PK Solutions

Pharmacokinetics Data Analysis

Version 2.0

User Guide

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Forward

User Guide for PK Solutions – Noncompartmental Pharmacokinetics Data Analysis

PK Solutions – Noncompartmental Pharmacokinetics Data Analysis Excel Template Software Program

PK Solutions 2.0.x

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1. Introduction

1.1 About PK Solutions

Pharmacokinetics is the study of the time course of absorption, distribution, metabolism, and excretion of a drug or other substance in the body. *PK Solutions* is designed to provide a fast and easy means of computing and graphing the basic and most commonly reported pharmacokinetic parameters associated with blood (plasma, serum) concentration-time data following oral (extravascular) and intravenous dosing. Approximately 75 pharmacokinetic parameters are computed for each data set, including tables and graphs projecting multiple dose regimes based on single dose results. A modest understanding of the principles of pharmacokinetics is presumed, but no programming or construction of equations is required. The program is automated, operating mainly by point-and-click methods. In addition to concentration-time data, *PK Solutions* can independently or simultaneously compute and graph results using disposition constants imported, for example, from a curve-fitting program or other source. Once the calculation mode is selected, a few mouse selections are all that is needed to analyze either type of data and produce graphs and parameter tables ready for printing and saving. Dynamic updating of tables and graphs provides a means of trying out “what if” cases and makes *PK Solutions* an ideal tool for learning the principles of pharmacokinetic analysis.

1.2 Methodology

PK Solutions relies on the use of noncompartmental methods of analysis for the estimation of pharmacokinetic parameters. Two noncompartmental techniques are employed and their results compared where appropriate in the parameter tables. One technique is based on the estimation of the area associated with the curve described by the concentration-time profile. In this case, the classic trapezoidal rule is used to compute the area under the curve (AUC).

The second and more extensively used method is based on curve stripping, which resolves a curve into a series of exponential terms corresponding to the absorption, distribution, and elimination phases occurring during the time course of the drug in the blood. These exponential terms are used to calculate the various single and multiple dose pharmacokinetic parameters following well established textbook calculations. The curve stripping approach assumes that the disposition phases of the drug follow apparent first-order rate processes, which is evidenced by linearity in the terminal portion of a semi-log plot. This is, in fact, the case for the overwhelming majority of drugs, making *PK Solutions* a widely useful tool.

Calculation of PK parameters based on curve area and curve stripping results are often called “model-independent” because they are free of any assumption about the underlying compartmental model that the drug obeys. Nonetheless, noncompartmental methods can yield results that confer specific model characteristics on a drug's behavior. The model-independent approach, which serves as the basis for *PK Solutions*, can be contrasted with mathematical curve-fitting programs that are specifically designed to elaborate compart-mental models and their descriptive. The calculations produced by *PK Solutions* are those most commonly reported in the tables of drug metabolism and pharmacokinetics literature.

1.3 Typical Users

PK Solutions is intended to be used by researchers, by those who need and publish basic pharmacokinetic parameters, by students and educators who can make use of the program's flexibility to study the principles of PK analysis, and by pharmacists and physicians to view the effects of dosing regimens on blood levels. It is designed to augment rather than replace or compete with compartmental analysis based on experimental curve-fitting, or clinical dose prediction software. Any one who works with blood level data in pharmaceutical or other product discovery and development or in other bio-research and teaching areas will benefit from the ease of operation, interactivity and comprehensiveness provided by *PK Solutions*.

1.4 Excel-Based Program

PK Solutions operates as a Microsoft Excel™ template using built-in formulas and Visual Basic for Application (VBA) programming. *PK Solutions* is intentionally developed as a Microsoft Excel template in order to make use of Excel's extensive feature set, its cross-platform compatibility between Windows and Macintosh computers, and its ready integration with other Microsoft Office, internet, and VBA supported software. Unlike proprietary programs that require special learning and are limited to the effort programmers expend on creating a graphical environment, *PK Solutions* gives you a programmed solution that takes full advantage of the power, flexibility, and integration of Excel and allows you to enhance, customize, and integrate your PK results with your other digital documents and applications.

1.5 System Requirements

PK Solutions is available for both Windows-based and Macintosh computers. For Windows-based computers, the minimum requirements are a 486 PC, Microsoft Windows 3.1 operating system, and a working version of Microsoft Excel that supports the workbook format (i.e., Excel versions 5.0 and Microsoft Office 5 and above). The program will run under any Windows operating system (3.x/9x/ME/NT/2000/XP/2003). For Macintosh computers, the minimum requirement is a 68040 CPU running under system 7.1 or greater. Microsoft Excel 5.0 for the Macintosh (Office 4.0) or a later version is required. Approximately 1 MB of hard disk space is needed. A 17" color monitor (256 colors or greater at a resolution of 1152x864 pixels or greater) is recommended for optimum spreadsheet viewing. In short, if your computer runs Excel, it will run PK Solutions.

