A User's Guide to

ALLFIT

Simultaneous fitting of families of sigmoidal dose response curves using the four-parameter logistic equation

Andre DeLean, Peter J. Munson
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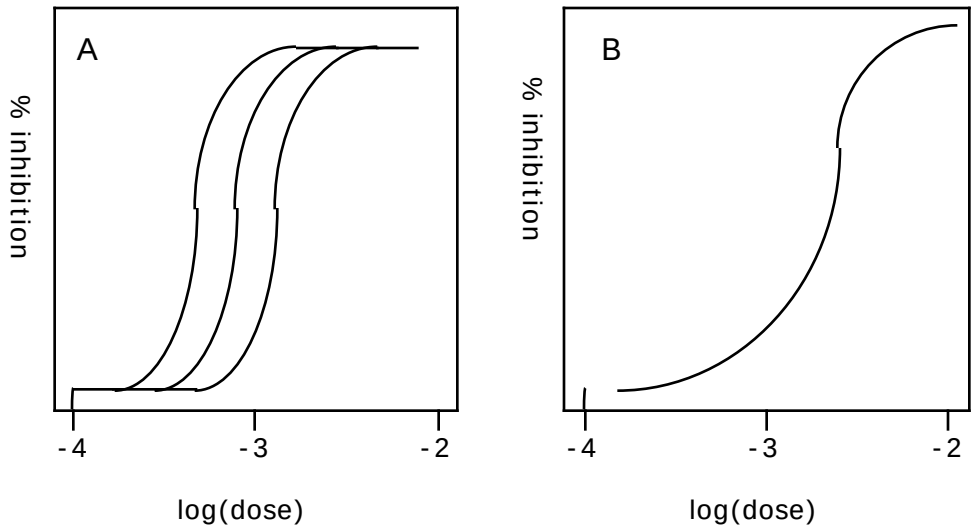

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November 1988

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This manual is oriented primarily towards the IBM-PC and equivalents. Users of versions for other computers (VAX) should also use this manual, taking into account differences relating to system operations. The first version of the program ALLFIT on the DEC10 and the User's Guide were prepared by A.D.L., P.J.M. , and D.R. in 1977. Graphics capabilities added by P.J.M. in 1982. The version 2.0 of the program (adding multiple input files, session file, covariance matrix for parameters and other changes), and the first revision of the User's Guide, were prepared by V.G. during 1984-1986. The microcomputer version was developed by V.G. during 1985-1986. The DEC-VAX version was developed by V.G. in December, 1984, on the DEC-VAX 750 system at the "Mario Negri" Institute for Pharmacological Research, Milano, Italy.

1 Introduction

This program is used for the statistical analysis of a family of sigmoidal (S-shaped[1]) dose-response curves as often encountered in pharmacological studies of a series of drugs hormones, neurotransmitters, etc. The four parameter logistic equation often describes such dose-response curves:

$$Y = \frac{A - D}{1 + (X/C)^B} + D$$

where X and Y are the dose and the response, respectively. The four fitted parameters represent the expected maximal response ("A"), slope factor ("B"), ED_{50} or ID_{50}, ie. dose with expected response half way between "A" and "D" ("C"), and minimal response ("D"). Note that this definition of "A" and "D" differs from that used in previous versions of ALLFIT and in the attached reprint. The current definition ensures that (A-D) is always positive. It also implies that "B" is negative when the curve rises and positive when the curve falls. The adequacy of the logistic equation or similar forms for fitting many sigmoidal dose-response curves has been already recognized and its use has been advocated for bioassay, radioimmunoassay, and radioreceptor assay data. When two or more dose-response curves are considered simultaneously, each curve can be fitted with the logistic equation and the estimated parameters for each curve are compared. Fitting each member of a family of curves individually is not always appropriate. When fitting curves individually, the parameters which should theoretically be identical (e.g. often A, D, and

[1] The sigmoidal shape is a general pattern of dose-response curves when they are portrayed on a logarithmic scale (log dose). All references to the graphical properties of dose-response curves will be made in this coordinate system. However, all calculations involve the original dose levels (X).

possibly B), are not pooled or shared by members of the family. Simultaneous curve fitting provides a basis for refining the data analysis of families of dose-response curves: it permits one to pool information from several curves, e.g. a common slope (B), or upper and lower plateaus (A and D) may be present. Using ALLFIT, one can interactively specify which parameters are common (shared). Some of the curves may share the same response level at zero dose (parameter "A") or some of the curves may be parallel (same parameter "B"). The choice of which parameters to be shared among the curves can be based on a prior considerations and experience. The statistical hypotheses that two or more curves share a common parameter value can be tested by first forcing the curves to share these parameters and then verifying that such constraints have only minimal effects on several indicators of "goodness of fit". The following documentation describes the operation of the program using an IBM PC-XT or compatible computer, and discusses statistical and numerical methods used. The program can be modified to use equations other than the four parameter logistic, if required, for other purposes.

2 Preparing the Data Files

A data file should contain the list of the pairs of X, Y (dose, response) for one or more curves. The file name must have extension ".ALL". Each line will contain one pair of X and Y values. The X and Y values must be *separated by a comma*. If the response for each dose was measured more than once (replicate Y) , the mean of the replicates should be entered together with the common dose. Alternately, the individual Y values may be entered on separate lines, repeating the appropriate X value. The data points for each curve should be in sequence and separated from those of other curves in the same data file by a "header line". This "header line" precedes the data for its respective curve. The "header line" must not begin with a digit (0,1,2...) and must not be a blank line. Usually, this line will contain the name of the curve; e.g. DRUG NO. 1. A listing of the data file SAMPLE.ALL appears on the following page.

Listing of SAMPLE.ALL

```
***APOMORPHINE-TER***
0,    12570
1E-9,      12208
1E-8,      11789
3E-8,      11273
1E-7,      10382
3E-7,      9828
1E-6,      7918
3E-6,      6351
1E-5,      6135
1E-4,      5724
***DOPAMINE-TER***
0,    12556
1E-9,      12421
1E-8,      11743
3E-8,      11287
1E-7,      11333
3E-7,      10328
```

```
1E-6,     9443
3E-6,     8610
1E-5,     7853
1E-4,     5984
***EPINEPHRINE-TER***
0,   12940
1E-8,     12090
1E-7,     11248
3E-7,     10628
1E-6,     10239
3E-6,     9282
1E-5,     8526
3E-5,     7560
1E-4,     6788
1E-3,     6140
***NOREPINEPHINE-TER***
0,   12923
1E-8,     12421
1E-7,     11625
3E-7,     10955
1E-6,     10196
3E-6,     9799
1E-5,     8905
3E-5,     8202
1E-4,     7289
1E-3,     6416
```

3 Running ALLFIT

Once the data file has been created, the program ALLFIT can be executed by typing 'ALLFIT' at the DOS prompt. You will first be asked if you want to use a response file. Ignore this for now by answering 'NO' (or just hitting the ENTER key). See the section of this manual on using a response file to learn how to use this feature (Chapter 7). The following is a step-by-step example of working through ALLFIT with a sample set of data. Note that anything that is underlined indicates a response from the user. Default responses, obtained by simply pressing the carriage return key (enter key), are indicated inside the square brackets.

```
C:> ALLFIT

--------------- ALLFIT 2.7, LTPB/NICHD/NIH ---------------

DO YOU WANT TO USE A RESPONSE FILE [N] ? <return>
                  This is an advanced feature of the program, To learn
                  how to use it, see Chapter 7 on using a response file

TYPE FILE NAME FOR SESSION FILE [NO EXTENSION]
a previous file with same name will be scratched!

SESSION FILE NAME ? test
                  Give a file name to the session file that is always
                  created as ALLFIT runs.  Any legal DOS file name is
                  allowed.  Do not use a file name extension (.SES is
                  assumed).

you can now enter a description
TEXT ? sample session file
                  This is a comment line that goes at the top of the
                  session file.  Put anything you want on this line.

ENTER FILE NAME(S); extension .ALL is assumed
one file name per line

DATA FILE ? SAMPLE
```

