	20	20	20			
40	20	20	20	20			
56	20	20	20	20			
73	20	20	20	20			

CHRONIC TOXICITY

Test type: ELS Test duration (days): 60
Observed no-effect concentrations (ug/L):
Lethality: 1.2-3.1 Growth: 1.2-3.1 Reproduction:

Reference:

Buckler, D.R., A. Witt, Jr., F.L. Mayer, and J.N. Huckins. 1981. Acute and chronic effects of Kepone and mirex on the fathead minnow. Trans. Am. Fish. Soc. 110:270-280.

FRESHWATER FISH TESTS

ACUTE TOXICITY

Chemical: **Methoxychlor**
Species: Brook trout
Number/Concentration: 20
Age (days): Mean=

Length (mm): Mean=
Weight (g): Mean= 0.97
Temperature (C): Mean= 12
pH: Mean=
Hardness (mg/L): Mean=

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	24	48	72	96			
0	0	0	0	0			
4.8	0	0	0	0			
9.4	0	0	3	14			
24	3	11	20	20			

CHRONIC TOXICITY (Rainbow trout)

Test type: ELS Test duration (days): 90
Observed no-effect concentrations (ug/L):
Lethality: 1.1-3.1 Growth: 1.1-3.1 Reproduction:

Reference:

Unpublished data, Columbia National Fisheries Contaminant Research Center.

MARINE FISH TESTS

ACUTE TOXICITY

Chemical: **Methoxychlor**
Species: Sheepshead minnows 1
Number/Concentration: 20
Age (days): Mean= 47

Length (mm): Mean = 15
Weight (g): Mean= 0.04
Temperature (C): Mean= 25
Salinity (o/oo): Mean= 22

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	24	48	72	96			
0	0	0	0	0			
13	0	0	0	0			
20	2	2	3	3			
43	0	8	18	20			
60	5	20	20	20			
98	20	20	20	20			

Reference:

This study

CHRONIC TOXICITY

Test type: PLC, ELS Test duration (days): 112,28
Observed no-effect concentrations (ug/L):
Lethality: 23-48 Growth: ---- Reproduction: 12-23
12-23 >12

Reference:

Hansen, D.J. and P.R. Parrish. 1977. Suitability of sheepshead minnows (Cyprinodon variegatus) for lifecycle toxicity tests. Pages 117-126 in F.L. Mayer and J.L. Hamelink, eds. Aquatic Toxicology and Hazard Evaluation. American Society for Testing and Materials STP 634, Philadelphia, PA.

MARINE FISH TESTS

ACUTE TOXICITY

Chemical: **Methoxychlor**
Species: Sheepshead minnows 2
Number/Concentration: 20
Age (days): Mean= 21

Length (mm): Mean = 9
Weight (g): Mean= 0.007
Temperature (C): Mean= 25
Salinity (o/oo): Mean= 21

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	24	48	72	96			
0	0	0	0	0			
13	0	0	0	0			
20	0	0	0	0			
35	0	6	19	20			
61	9	20	20	20			
120	20	20	20	20			

Reference:

This study

CHRONIC TOXICITY

Test type: PLC,ELS Test duration (days): 112,28
Observed no-effect concentrations (ug/L):
Lethality: 23-48 Growth: ---- Reproduction: 12-23
12-23 >12

Reference:

Hansen, D.J. and P.R. Parrish. 1977. Suitability of sheepshead minnows (Cyprinodon variegatus) for lifecycle toxicity tests. Pages 117-126 in F.L. Mayer and J.L. Hamelink, eds. Aquatic Toxicology and Hazard Evaluation. American Society for Testing and Materials STP 634, Philadelphia, PA.

MARINE FISH TESTS

ACUTE TOXICITY

Chemical: **Methoxychlor**
Species: Sheepshead minnows 3
Number/Concentration: 20
Age (days): Mean= 27

Length (mm): Mean = 9
Weight (g): Mean= 0.007
Temperature (C): Mean= 25
Salinity (o/oo): Mean= 20

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	24	48	72	96			
0	0	0	0	0			
15	0	0	0	0			
31	0	0	1	1			
49	0	14	15	15			
57	4	17	20	20			
86	19	20	20	20			

Reference

This study

CHRONIC TOXICITY

Test type: PLC, ELS Test duration (days): 112,28
Observed no-effect concentrations (ug/L):
Lethality: 23-48 Growth: ---- Reproduction: 12-23
12-23 >12

Reference:

Hansen, D.J. and P.R. Parrish. 1977. Suitability of sheepshead minnows (Cyprinodon variegatus) for lifecycle toxicity tests. Pages 117-126 in F.L. Mayer and J.L. Hamelink, eds. Aquatic Toxicology and Hazard Evaluation. American Society for Testing and Materials STP 634, Philadelphia, PA.