1.6 File Description

For Windows-based systems, *PK Solutions* is shipped as both an Excel 7.0 (Office 95) and an Excel 2002 (Office XP) template. For Macintosh systems, *PK Solutions* is shipped as an Excel 5.0 for Macintosh template. The two versions are identical except that the font selections and a few other format options are optimized for their respective operating systems. The Excel templates are forward compatible with all versions of Excel. Since this is a template file, it will open as a copy of the original. To open a copy of *PK Solutions*, click on the template file then use the File | Save As menu to rename and save the current copy to your computer.

1.7 Using the Program - Please Read

Installation. Copy the file PKSOLN.xlt (named "PK Solutions" on Mac versions) from the supplied diskette to any desired directory or folder on your hard drive. The *PK Solutions* program file is a standard Excel template and can be started either by double clicking on its icon or by using the File|Open menu selection, if Excel is already running.

Allow Macros Upon Opening. If you get the security warning "This file contains macros," you can safely proceed. PK Solutions runs using Visual Basic for Applications (VBA) code composed of macros. You should consider turning your security level to a lower option to prevent this warning. The Excel macro security only warns of the presence of macro, but does not detect malicious code as anti-virus programs effectively do.

Saving a New File. Since PKSOLN.xlt is an Excel template, only a copy of the file is opened by Excel. The original template serves as a backup of the program since it remains intact and independent of any copies made from it. The first thing you should do when a new copy is made is give it a name and save it using the File|Save As menu selection.

Red Color for User Inputs. Red color is used to highlight places where user actions or inputs are allowed or required. Cells containing red text signify places where user inputs or changes are allowed. Red buttons on the worksheets indicate required user actions or selections. Buttons with red text in the header bar invoke programmatic changes to the data. Blue text header buttons simply change the focus of the display to the area indicated.

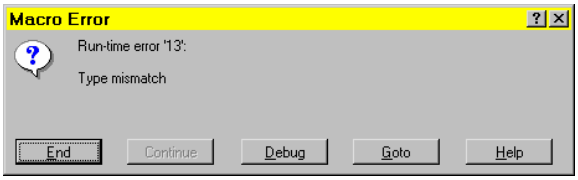
Restrictions. Except as provided on the Data Setup sheet, please do not use the margins or free space in any of the worksheets as a scratch pad. This space is reserved for program use and any entries you make could affect or impede the functioning of the program. Directly overtype or use copy-and-paste to modify data or entries in allowable cells (designated with red). Do not rename or alter the structure of spreadsheets. Use Copy and Paste techniques for moving data internally, and avoid using Cut, Insert or Delete.

Protection Control. The Data Setup and Curve Areas worksheets are programmed to make use of Excel's sheet protection feature (no password) to avoid accidental changes and should not be disabled. The Summit sheet, Test Data sheet, and internal programming modules (hidden) are password protected using a password created by SRS.

Unprotected Worksheets. The Data Analysis, Curve Fit, Single Dose PK, and Multiple Dose PK worksheets are operated in an unprotected mode. This gives the user the option of overriding or changing any of the display and formatting features. Normally, this freedom should be restricted to changing the decimal point display or adjusting chart axis or formats (in the rare instances where such are needed) using Excel's built-in features. Caution should be used to avoid unintentional changes to objects (i.e., buttons, graphic elements, and charts) or data cells that may contain formulas. Except for the above conventions and restrictions, *PK Solutions* may be used like any other Excel file.

Default Data. A new workbook always opens with default data serving as placeholders for the new data. The default data represents the results of running *PK Solutions* on the "ORAL 3" data set found in the Test Data worksheet. There are two reasons for using default data instead of starting with blank cells and featureless graphs. First, since the program employs dynamic formulas throughout, the presence of default data in the results and graphs prevents the display of apparent error notes, which would occur if all the cells were initially blank. Second, default data serves as a built-in guide to illustrate the setup and normal display of the program.

Excel Setup Recommendations. *PK Solutions* uses Visual Basic for Applications (VBA) for programmed tasks. The VBA module is hidden from view and password protected to prevent user changes. When a VBA procedure is unable to complete a task, the program stops and a run-time error dialog box similar to the one depicted below is presented. If this occurs, the best response is to select "End". This will return control to the user at the place where the procedure stopped. Under most circumstances, the interruption will not affect the status of the workbook and use of the program can continue. As with all programs, the working file should be saved to disk frequently during use. This practice will increase the possibility of recovering a usable file following a program error or power outage.



The VBA procedures in *PK Solutions* employ error-trapping routines specifically designed to prevent program interruption and to handle most error conditions that might arise. In order to allow these routines to function, Excel's error interruption option must be disabled before running *PK Solutions*. The option is automatically disabled in Excel 97 and above. For users running Excel 95, follow these steps to disable the error interruption:

1. Select Tools | Options from the menu.
2. Select the Module General tab in the Options dialog box.
3. Make sure that the check box for Break on All Errors is *not* checked.
4. Select "Okay" to close the Options box.