This is the name of the data file that contains the dose, response pairs. Do not use a file name extension (.ALL is assumed). One or more files can be read in, supply each name on a seperate line. The program will print any header lines as it reads the files.

```
***APOMORPHINE-TER***
CURVE # 1
***DOPAMINE-TER***
CURVE # 2
***EPINEPHRINE-TER***
CURVE # 3
***NOREPINEPHINE-TER***
CURVE # 4

DATA FILE ?  <return>
```

Hit the carriage return to enter a blank line when there are no more data files.

```
CURVE # 1 HAS 10 POINTS
CURVE # 2 HAS 10 POINTS
CURVE # 3 HAS 10 POINTS
CURVE # 4 HAS 10 POINTS
```

Remember that ALLFIT allows a maximum of 15 curves.

```
DO YOU WANT A WEIGHTED FIT (YES/NO) [NO] ? <return>
```

If there is a significant non-uniformity of variance (e.g. if the variance for large response values is much larger than the variance for small responses) then answer 'YES'. Then provide the estimates for the general weighting function:

$$\text{weight} = \frac{1}{\text{Var}\ (Y)} = \frac{1}{a_0 + a_1 Y + a_2 Y^2 + a_3 Y^{a_4}}$$

If the power function is used, then parameters a_0, a_1, a_2 should be set to zero. See chapter 8 for a fuller discussion of weighting.

```
THE FOLLOWING PARAMETERS WILL BE FIT :

A1   B1   C1   D1
A2   B2   C2   D2
A3   B3   C3   D3
A4   B4   C4   D4

LIST THE GROUPS OF SHARED PARAMETERS
ONE GROUP PER LINE (NO COMMA)
<return>
```

No shared parameters for this fit. It is advisable to perform the first fit with a minimum number of

constraints of shared or constant parameters

```
LIST THE CONSTANT PARAMETERS (NO COMMA) : <return>
```

No constant parameters for this fit.

```
DO YOU WANT AUTOMATIC INITIAL PARAMETER ESTIMATES (YES/NO) [YES] ?
<return>
```

Answer 'NO' if you want to provide your own initial values. The program will iteratively fit the curves until convergence is reached. It will print the results of some statistical tests which will be discussed later.

```
IT.   A          B     C                D          RES.VAR.

0     13254.6    1     4.608407E-07     5039.4
      13213.2    1     1.256289E-06     5326.8
      13620      1     2.230968E-06     5460
      13573.7    1     3.571602E-06     5765.3     2407106

TYPE C TO CONTINUE
     X TO STOP       ?  C
```

Type 'X' to halt the iterative process, or 'C' to continue iterations.

```
NUMBER OF ITERATIONS ?  9
```

Enter the number of iterations to do.

```
1     12203.68    .7978256    4.660659E-07     5503.16
      12261       .42170161   501487E-06       5769.673
      12456.12    .3746998    2.57011E-06      6123.407
      12566.1     .297518     3.81663E-06      6330.942    262864.3

2     12285.89    .7463442    4.451047E-07     5462.252
      12615.66    .357653     5.263239E-06     4160.795
      12898.48    .3542877    5.08518E-06      5042.865
      12947.8     .3652002    8.662908E-06     5280.69     61898.34

3     12309.7     .7366832    4.385167E-07     5451.463
      12605.34    .3730461    5.561624E-06     3777.533
      12888.47    .3658086    4.356114E-06     5089.731
      12980.03    .355592     6.615331E-06     5292.985    46324.79

4     12314.34    .734037     4.375398E-07     5448.48
      12609.26    .3719321    5.562808E-06     3768.151
      12887.77    .3650703    4.4476E-06       5070.142
      12976.79    .3588125    6.431462E-06     5323.241    46298.47

FIT 1: FINAL PARAMETER ESTIMATES +/- STANDARD ERROR (APPROX) AND %CV

       A1 = 12314.34          +/-     149.9612         ( 1.2 %)
       B1 = .734037           +/-     7.733138E-02     ( 10.5 %)
```

```
        C1 = 4.375398E-07     +/-    7.253546E-08    ( 16.6 %)
LOG OF  C1 = -6.358983        +/-    7.198448E-02
        D1 = 5448.48          +/-    231.7276        ( 4.3 %)

        A2 = 12609.26         +/-    189.3325        ( 1.5 %)
        B2 = .3719321         +/-    5.746045E-02    ( 15.4 %)
        C2 = 5.562808E-06     +/-    4.387386E-06    ( 78.9 %)
LOG OF  C2 = -5.254706        +/-    .3424663
        D2 = 3768.151         +/-    1190.765        ( 31.6 %)

        A3 = 12887.77         +/-    203.1832        ( 1.6 %)
        B3 = .3650703         +/-    4.502269E-02    ( 12.3 %)
        C3 = 4.4476E-06       +/-    2.029435E-06    ( 45.6 %)
LOG OF  C3 = -5.351874        +/-    .1981324
        D3 = 5070.142         +/-    535.5873        ( 10.6 %)

        A4 = 12976.79         +/-    202.3828        ( 1.6 %)
        B4 = .3588125         +/-    4.734439E-02    ( 13.2 %)
        C4 = 6.431462E-06     +/-    3.356905E-06    ( 52.2 %)
LOG OF  C4 = -5.19169         +/-    .2266394
        D4 = 5323.241         +/-    604.795         ( 11.4 %)

        C2/C1 = 12.71384      +/-    10.24652        ( 80.6 %)
        C1/C2 = 7.865444E-02  +/-    6.339034E-02    ( ' %)
        C3/C1 = 10.16502      +/-    4.934925        ( 48.5 %)
        C1/C3 = 9.837658E-02  +/-    4.775997E-02    ( ' %)
        C4/C1 = 14.69915      +/-    8.049924        ( 54.8 %)
        C1/C4 = 6.803113E-02  +/-    3.725693E-02    ( ' %)
```

The standard errors for parameters in the foregoing table are asymptotically correct based on a linearization of the model. The '%CV' values are the standard errors divided by the parameter estimates, expressed as a percentage. The ratios C2/C1 or C1/C2 are the relative potency estimates.

CURVE	SUM OF SQUARES	D.F.	MEAN SQUARE	F		RESIDUALS (+)	(-)	RUNS
1	607072.1	6	101178.7	3.61	(P= .032)	5	5	5 (good)
2	244130.5	6	40688.43	.84	(P= .71)	3	7	7 (good)
3	150472.3	6	25078.72	.47	(P= .2)	5	5	5 (good)
4	109488	6	18248	.33	(P= .064)	6	4	6 (good)
TOTAL	1111163	24	46298.47			19	21	

The foregoing table gives the residual sum of squares for each curve, an estimate of the degrees-of-freedom attributable to each curve and the corresponding mean square. The "F" column gives a ratio of the mean

*square for an individual curve to the average mean-square error for all the other curves. The corresponding significance level (p-value) for this ratio is also given. The F ratio can be used as an indication of a single outlying observation. Finally the number of positive (+) and negative (-) residuals is tabulated, and the number of runs of signs of residuals together with a measure of its statistical significance **[**good (p>0.05), poor (p< 0.05), bad (p< 0.01)**]**, an indication of the quality of the curve-fit. Many runs indicate a good fit, few runs a bad fit. In the Total row we see that there are 40 (19+21) points in the entire data set and that the degrees-of-freedom are 24 (40 data points minus 16 parameters).*

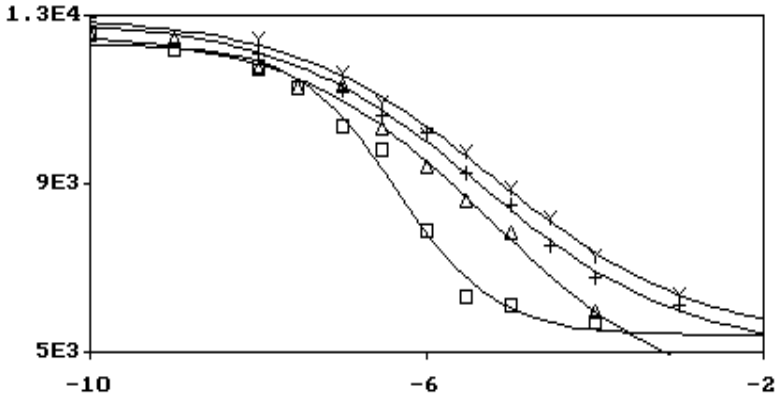
DO YOU WANT A GRAPH FILE (YES/NO) [NO] ? Y

This option creates a graphics file so that these curves can be viewed later (See chapter 6 on using GRAFIT). All fits made with the same set of data will be in the same graph file. If you use a new set of data then you will be prompted for a new graphics file name.