FRESHWATER FISH TESTS

ACUTE TOXICITY

Chemical: **Pentachlorophenol-P**
Species: Fathead minnows
Number/Concentration: 20
Age (days): Mean= 40

Length (mm): Mean= 26
Weight (g): Mean= 0.02
Temperature (C): Mean= 22
pH: Mean= 7.4
Hardness (mg/L): Mean= 272

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	24	48	72	96			
0	0	0	0	0			
237	0	0	0	0			
311	2	4	4	4			
414	20	20	20	20			

CHRONIC TOXICITY

Test type: PLC Test duration (days): 90
Observed no-effect concentrations (ug/L):
Lethality: >142 Growth: 36-85 Reproduction:

Reference:

Cleveland, L., D.R. Buckler, F.L. Mayer, and D.R. Branson. 1982. Toxicity of three preparations of pentachlorophenol to fathead minnows-A comparative study. Environ. Toxicol. Chem. 1:205-212.

MARINE FISH TESTS

ACUTE TOXICITY

Chemical: **Phorate**
Species: Sheepshead minnows
Number/Concentration: 20
Age (days): Mean=

Length (mm): Mean = 7
Weight (g): Mean=
Temperature (C): Mean= 25
Salinity (o/oo): Mean= 27

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	24	48	72	96			
0	0	0	0	0			
0.12	0	0	0	0			
0.22	0	0	0	0			
0.50	0	0	0	1			
0.83	2	2	2	2			
1.1	2	4	4	5			
1.5	4	4	5	14			
4.2	20	20	20	20			
6.3	20	20	20	20			
10	20	20	20	20			

CHRONIC TOXICITY

Test type: ELS Test duration (days): 28
Observed no-effect concentrations (ug/L):
Lethality: 0.24-0.41 Growth: 0.24-0.41 Reproduction:

Reference:

U.S. Environmental Protection Agency. 1981. Acephate aldicarb, carbophenothion, DEF, EPN, ethoprop, methyl parathion, and phorate: Their acute and chronic toxicity, bioconcentration potential, and persistence as related to marine environments. EPA-600/4-81-023. U.S. Environmental Protection Agency, Gulf Breeze, FL.

FRESHWATER FISH TESTS

ACUTE TOXICITY

Chemical: **Pydraul 50E**
Species: Fathead minnows
Number/Concentration: 20
Age (days): Mean=

Length (mm): Mean=
Weight (g): Mean= 1.6
Temperature (C): Mean= 16
pH: Mean= 7.7
Hardness (mg/L): Mean= 272

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	24	48	72	96			
0	0	0	0	0			
754	0	0	0	0			
1034	0	0	1	3			
1301	0	1	6	12			
1830	13	19	20	20			

CHRONIC TOXICITY

Test type: PLC Test duration (days): 90
Observed no-effect concentrations (ug/L):
Lethality: 317-752 Growth: 317-752 Reproduction:

Reference:

Mayer, F.L., W.J. Adams, M.T. Finley, P.R. Michael, P.M. Mehrle, and V.W. Saeger. 1981. Phosphate ester hydraulic fluids: An aquatic environmental assessment of Pydrauls 50E and 115E. Pages 103-123 in D.R. Branson and K.L. Dickson, eds. Aquatic Toxicology and Hazard Assessment. American Society for Testing and Materials STP 737, Philadelphia, PA.

FRESHWATER FISH TESTS

ACUTE TOXICITY

Chemical: **TFM**
Species: Brook trout
Number/Concentration: 10
Age (days): Mean= Adult

Length (mm): Mean= 261
Weight (g): Mean= 212
Temperature (C): Mean= 10
pH: Mean= 7.4
Hardness (mg/L): Mean= 272

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	1	3	6	24	48	72	96
0	0	0	0	0	0	0	0
4500	0	0	0	0	0	0	0
6500	0	0	1	1	1	5	7
8600	0	0	5	7	7	7	8
11700	0	5	8	10	10	10	10
14400	1	8	10	10	10	10	10

CHRONIC TOXICITY

Test type: PLC (adult) Test duration (days): 120
Observed no-effect concentrations (ug/L):
Lethality: 4000-8800 Growth: 4000-8800 Reproduction: 1600-4000

Reference:

Dwyer, W.P., F.L. Mayer, J.L. Allen, and D.R. Buckler. 1978. Chronic and simulated use-pattern exposures of brook trout (Salvelinus fontinalis) to 3-trifluromethyl-4-nitrophenol (TFM). Investigations in Fish Control No. 84. U.S. Fish and Wildlife Service, Washington, DC.

FRESHWATER FISH TESTS

ACUTE TOXICITY

Chemical: **Toxaphene**
Species: Brook trout 1
Number/Concentration: 20
Age (days): Mean= 480

Length (mm): Mean= 231
Weight (g): Mean= 133
Temperature (C): Mean= 10
pH: Mean= 7.4
Hardness (mg/L): Mean= 272

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	72	96	120	144			
0	0	0	0	0			
3.8	0	0	0	0			
5.1	0	0	0	3			
6.2	0	1	4	15			
8.8	0	4	15	20			
12	16	20	20	20			

CHRONIC TOXICITY

Test type: PLC (adult) Test duration (days): 180
Observed no-effect concentrations (ug/L):
Lethality: 0.14-0.29 Growth: 0.14-0.29 Reproduction: 0.039-0.068

Reference:

Mayer, F.L., P.M. Mehrle, and W.P. Dwyer. 1975. Toxaphene effects on reproduction, growth, and mortality of brook trout. EPA-600/3-75-013. U.S. Environmental Protection Agency, Duluth, MN.

FRESHWATER FISH TESTS

ACUTE TOXICITY

Chemical: **Toxaphene**
Species: Brook trout 2
Number/Concentration: 26
Age (days): Mean=

Length (mm): Mean=
Weight (g): Mean= 9.2
Temperature (C): Mean= 12
pH: Mean= 7.4
Hardness (mg/L): Mean= 272

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	24	48	72	96			
0	0	0	0	0			
2.0	0	0	0	0			
2.9	0	0	0	4			
4.2	0	0	9	22			
6.2	0	10	26	26			
8.2	0	23	26	26			
11	2	26	26	26			
16	25	26	26	26			

CHRONIC TOXICITY

Test type: ELS Test duration (days): 90
Observed no-effect concentrations (ug/L):
Lethality: 0.068-0.14 Growth: 0.068-0.14 Reproduction:

Reference:

Mayer, F.L., P.M. Mehrle, and W.P. Dwyer. 1975. Toxaphene effects on reproduction, growth, and mortality of brook trout. EPA-600/3-75-013. U.S. Environmental Protection Agency, Duluth, MN.

FRESHWATER FISH TESTS

ACUTE TOXICITY

Chemical: **Toxaphene**
Species: Fathead minnows
Number/Concentration: 10
Age (days): Mean=

Length (mm): Mean=
Weight (g): Mean= 0.3
Temperature (C): Mean= 25
pH: Mean= 7.4
Hardness (mg/L): Mean= 272

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	24	48	72	96			
0	0	0	0	0			
2.8	0	0	0	0			
4.2	0	0	0	0			
6.0	0	1	2	4			
7.8	0	2	3	4			
11	0	6	9	10			
15	2	10	10	10			
20	7	10	10	10			

CHRONIC TOXICITY

Test type: PLC,ELS Test duration (days): 98,30
Observed no-effect concentrations (ug/L):
Lethality: >0.17 Growth: 0.054-0.097 Reproduction: >0.17
0.097-0.17

Reference:

Mayer, F.L., P.M. Mehrle, and W.P. Dwyer. 1975. Toxaphene: Chronic toxicity to fathead minnows and channel catfish. EPA-600/3-77-069. U.S. Environmental Protection Agency, Duluth, MN.

FRESHWATER FISH TESTS

ACUTE TOXICITY

Chemical: **Toxaphene**
Species: Channel catfish
Number/Concentration: 20
Age (days): Mean=

Length (mm): Mean=
Weight (g): Mean=
Temperature (C): Mean= 20
pH: Mean= 7.4
Hardness (mg/L): Mean= 272

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	24	48	72	96			
0	0	0	0	0			
0.56	0	0	0	0			
1.0	0	0	4	10			
1.8	0	16	18	20			
3.2	20	20	20	20			

CHRONIC TOXICITY

Test type: ELS of PLC Test duration (days): 90
Observed no-effect concentrations (ug/L):
Lethality: 0.070-0.13 Growth: 0.070-0.13 Reproduction: 0.13-0.30

Reference:

Mayer, F.L., P.M. Mehrle, and W.P. Dwyer. 1975. Toxaphene: Chronic toxicity to fathead minnows and channel catfish. EPA-600/3-77-069. U.S. Environmental Protection Agency, Duluth, MN.