GRAPHICS FILE NAME [NO EXTENSION] ? SESSION

Enter graphics file name. Again, do not use a file extension (.GRF assumed). The graph, subsequently obtained using GRAFIT, should look like this.

```
ALLFIT-PC: SAMPLE.ALL
FIT #  1   SESSION: TEST.SES
```



ANOTHER FIT (YES/NO) [NO] ? Y

A new fit can be performed on the same data. You again have the option of sharing and constraining parameters. Each additional fit will be compared with the first fit. We continue in the next section, trying different

parameter constraints.

```
LIST THE GROUPS OF SHARED PARAMETERS
ONE GROUP PER LINE (NO COMMA)
A1 A2 A3 A4 <return>
D1:D4 <return>
<return>
```

Enter any group of common parameters, one group per line. Notice that the 'A' parameters are entered individually with a space between each one, and the 'D' parameters are entered as a range using a colon. Alternately, we could have typed D1 D2 D3 D4 <return>. The range colon notation is very useful when dealing with large numbers of curves. Then enter an empty line. All parameters listed on the same line are "locked" together and will always assume the same value during the iterative fitting process. By appropriately choosing the shared and the constant parameters, it is possible to configure the fitting process to conform to almost any desired requirements. As a result of the previous fit, and because the four curves arose in the same assay dose-response system, we suspect that all the A's and D's may have a common value. To test this we now fit with the constraint A1=A2=A3=A4 and D1=D2=D3=D4, then compare the quality of the two fits.

```
LIST THE CONSTANT PARAMETERS (NO COMMA) : <return>
```

No constant parameters for this fit.

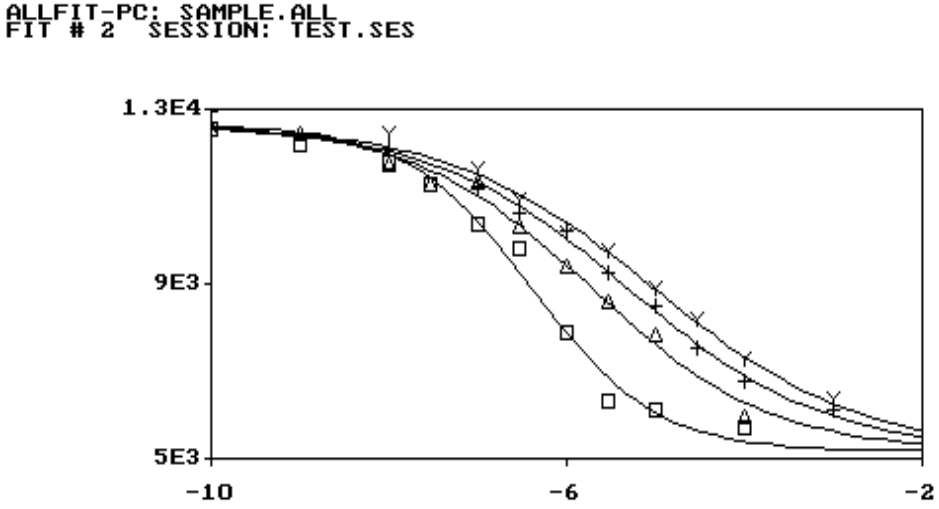
```
DO YOU WANT AUTOMATIC INITIAL PARAMETER ESTIMATES (YES/NO) [YES] ?
<return>


IT.   A          B     C                D          RES.VAR.

0     13415.38   1     4.608407E-07     5397.875
      13415.38   1     1.256289E-06     5397.875
      13415.38   1     2.230968E-06     5397.875
      13415.38   1     3.571602E-06     5397.875   2046596


TYPE C TO CONTINUE
     X TO STOP       ?  C

NUMBER OF ITERATIONS ?  9


1     12354.35   .8687935   3.715281E-07     5975.473
      12354.35   .4471533   1.347482E-06     5975.473
```

```
      12354.35    .3491982    2.82191E-06       5975.473
      12354.35    .2802504    4.687583E-06      5975.473    261076.9

2     12576.06    .5810638    4.290113E-07      5257.843
      12576.06    .4406176    2.069128E-06      5257.843
      12576.06    .4027172    5.724121E-06      5257.843
      12576.06    .3870961    1.349396E-05      5257.843    72919.62

3     12653.23    .614075     4.05517E-07       5194.295
      12653.23    .4333872    1.928338E-06      5194.295
      12653.23    .3918148    4.895612E-06      5194.295
      12653.23    .372896     9.609847E-06      5194.295    56338.07

4     12647.35    .6195432    4.083021E-07      5202.583
      12647.35    .4343239    1.924601E-06      5202.583
      12647.35    .3932463    4.886694E-06      5202.583
      12647.35    .3776013    9.572694E-06      5202.583    56254.79
```

FIT 2: FINAL PARAMETER ESTIMATES +/- STANDARD ERROR (APPROX) AND %CV

	A1 = 12647.35	+/-	99.9922	(.8 %)
	B1 = .6195432	+/-	.0563784	(9.1 %)
	C1 = 4.083021E-07	+/-	6.905803E-08	(16.9 %)
LOG OF	C1 = -6.389019	+/-	7.344101E-02	
	D1 = 5202.583	+/-	230.5065	(4.4 %)
	A2 = 12647.35	+/-	99.9922	(.8 %)
	B2 = .4343239	+/-	3.589449E-02	(8.3 %)
	C2 = 1.924601E-06	+/-	4.226657E-07	(22 %)
LOG OF	C2 = -5.715659	+/-	9.535913E-02	
	D2 = 5202.583	+/-	230.5065	(4.4 %)
	A3 = 12647.35	+/-	99.9922	(.8 %)
	B3 = .3932463	+/-	3.211448E-02	(8.2 %)
	C3 = 4.886694E-06	+/-	1.150023E-06	(23.5 %)
LOG OF	C3 = -5.310985	+/-	.1021874	
	D3 = 5202.583	+/-	230.5065	(4.4 %)
	A4 = 12647.35	+/-	99.9922	(.8 %)
	B4 = .3776013	+/-	3.034755E-02	(8 %)
	C4 = 9.572694E-06	+/-	2.322799E-06	(24.3 %)
LOG OF	C4 = -5.018966	+/-	.1053619	
	D4 = 5202.583	+/-	230.5065	(4.4 %)
	C2/C1 = 4.713668	+/-	.8570216	(18.2 %)
	C1/C2 = .212149	+/-	3.857214E-02	(' %)
	C3/C1 = 11.96832	+/-	2.243532	(18.7 %)
	C1/C3 = 8.355388E-02	+/-	1.566266E-02	(' %)
	C4/C1 = 23.44513	+/-	4.549345	(19.4 %)
	C1/C4 = 4.265278E-02	+/-	8.276438E-03	(' %)

The graph, subsequently obtained using GRAFIT, should look like this.