MARINE FISH TESTS

ACUTE TOXICITY

Chemical: **Toxaphene**
Species: Sheepshead minnows
Number/Concentration: 20
Age (days): Mean= 23

Length (mm): Mean = 7
Weight (g): Mean= 0.004
Temperature (C): Mean= 27
Salinity (o/oo): Mean= 22

Measured [conc] (ug/L)	Number dead at selected observation time (hours)						
	24	48	72	96			
0	0	0	0	0			
1.7	0	0	0	1			
2.4	0	1	2	6			
4.4	1	6	18	20			
6.4	0	18	20	20			
9.7	18	20	20	20			

Reference:

This study

CHRONIC TOXICITY

Test type: ELS Test duration (days): 28
Observed no-effect concentrations (ug/L):
Lethality: 1.1-2.5 Growth: >2.5 Reproduction:

Reference:

Goodman, L.R., D.J. Hansen, J.A. Couch, and J.Forester. 1976. Effects of heptachlor and toxaphene on laboratory-reared embryos and fry of the sheepshead minnow. Southeast Assoc. Game and Fish Comm. 30:192-202.

BIRD TESTS

ACUTE TOXICITY (Subacute)

Chemical: **Mercury ($HgCl_2$)**
Species: Coturnix quail
Number/Concentration: 15
Age (days): Mean= 14

Measured [conc] (ug/g)	Number dead at selected observation time (hours)									
	24	48	72	96	120	144	168	192	216	240
0	0	0	0	0	0	0	0	0	0	0
2500	0	0	1	2	2	2	2	2	2	2
3535	0	0	0	0	1	4	5	5	5	5
5000	0	0	0	1	5	7	7	7	7	7
7070	0	1	1	5	8	11	11	11	11	11
10000	0	2	4	5	8	11	11	12	12	12

Chemical was presented at various concentrations in turkey starter mash for 5 days. Daily observations for evidence of toxicity were made from first presentation of treated feed until clinical signs were no longer detectable.

CHRONIC TOXICITY

Test type: sublethal Test duration (days): 63(hatch-9wks)
Observed no-effect concentrations (ug/g):
Lethality: >32 Growth: >32 Reproduction: >32

Reference:

Hill, E.F. and J.H. Soares, Jr. 1984. Subchronic mercury exposure in coturnix and a method of hazard evaluation. Environ. Toxicol. Chem. 3:489-502.

BIRD TESTS

ACUTE TOXICITY (Subacute)

Chemical: **Methyl mercury (CH_3HgCl)**
Species: Coturnix quail
Number/Concentration: 15 (exceptions: 0=10,30 ug/g=16)
Age (days): Mean= 14

Measured [conc] (ug/g)	Number dead at selected observation time (hours)									
	24	48	72	96	120	144	168	192	216	240
0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	0	1
30	0	0	0	0	0	1	1	2	2	2
42	0	0	0	0	1	1	2	3	3	6
60	0	0	0	0	0	0	1	4	7	11

Chemical was presented at various concentrations in turkey starter mash for 5 days. Daily observations for evidence of toxicity were made from first presentation of treated feed until clinical signs were no longer detectable.

CHRONIC TOXICITY

Test type: sublethal Test duration (days): 63(hatch-9wks)
Observed no-effect concentrations (ug/g):
Lethality: 2-8 Growth: >32 Reproduction: 2-8

Reference:

Hill, E.F. and J.H. Soares, Jr. 1984. Subchronic mercury exposure in coturnix and a method of hazard evaluation. Environ. Toxicol. Chem. 3:489-502.