CURVE	SUM OF SQUARES	D.F.	MEAN SQUARE	F	RESIDUALS (+)	(-)	RUNS	
1	793619.6	7.5	105816	2.66 (P= .066)	4	6	4	(good)
2	413086.2	7.5	55078.16	.97 (P= .886)	4	6	7	(good)
3	214447.6	7.5	28593.01	.44 (P= .122)	6	4	5	(good)
4	266490.4	7.5	35532.06	.56 (P= .268)	7	3	5	(good)
TOTAL	1687644	30	56254.79		21	19		

There are now 30 (40 data points minus 10 parameters) degrees-of-freedom The parameters are A, B1, B2, B3, B4, C1, C2, C3, C4, D.

FIT	SUM OF SQUARES	D.F.	MEAN SQUARE	F
1	1111163	24	46298.47	---
2	1687644	30	56254.79	2.08 (P= .094)

This table compares the quality of the fit of the first two fits or models. The F column contains the "extra sum of squares F-statistic" and its appropriate significance level. It is calculated as :

F = (delta-SS) / (delta-df) / MS1 or

(1687643-1111164) / (30-24) / 46298

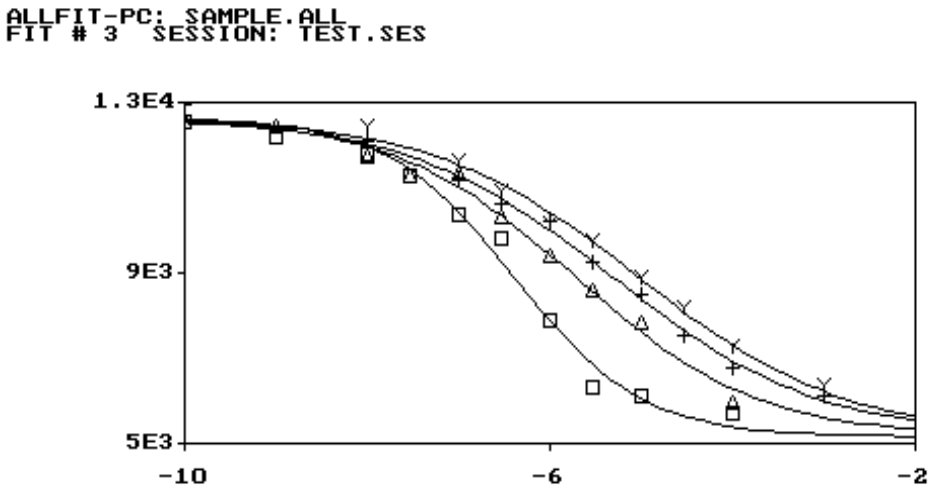
The value MS1 is chosen in the denominator since it corresponds to the more complex model (more parameters, fewer degrees-of-freedom). The indication here is that adding the constraints A1=A2=A3=A4 and D1=D2=D3=D4 did not significantly degrade the fit (increase the mean square). Thus, it is plausible that the data arise from a system with common plateau values. If more than two fits are being compared, the program calculates the comparison with reference to the fit with fewest degrees-of-freedom.

```
ANOTHER FIT (YES/NO) [NO] ? Y

LIST THE GROUPS OF SHARED PARAMETERS
ONE GROUP PER LINE (NO COMMA)
A1:A4 <return>
B3 B4 <return>
D1:D4 <return>
<return>

LIST THE CONSTANT PARAMETERS (NO COMMA) : <return>
                  No constant parameters for this fit.

DO YOU WANT AUTOMATIC INITIAL PARAMETER ESTIMATES (YES/NO) [YES]
<return>


IT.   A           B     C                 D           RES.VAR.

0     13415.38    1     4.608407E-07      5397.875
      13415.38    1     1.256289E-06      5397.875
      13415.38    1     2.230968E-06      5397.875
      13415.38    1     3.571602E-06      5397.875    1980576

TYPE C TO CONTINUE
     X TO STOP       ?  C

NUMBER OF ITERATIONS ?  9

1     12354.16    .8686034    3.716883E-07      5974.486
      12354.16    .447001     1.34804E-06       5974.486
      12354.16    .3142321    2.823306E-06      5974.486
      12354.16    .3142321    4.688041E-06      5974.486    245514.5

2     12579.62    .5808339    4.277121E-07      5260.051
      12579.62    .4403395    2.058504E-06      5260.051
      12579.62    .3953709    5.980047E-06      5260.051
      12579.62    .3953709    1.222166E-05      5260.051    68291.77
```

```
3     12653.01    .6134252    4.068154E-07      5189.699
      12653.01    .433083     1.935502E-06      5189.699
      12653.01    .3823098    4.92642E-06       5189.699
      12653.01    .3823098    9.565799E-06      5189.699    54882.95

4     12648.05    .6187757    4.092013E-07      5197.932
      12648.05    .4339651    1.930172E-06      5197.932
      12648.05    .3848522    4.916835E-06      5197.932
      12648.05    .3848522    9.522883E-06      5197.932    54832.53
```

```
FIT 3: FINAL PARAMETER ESTIMATES +/- STANDARD ERROR (APPROX) AND %CV

        A1 = 12648.05          +/-    98.72836         ( .8 %)
        B1 = .6187757          +/-    5.560079E-02     ( 9 %)
        C1 = 4.092013E-07      +/-    6.842111E-08     ( 16.7 %)
LOG OF  C1 = -6.388063         +/-    7.260378E-02
        D1 = 5197.932          +/-    228.2123         ( 4.4 %)

        A2 = 12648.05          +/-    98.72836         ( .8 %)
        B2 = .4339651          +/-    3.540852E-02     ( 8.2 %)
        C2 = 1.930172E-06      +/-    4.189558E-07     ( 21.7 %)
LOG OF  C2 = -5.714404         +/-    9.424935E-02
        D2 = 5197.932          +/-    228.2123         ( 4.4 %)

        A3 = 12648.05          +/-    98.72836         ( .8 %)
        B3 = .3848522          +/-    2.576263E-02     ( 6.7 %)
        C3 = 4.916835E-06      +/-    1.159706E-06     ( 23.6 %)
LOG OF  C3 = -5.308314         +/-    .1024161
        D3 = 5197.932          +/-    228.2123         ( 4.4 %)

        A4 = 12648.05          +/-    98.72836         ( .8 %)
        B4 = .3848522          +/-    2.576263E-02     ( 6.7 %)
        C4 = 9.522883E-06      +/-    2.250925E-06     ( 23.6 %)
LOG OF  C4 = -5.021232         +/-    .1026357
        D4 = 5197.932          +/-    228.2123         ( 4.4 %)

        C2/C1 = 4.716924       +/-    .8467352         ( 18 %)
        C1/C2 = .2120026       +/-    3.805659E-02     ( ' %)
        C3/C1 = 12.01568       +/-    2.25031          ( 18.7 %)
        C1/C3 = 8.322456E-02   +/-    1.558638E-02     ( ' %)
        C4/C1 = 23.27187       +/-    4.390616         ( 18.9 %)
        C1/C4 = 4.297033E-02   +/-    8.107048E-03     ( ' %)
```

The graph, subsequently obtained using GRAFIT, should look like this.



CURVE	SUM OF SQUARES	D.F.	MEAN SQUARE	F		RESIDUALS (+)	(-)	RUNS
1	794997	7.5	105999.6	2.75	(P= .054)	3	7	4 (good)
2	412104.7	7.5	54947.29	1	(P= .924)	4	6	7 (good)
3	220309.8	8	27538.72	.43	(P= .108)	6	4	5 (good)
4	272397.1	8	34049.64	.55	(P= .25)	7	3	5 (good)
TOTAL	1699809	31	54832.53			20	20	

FIT	SUM OF SQUARES	D.F.	MEAN SQUARE	F	
1	1111163	24	46298.47	---	
2	1687644	30	56254.79	2.08	(P=.094)
3	1699809	31	54832.53	1.82	(P=.129)

The third fit was made with the constraints A1=A2=A3=A4, D1=D2=D3=D4 and B3=B4. It appeared from fit #3 that the values for B3 and B4 may have been identical. Unfortunately, the table compares fit #3 with fit #1 (the most complex model), so that we must calculate the F statistic comparing fits #2 and #3 by hand.

delta SS = 1699810 - 1687643 = 12167
delta df = 31 - 30 = 1

So that

F = (12167 / 1) / 56254 = .216

which is less than 1.0 so the p-value is > 0.5 and the added constraint does not significantly degrade the fit. Thus we conclude that B3 = B4.