Computer Product Information Sheet

NTIS Federal Computer Products Center
UNITED STATES DEPARTMENT OF COMMERCE
National Technical Information Service
5285 Port Royal Road, Springfield, VA 22161

Software

TO ORDER: **Phone: (703) 487-4650** **FAX: 703-321-8547** **Telex: 64617**
(Also available on a Rush basis for an added fee)

Title:
Statistical Approach to Predicting Chronic Toxicity of Chemicals to Fishes from Acute Toxicity Test Data (for microcomputers)

Source: Environmental Protection Agency
NTIS Order Number: **PB92-503119** Product Type: **Software-Diskette**
Date: as of June 1992
Price Code: D02 U.S., Canada, & Mexico: $90.00, all other addresses: $180.00

(Price includes documentation, add $3 to each order for handling)

Summary:
A methodology was developed to predict chronic toxicity based on acute data. This method is called Multifactor Probit Analysis (MPA) and uses the iteractive reweighed least squares method to estimate parameters of the probit surface. The independent variables are time of exposure and probit of the proportion responding to a concentration. MPA allows the user to predict the concentration of toxicant at any time and percent mortality, LC t,p. MPA calculates a point estimate and a measure of dispersion (95% Confidence limits). The MPA is versatile, entirely menu-driven and offers 7 probit models and transformation combinations of the independent variables

The software is on one 5 1/4 inch diskette, 360K double density. File format: ASCII. Documentation included; may be ordered separately as PB92-169655.

System: IBM PS2 Model 50; DOS 4.0 operating system, 640K. Language: True BASIC.

7/13/92

PB92-503119

COMPUTER DISKETTE FILE PROPERTIES

(FCPC#5075)

01. Completion Date			02. Long Title	03. Short Title
Year	Month	Day	**Statistical Approach to Predicting Chronic Toxicity of Chemicals to Fishes from Acute**	Multifactor Probit Analysis (MPA)

04. Copying Date			05. Subscription	06.	07. Number of Diskettes
Year	Month	Day	No	☒ New Product ☐ Replacement	1

08. Submitting Organization and Address	09. For information about the content, contact:
Environmental Protection Agency Office of Research and Development Environmental Research Laboratory Gulf Breeze, FL 32561	**Dr. F.L. Mayer () (904)-934-9380** **Dr. G.F. Krause () (314)-882-6663**

10. Host Computer/Model	11. Memory Requirement	12. Language/Format
IBM PS2 Model 50	640 K	True BASIC ASCII

13. Diskette Size	14. Diskette Capacity	15. Operating System/Version
5 1/4 inch	360K	DOS 4.0

16. Number of Files	17. Number of Records	18. Record Length
1		

19. Documentation

PB92-169655

20. Supplemental Information

System: IBM PS2 Model 50; DOS 4.0 operating system, 640K. Language: True BASIC.
The software is on one 5 1/4 inch diskette, 360K double density. File format: ASCII.
Documentation included; may be ordered separately as PB92-169655.

21. For Submitting Organization Use

Form NTIS-FCPC-01(1/89)

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before completing)

1. REPORT NO. EPA/600/R-92/091 [2] EPA/SW/DK-92/047a	3. RECIPIENT'S ACCESSION NO. PB92-169655
4. TITLE AND SUBTITLE STATISTICAL APPROACH TO PREDICTING CHRONIC TOXICITY OF CHEMICALS TO FISHES FROM ACUTE TOXICITY TEST DATA	5. REPORT DATE JUNE 1992 6. PERFORMING ORGANIZATION CODE EPA/ORD
7. AUTHOR(S) E.L. Mayer[1], G.F. Krause[2], M.R. Ellersieck[2], G. Lee	8. PERFORMING ORGANIZATION REPORT NO
9. PERFORMING ORGANIZATION NAME AND ADDRESS [2]University of Missouri Agricultural Experiment Station, Columbia, Missouri 65211	10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS U.S. ENVIRONMENTAL PROTECTION AGENCY ENVIRONMENTAL RESEARCH LABORATORY OFFICE OF RESEARCH AND DEVELOPMENT GULF BREEZE, FLORIDA 32561	13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE

15. SUPPLEMENTARY NOTES

FOR DISKETTE SEE: PB92-503119

16. ABSTRACT

A comprehensive approach to predicting chronic toxicity from acute toxicity data was developed in which simultaneous consideration is given to concentration, degree of response, and time course of effect. A consistent endpoint (lethality) and degree of response (0%) were used to compare acute and chronic tests. Predicted no-effect concentrations were highly accurate 92% of the time (within a factor of 2.0 of the limits of the maximum acceptable toxicant concentrations for lethality) and did not vary by more than a factor of 4.8 when the technique was applied to a data base of 18 chemicals and 7 fish species. Growth effects can be predicted from chronic lethality, but reproductive effects should not be.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS (This Report)	21. NO. OF PAGES 104
	20. SECURITY CLASS (This page)	22. PRICE

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