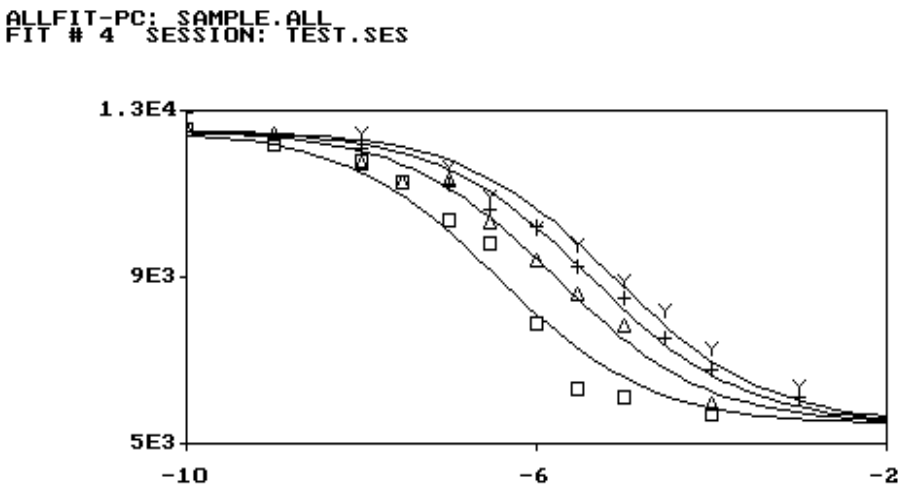
```
ANOTHER FIT (YES/NO) [NO] ? Y

LIST THE GROUPS OF SHARED PARAMETERS
ONE GROUP PER LINE (NO COMMA)
A1:A4 <return>
D1:D4 <return>
B1:B4 <return>
<return>

LIST THE CONSTANT PARAMETERS (NO COMMA) : B1
```

Enter the list of the parameters whose value will not change during fitting and which will keep a preassigned value. If a group of parameters which has already been declared as common (shared) should also remain constant during fitting, it is necessary to declare only one of them in the list for constants .

```
    B1 B2 B3 B4 [ 1 ] ?  .5

DO YOU WANT AUTOMATIC INITIAL PARAMETER ESTIMATES (YES/NO) [YES]
<return>

IT.   A            B    C                 D           RES.VAR.

0     13415.38     .5   4.608407E-07      5397.875
      13415.38     .5   1.256289E-06      5397.875
      13415.38     .5   2.230968E-06      5397.875
      13415.38     .5   3.571602E-06      5397.875    438794.8

TYPE C TO CONTINUE
     X TO STOP       ?  C

NUMBER OF ITERATIONS ?  9

1     12577.06     .5   3.21736E-07       5640.985
      12577.06     .5   1.421733E-06      5640.985
      12577.06     .5   3.301208E-06      5640.985
      12577.06     .5   5.855915E-06      5640.985    120999.6

2     12537.31     .5   3.595332E-07      5523.295
      12537.31     .5   1.628047E-06      5523.295
      12537.31     .5   4.046452E-06      5523.295
      12537.31     .5   7.42411E-06       5523.295    116648

3     12529.37     .5   3.625648E-07      5499.867
      12529.37     .5   1.668351E-06      5499.867
```

```
      12529.37    .5    4.18535E-06       5499.867
      12529.37    .5    7.737525E-06      5499.867    116499.9


FIT 4: FINAL PARAMETER ESTIMATES +/- STANDARD ERROR (APPROX) AND %CV

        A1 = 12529.37          +/-    111.1176         ( .9 %)
        B1 = .5
        C1 = 3.625648E-07      +/-    9.408099E-08     ( 25.9 %)
LOG OF  C1 = -6.440614         +/-    .1126736
        D1 = 5499.867          +/-    187.7857         ( 3.4 %)

        A2 = 12529.37          +/-    111.1176         ( .9 %)
        B2 = .5
        C2 = 1.668351E-06      +/-    4.325318E-07     ( 25.9 %)
LOG OF  C2 = -5.777712         +/-    .1125736
        D2 = 5499.867          +/-    187.7857         ( 3.4 %)

        A3 = 12529.37          +/-    111.1176         ( .9 %)
        B3 = .5
        C3 = 4.18535E-06       +/-    1.084428E-06     ( 25.9 %)
LOG OF  C3 = -5.378268         +/-    .1125059
        D3 = 5499.867          +/-    187.7857         ( 3.4 %)

        A4 = 12529.37          +/-    111.1176         ( .9 %)
        B4 = .5
        C4 = 7.737525E-06      +/-    1.997968E-06     ( 25.8 %)
LOG OF  C4 = -5.111398         +/-    .1121225
        D4 = 5499.867          +/-    187.7857         ( 3.4 %)

        C2/C1 = 4.601527       +/-    1.229475         ( 26.7 %)
        C1/C2 = .2173192       +/-    .0580652         ( ' %)
        C3/C1 = 11.54373       +/-    3.035063         ( 26.3 %)
        C1/C3 = 8.662713E-02   +/-    .0227759         ( ' %)
        C4/C1 = 21.34109       +/-    5.634641         ( 26.4 %)
        C1/C4 = 4.685796E-02   +/-    .0123718         ( ' %)
```

The graph, subsequently obtained using GRAFIT, should look like this.



CURVE	SUM OF SQUARES	D.F.	MEAN SQUARE	F		RESIDUALS (+)	(-)	RUNS	
1	1908405	8.5	224518.3	2.79	(P= .042)	6	4	2	(bad)
2	511995.6	8.5	60234.77	.45	(P= .112)	5	5	6	(good)
3	618004.9	8.5	72706.45	.55	(P= .23)	6	4	5	(good)
4	922592.3	8.5	108540.3	.91	(P= .798)	6	4	3	(poor)
TOTAL	3960998	34	116499.9			23	17		

FIT	SUM OF SQUARES	D.F.	MEAN SQUARE	F	
1	1111163	24	46298.47	---	
2	1687644	30	56254.79	2.08	(P=.094)
3	1699809	31	54832.53	1.82	(P=.129)
4	3960998	34	116499.9	6.16	(P=0)

In fit #4 we have added the constraint that B1=B2=B3=B4 to fit #2. The mean square value doubles, indicating a substantial degradation of the fit. Comparing fit #2 with fit #4,

F = ((3960996 - 1687643) / (34 - 30)) / 56254 = 10.1

which is significant at the p = .001 level. Thus, we reject the idea of common slope factors. This conclusion is also supported by the graph of the data, and by the indication of lack of fit in curves 1 and 4 where the number of runs is "bad" or "poor".

ANOTHER FIT (YES/NO) [NO] ? <return>

A new set of data files can be entered. The same session file is used. Before ending, the program outputs the full name of the session file as a reminder.

4 Interpreting the Results

For each fitting process, the program prints

1) The final parameter estimates.
2) The ratios of the ED_{50}'s (potency ratios relative to the first curve).
3) The covariance/correlation matrix for parameters (in the session file only).
4) The residual variance tests and run tests for the individual curves.
5) The "extra sum of squares" F test, to compare various fits.

(1) The final parameter estimates

These estimates are provided together with their approximate standard error and percent coefficient of variation (100 × standard error / parameter value). Unlike the case for straight line or polynomial regression, the standard errors do not provide exact confidence limits (2,5). They do provide an index of the accuracy of the estimates. For convenience, the logarithm of parameter C (ED_{50}) is also calculated and printed.

(2) The potency ratios (ratios of ED_{50})

If the first dose response curve is the standard or reference curve, these ratios will provide estimates of the potency of the test preparations; for convenience both ratios C_n/C_1 and the reciprocal C_1/C_n are printed. In the case of displacement curves of a labeled drug by analogs, these ratios usually do not equal the true ratios of the dissociation constants (K_d) unless both the binding site concentration and the labeled drug concentration are infinitesimal. The exact mathematical treatment for estimating K_d from displacement curves has been described elsewhere (7,8).

(3) The correlation/covariance matrix for errors in parameters.

The correlation matrix shows the degree of correlation between parameters. For each pair of parameters, a value between -1 and 1 is printed: the closer this value is to -1 or 1, the higher is the correlation between the two parameters.

(4) The residual variance test and the runs test

These tests provide indices of the goodness of fit for the individual dose response curves. The F ratio tests are obtained by calculating the ratio of the residual variance for one curve to the overall residual variance for all of the other curves. These ratios will be unexpectedly large or small if a) some curve contains an outlier which results in a larger residual variance for that curve than for the others; or b) the weighting function is inappropriate; or c) the constraints (choice of common or constant parameters) are inappropriate; or d) one or more curves do not conform to the sigmoidal model; or e) different curves have different degrees of random experimental error. The runs test for each curve is a test of randomness of the residuals. Excessively large or small numbers of runs suggest either an inappropriate choice of constraints or deviation of the data from the sigmoidal logistic model. These tests are discussed elsewhere (2,3). The words "bad, poor, good" correspond to $p<0.01$, $p<0.05$, and $p>0.05$), respectively. The test is insensitive and conservative when a curve consists of less than 10 points.

(5) Test for the "extra sum of squares"

Any constraint (choice of common and/or constant parameters) will increase the sum of squares of residuals because the curves are constrained away from the position they

would occupy if they were fit free of any constraint. However, with constraints there are fewer parameters to estimate since some of them have been "fused" together. If the decrease in the effective number of parameters compensates for the extra sum of squares due to the constraints, then the F ratio test is not significantly different from unity. A significant value for this test indicates that the data reject the hypothesis that the shared parameters are in fact compatible with each other. The program automatically selects the least constrained fit with the smallest weighted sum of squares of residuals as a reference for these tests, irrespective of the order of the fits.

5 Numerical and Statistical Methods

Let us consider the set of data points (X_{ij}, Y_{ij}) for n curves (i = 1 to n, j = 1 to m_i for each curve). We wish to fit simultaneous equations:

$$y_{ij} = F(X_{ij}, a_i, b_i, c_i, d_i) = \frac{a_i - d_i}{1 + (X_{ij} / c_i)^{b_i}} + d_i$$

so as to minimize the sum of squares of residuals:

$$SS = \sum_i \sum_j w_{ij} (Y_{ij} - y_{ij})^2$$

where y_{ij} is the predicted response and w_{ij} is the reciprocal of the predicted variance of the response. There is a maximum number (4 × n) of parameters to be estimated. If parameters are constrained to be equal, then the effective number of parameters will decrease accordingly to (4 × n - q) where q is the number of independent equality constraints and constant parameters. The predicted variance can be either a quadratic or a power function of the predicted response y according to:

$$w_{ij} = \frac{1}{var(y_{ij})} = \frac{1}{a_0 + a_1 y_{ij} + a_2 y_{ij}^2}$$

or

$$w_{ij} = \frac{1}{a_3 y_{ij}^{a_4}}$$

Least squares curve fitting is based on a Newton-Gauss linearization method with modification by Levenberg and Marquardt (4,5). For each curve, construct a matrix J_i (m_i

× 4) of the estimates of the partial derivatives $\partial F_{ij} / \partial P_k$ (i = 1 to n, j = 1 to m_i, k = 1 to 4) of the logistic equation F with respect to the parameters P_k. Assemble the matrices of partial derivatives for each curve into a global matrix J with $\sum_i m_i$ rows and (4 × n - q) columns. In the absence of any equality constraint, the matrix J will have 4 × n columns and will contain the matrices of partial derivatives for each curve (J_1, J_2, J_3 …J_n) in disjoint rows and columns:

$$\begin{array}{cccc} J_1 & 0 & 0 \ldots & 0 \\ 0 & J_2 & 0 \ldots & 0 \\ 0 & 0 & J_3 \ldots & 0 \\ \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots & & & \\ 0 & 0 & 0 & J_n \end{array}$$

When some variables are forced to be common (shared) between curves, the submatrices J_1 to J_n are still in disjoint rows but they are now share common columns corresponding to the shared parameters. As an example, if two curves are to be fit subject to the constraints A1=A2 and D1=D2, then the resulting global matrix will be the following:

Parameter	A	B1	C1	D	B2	C2
	……					
	X	X	X	X	0	0
Curve 1	X	X	X	X	0	0
	X	X	X	X	0	0
	……					
	X	0	0	X	X	X
Curve 2	X	0	0	X	X	X
	X	0	0	X	X	X
	……					

where X stands for estimates of partial derivatives and 0 stands for zero elements.

Except for how the matrix J is constructed, the method described is identical to that extensively studied by Fletcher and Shrager (4). If some parameters are set to a constant value, the corresponding columns in the matrix J are removed and the effective number of

parameters to be fit is decreased accordingly. When convergence has been obtained, then the final estimates of the parameters are used to calculate the overall residual variance:

$$S^2 = \frac{SS}{\sum_i m_i - 4n + p}$$

where p is the number of independent equality constraints. The overall sum of squares of residuals and the total number of degrees-of- freedom can be decomposed into individual values for each curve. The approximate number of degrees-of-freedom for each curve is calculated by subtracting from the number of data points m_i the effective number of parameters fitted. Each constant parameter is not considered in this calculation; each shared parameter is weighted as the reciprocal of the number of parameters in its pool, so the number of degrees-of-freedom can be a fraction. The resulting table is analyzed with ANOVA in order to test for homogeneity of the residual variance for the several curves.

If SS_0 and SS_1 are the sum of squares of residuals obtained in the absence and the presence of some constraint and df_0 and df_1 are the corresponding degrees-of-freedom, an estimate of the variance based on the excess component of the sum of squares due to the constraints is:

$$S^2_{diff} = \frac{SS_1 - SS_0}{df_1 - df_0}$$

The estimate for the variance of the response in the absence of constraints is:

$$S^2 = \frac{SS_0}{df_0}$$

The ratio of these estimates provides an approximate F test:

$$F = \frac{S^2_{diff}}{S^2}$$

The probability level of the F ratio is automatically calculated by the program (6). During the iterative calculations of ALLFIT, some messages warning of "numeric errors" (due to square root of negative number, overflow, division by 0) can occur. This can happen transitorily during intermediate calculations. However, a large number of warning messages is a likely sign of "ill-conditioning" due to too many parameters, too few data points, erroneous constraints, severely "out of bounds" initial estimates of parameters, or other reasons.

6 Creating Graphs

The GRAFIT program is an IBM-PC based program designed to assist users in plotting the results obtained from ALLFIT. (GRAFIT is also used with EXPFIT, FLEXIFIT and PULSEFIT).

GRAFIT has been designed to work on machines with the following graphics cards.

Hercules
CGA
EGA
VGA
MCGA

The type of video card is automatically detected by the program.

GRAFIT now supports laser printers that use the picture drawing language PostScript. The best hard copy plots are achieved using a laser printer, however, dot matrix printers are still supported. Previously, the plots were produced at screen resolution. The software has now been modified to take advantage of the higher resolution available on 9 pin dot matrix printers.

In order to support still more printers, GRAFIT is capable of producing a Lotus 123 .PIC file. This file can be printed using the PrintGraph program distributed with Lotus 123 version 2.

The GRAFIT program exits as a single executable file. Your may run it from a floppy drive or copy it to your hard disk.

Start the program by typing grafit at the DOS prompt.

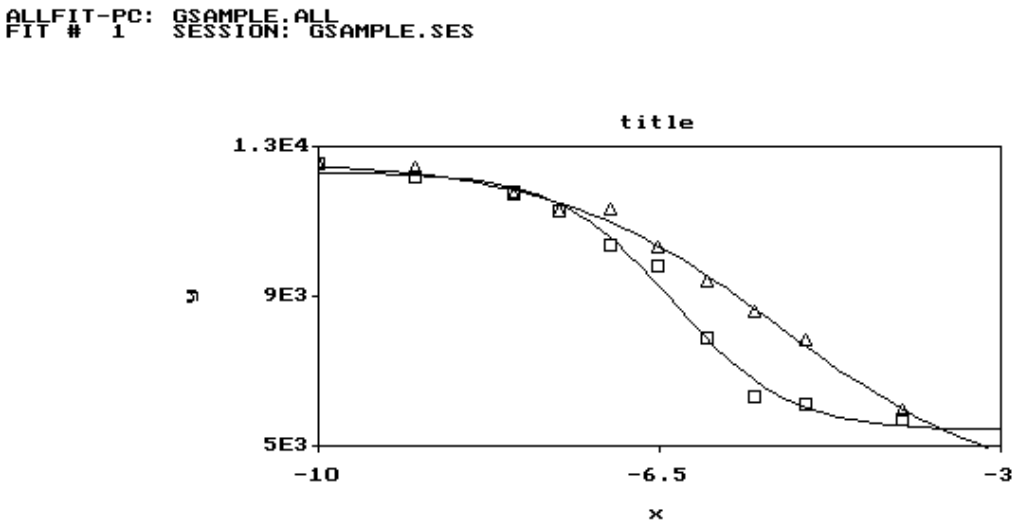
```
> grafit
```

You will be prompted for a .grf file name.

```
Graphics file name (no extension) ? gsample
```

(Don't enter the .grf file extension, it is assumed.)
The file gsample.grf, found on the Allfit distribution diskette, will be used to demonstrate GRAFIT operation.

The following plot will be displayed on the screen.



The line at the bottom of the screen contains 7 different commands...

```
C:Change N:Next F:First K:Key P:Print R:Restart Q:Quit
```

A command is entered by typing the first letter of the command. No return is necessary. These commands are only active while a plot is on the screen and the commands are displayed at the bottom of the screen.

Change Command

Type the letter 'C', the following screen will be displayed.

```
Graph Set Up Screen

      Label 1:    ALLFIT-PC: GSAMPLE.ALL
      Label 2:    FIT   #   1      SESSION: GSAMPLE.SES
      Title:      title
      X label:    x
      Y label:    y

      x min:      -1.0000E+01
      x max:      -3.0000E+00
      y min:      5.0000E+03
      y max:      1.3000E+04
      X divisions (1-15):   2
```

```
      Y divisions (1-15):   2
      Size of plotted points  (1-10):   3


F2:Accept new values   Esc:Cancel
```

You can change the plot labels or axis limits. The number of tick marks on the X and Y axis can be adjusted along with the size of the symbols used to plot the data points. Strike the Esc key to ignore your changes and return to the plot. F2 will register your changes and display the updated plot.

Next Command

To display the next plot in the file, type the letter 'N'. The graph file will be read and the next plot will be displayed on the screen. If no more plots are in the file a message to this effect will be displayed.

First Command

You can display the first plot in the file at any time by typing the letter 'F'.

Key (Legend) Command

After entering 'K' from the keyboard you will be presented with the following screen.

```
Key Set Up Screen

      Write curve labels? (Y/N):   N

      Name of curve 1:  Draw curve 1? (Y/N):   Y
      Name of curve 2:  Draw curve 2? (Y/N):   Y


F2:Accept new values   Esc:Cancel
```

You may enter a label for each curve in the plot. Initially the key or legend is not displayed. Display the key by answering 'Y' to the question 'Write curve labels?' . The key consisting of the curve labels and associated symbols will be displayed to the right of the plot. Be careful when printing not to position the plot too far to the right as this may push the key off of the page.

You may choose to display or not display any particular curve. Answering yes to the question 'Draw curve 1?' will cause the curve to be drawn; answer no and the curve will not be drawn.

Print Command

GRAFIT supports several methods of printing your plots. Printing may be done directly to a 9 pin dot matrix printer. Alternately a PostScript file may be created and later sent to a PostScript printer. Lastly a Lotus 123 .PIC format file may be created. All of these methods are initiated from the print screen.

While a plot is displayed on the screen, entering 'P' will result in the display of the print menu.

```
Printer Set Up Screen

These coordinates alter the size and position of the plot box.
The lower left hand corner of the page is (0,0).

      Upper Left x page coordinate   (inches):   2.00
      Upper Left Y page coordinate   (inches):   9.00
      Lower Right x page coordinate  (inches):   7.00
      Lower Right y page coordinate  (inches):   6.00

      Post Script File (.ps will be appended):   gsample
      Lotus 123 file   (.pic will be appended):..gsample

F1:PostScript F2:Save F3:Dot Matrix F4:Lotus  Esc:Cancel
```

Creating a PostScript File

Entering F1 will result in the creation (or replacement) of the PostScript file gsample.ps. The default PostScript file name is the prefix of the .grf file. You may enter your own file name instead of using the default. To print this file you will have to exit this program (i.e. hit escape and then Quit), and then download the PostScript file gsample.ps to your laser printer. The page coordinates apply to PostScript file.

Dot Matrix Printing

Enter F3 to print the plot on a dot matrix printer attached to printer port LPT1. Once printing has started you may interrupt it by striking any key. GRAFIT supports 9 pin dot matrix printers which emulate the Epson FX series of printers. This method creates a plot at a much higher resolution than available with screen dumps. You may alter the size of the plot using the page coordinates.

Creating Lotus 123 Format .PIC Files

Entering F4 will result in the creation of the Lotus 123 .PIC file gsample.pic. (You may enter a different file name. .PIC is always appended.) This .PIC file may be printed using the Lotus 123 program Printgraph which is supplied with version 2 of Lotus 123. This enables GRAFIT to indirectly support a wide range of printers. Note, the page coordinates do not apply to this method.

F1, F2, F3 or F4 will result in your changes being saved. If you make changes in this screen and then enter Esc, without having entered F1, F2 or F3, then your changes will not

be saved.

Restart Command

The restart command is used to change the .grf file.

Quit Command

Enter 'Q' to exit the program. Again, this command is only active when a plot is displayed.

Prt-Screen Keyboard Key

The prt-scr key is supported only when a plot is being displayed. Screen dumps are supported in Hercules, CGA, EGA and VGA video modes. You do not have to have the MS-DOS graphics driver loaded to use this function.

GRAFIT file format

The file format for GRAFIT is discussed below, along with a listing of a sample graph file. This file is included on the disk (gsample.grf). The number of lines in any graph file is variable, depending on the number of curves. Here is a listing of gsample.grf.

```
ALLFIT-PC: GSAMPLE.ALL FIT #  1
SESSION: GSAMPLE.SES
```

These first two lines are graph labels. They will appear at the top of the graph.

```
2
```

This line is the number of curves.

```
10   10
```

These two numbers indicate how many points are in each curve respectively. Remember that if the number of curves were 3, then there would be three numbers here.

```
-10  -3   1
5500   13000   999.9999
```

The first line is the minimum x value, the maximum x value, and the default step value for the x axis. The second line contains the same values for the y axis. These values are for the whole graph, not any specific curve.

```
-10  12570
-9  12208
-8  11789
-7.522879  11273
-7  10382
-6.522879  9828
-6  7918
-5.522879  6351
-5  6135
-4  5724
```

These points are the x,y pairs for the 10 points in curve 1.

```
-10  12556
-9  12421
-8  11743
-7.522879  11287
-7  11333
-6.522879  10328
-6  9443
-5.522879  8610
-5  7853
-4  5984
```

These points are the x,y pairs for the 10 points in curve 2.

```
55
```

This number is the number of lines to follow. The lines contain x,y pairs for the two endpoints of line segments. These line segments make up the curve on the graph. These are for curve 1.

```
-10        12299.78   -9.872727 12296.29
-9.872727  12296.29   -9.745455 12291.98
-9.745455  12291.98   -9.618182 12286.63
-9.618182  12286.63   -9.49091  12280.02
-9.49091   12280.02   -9.363637 12271.83
           .
           .
```

Not all 55 lines of numbers are shown here.

```
           .
           .
-3.509097  5503.595   -3.381825 5492.997
-3.381825  5492.997   -3.254552 5484.425
-3.254552  5484.425   -3.127279 5477.498
-3.127279  5477.498   -3.000007 5471.9

55
```

This number is the number of lines to follow. The lines contain x,y pairs for the two endpoints of line segments. These line segments make up the curve on the graph. These are for curve 2.

```
-10         12459.92   -9.872727 12443.04
-9.872727   12443.04   -9.745455 12424.3
-9.745455   12424.3    -9.618182 12403.5
-9.618182   12403.5    -9.49091  12380.42
-9.49091    12380.42   -9.363637 12354.82
            .
            .
```

Not all 55 lines of numbers are shown here.

```
            .
            .
-3.509097   5387.667   -3.381825 5248.426
-3.381825   5248.426   -3.254552 5118.917
-3.254552   5118.917   -3.127279 4998.845
3.127279    4998.845   -3.000007 4887.851
```

End of graph file. GRAFIT can read multiple sets of curve data. For another graph, the above format of input can be repeated in the same file (see the file session.grf on the distribution disk). Make sure not to put any blank lines in the graph file.

7 Using a Response File

ALLFIT has an alternative way of providing input to the program through a response file. This is a file (ASCII format) that contains the responses to the ALLFIT program prompts. Responses to all but two questions asked by the program can be provided by a response file (see last page of this section on how to fully automate ALLFIT). When using ALLFIT with a response file, the program terminates after one fit. Notice that there are two sample response files on the ALLFIT distribution disk (TEST1.RSP and TEST2.RSP). Think of the line numbers you see below as positions within the response file, and not actual line numbers. The actual number of lines in your response file will vary depending on the number of data files, parameter constraints, etc. Below is a listing of the response file TEST1.RSP:

```
1:  TEST1
2:  RESPONSE FILE SESSION  1
3:  SAMPLE
4:  <blank line>
5:  N
6:  A1:A4
7:  B1:B4
8:  <blank line>
9:  B1
10: .5
11: N
12: 13254.6
13: 4e-7
14: 5039.4
15: 1e-6
16: 5362.8
17: 2e-6
18: 5460
19: 3e-6
```

```
20: 5765.3
21: 10
22: Y
23: TEST1GRF
24: <blank line>
```

Explanation of response file input sequence

"Line" #

1 Name of the session file.
2 Session file comment line.
3 data file name.

If there are two or more data files, then each of the data file names will appear on consecutive lines, one to a line. After the data files have been listed, the line immediately following the last one must be blank.

4 Blank line required after list of data files.
5 Answer to 'Do you want a weighted fit ?'.

If answer is 'No', then continue. If answer is 'Yes' (or 'Y') then the next line must contain all five of the values for the weighting function (a_0 a_1 a_2 a_3 a_4). These should be all on one line, each one separated by a blank space or a comma.

6 Groups of shared parameters.

One group per line. There can be as many lines as you wish of shared groups, but the last line must be blank. No commas.

7 Blank line required after list of shared groups.
8 One line only of constant parameters.
9 Value for the constant parameter given above.

The number of lines to follow the list of constant parameters in the response file depends upon the number of constant parameters entered. Each constant parameter will have its value listed (in order of input) on a separate line.

10 Answer to 'Do you want automatic initial estimates ?'.

If answer is 'Yes' then the program will generate initial estimates and "line" 21 will be the next line in the response file. If answer is 'No', then the remaining lines of the response file will contain the initial values for the non-constant parameters. The values should appear on separate lines in the following order (parameter constraints will alter this order):

A1
B1

C1
D1
A2
B2
.
.
C[# of curves]
D[# of curves]

Any parameter whose value is constant parameter should not be included in this list. For example, if A1 is declared constant, the first value that would be supplied by the response file would be for B1. If all the A parameters were shared (ex. A1 A2 A3 A4) and A1 was declared constant, then none of the A's would appear in the list. Also if all the A parameters were shared, but none of the A's were constant, then the response file would be required to supply only a value for A1 since A2,A3 and A4 all share this value. Using the above constraints (all A's shared, A1 constant) the lines in the response file would contain values for the following parameters :

B1
C1
D1
B2
C2
D2
B3
C3
D3
B4
C4
D4

21 Number of iterations
22 Answer to 'Do you want a graph file'.
If answer is 'Y' the next line in the response file will be the graph file name. If the answer is 'N' then a blank line will follow (end of response file).
23 Name of graph file
24 <blank line>
The last line of the response file must be a blank line.

The second response file on the disk is TEST2.RSP. It is listed below and is provided as another example for you to try. It is not discussed further in this documentation.

```
1:   TEST2
2:   RESPONSE FILE SESSION2
3:   SAMPLE
4:   <blank line>
5:   N
6:   <blank line>
7:   <blank line>
8:   N
9:   13254.6
10:  1
11:  4.608407E-7
12:  5039.4
13:  13213.2
14:  1
15:  1.256289E-6
16:  5362.8
17:  13620
18:  1
19:  2.230968E-6
20:  5460
21:  13573.7
22:  1
23:  3.571602E-6
24:  5765.3
25:  10
26:  N
27:  <blank line>
```

Completely Automatic ALLFIT

Though a response file significantly reduces user interaction, there is a way to run ALLFIT with no user interaction. This is useful for running ALLFIT over and over again from a batch file for many data sets. This technique involves using a response file just like it is detailed above. The rest of the user-required input is provided to the program through input redirection. When using a response file, the program still requires you to answer the following two questions.

1) Do you want a response file ?
2) Response file name ?

The answers to these questions will be in an ASCII disk file. Below is a sample of a file like this (do not enter the comments to the right in your file) :

```
Y           answer yes to question 1
RESPFIL     response file name
```

For this example, the above file will be named EX.DAT. From the DOS command line, run the ALLFIT program as shown below.

```
C:\ ALLFIT < EX.DAT
```

and ALLFIT will run to completion. The above command line may also be used in a batch file. All output from the program will be in a session file like it normally would be. Make sure that the number of iterations you supply is more than enough for convergence. The program will terminate prematurely if this number is too low for convergence.

8 A Note About Weighting

If you select weighting, then you must specify the five coefficients used in a variance model :

$$Var(Y) = a_0 + a_1 Y + a_2 Y^2 + a_3 Y^{a_4}$$

The weights will be calculated as weight = $\frac{1}{Var(Y)}$,where Y is the predicted Y value for any given value of X. This is called "iterative re-weighting", and ensures that all Y values for a given value of X will receive the same weight. The method for estimation of the coefficients for the weighting or variance model are given in (9). Usually, one uses a_0, a_1, and a_2 (setting $a_3 = a_4 = 0$), or one uses a_3 and a_4 (setting $a_0 = a_1 = a_2 = 0$). For example:

a_0	a_1	a_2	a_3	a_4	
1	0	0	0	0	Unweighted regression
100	0	0	0	0	Unweighted regression
0	0	0	1	0	Unweighted regression
0	0	0	1	1	Poisson-like error. $\sigma_y \quad \sqrt{y}$
0	0	0	.0001	2	Constant %CV (1% error) σ_y = .01 × Y or σ_y^2 = .0001 × Y^2
0	0	.0001	0	0	Constant %CV (1% error)
100	0	.0025	0	0	5% error plus standard deviation never goes less than 10 = $\sqrt{100}$.

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