Worst Case Scenario. *PK Solutions* is very robust and will tolerate most any entry or mistake you make. It will tell you if the calculations do not make sense or are not valid by displaying results like DIV/0! or VALUE!. These can probably be readily corrected by reviewing and changing the few input options that are available to you. If you ever get a "Run-time Error" box, simply select the "End" button, which will return you to program control where you can review your steps and try again. After you have become familiar with *PK Solutions*, you will find it possible to exploit all its features quickly and easily. In the worst case, you can simply discard the current copy and start over with a fresh template copy.

1.8 Customization

You may use whatever Excel capabilities available to customize PK Solutions' charts and tables, to apply statistical or the functions to the data, or to integrate worksheet contents with other Office applications. Contact Summit Research Services for custom modification of *PK Solutions* to meet specific data exchange, calculation, and reporting requirements. User comments and suggestions are always welcome.

2. Data Setup Worksheet

2.1 Description

The Data Setup worksheet (Fig. 1) is the starting point for using *PK Solutions*. The worksheet provides for entry or selection of global information such as the description of the current data set, the measurement units to be used in the calculations and headings, and dosage information. Following the general convention, required user entries or actions are indicated in red. Two sections are provided for placement of the source data that are to be analyzed and graphed. Concentration-time data obtained from blood level analyses are placed in the New Data columns. Alternatively, pairs of intercept and rate constants for up to three exponential terms describing a blood level curve are placed in the Imported Exponential Terms box. User control buttons instruct the program as to which of these data types and what dose route to use in calculating PK parameter tables and preparing graphs.

The worksheet is protected to prevent accidental changes to non-entry cells. For convenience, an unprotected scratch pad area is provided that covers the entire bottom of the worksheet.

A new workbook always opens with a completely analyzed default data set taken from the "ORAL 3" example found in the Test Data worksheet. The default button selections and filled in data sections should be changed or overwritten as needed.

The next section provides more detailed instructions on using the Data Setup sheet to initiate an analysis.

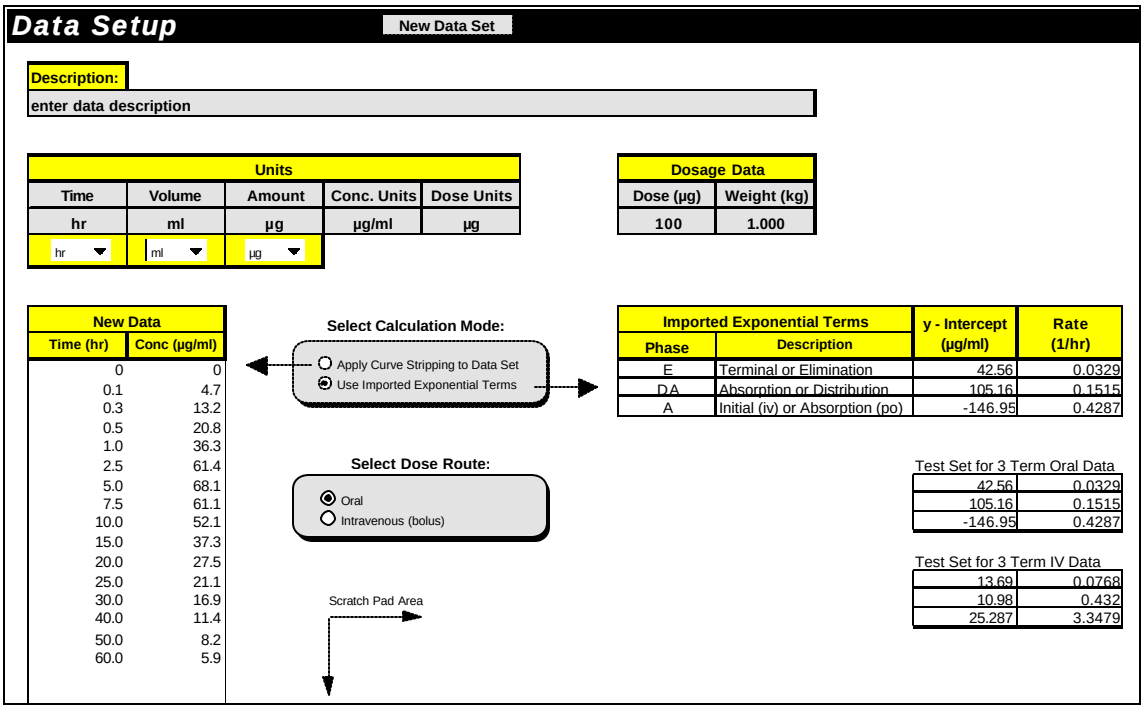


Figure 1. Example Data Setup Worksheet with Default Data

2.2 Components

Description Input

Type a description of the data set to be analyzed and displayed in the workbook. This description will appear at the top of each worksheet in the workbook. If you do not want a description to appear, delete the default "data description" entry.

Units Input

Use the drop-down box controls to select the appropriate units for the Time after dose of the samples analyzed and the units for the Volume and Amount to be used in the concentration expression. If you want to use units other than those available in the drop-down list, type the new unit in the space directly below any drop-down box (on the line with the red indicator). You can then select the new unit, which will appear as the first item in the drop-down list. The selected units are automatically applied throughout the workbook both in the column titles and alongside each calculated pharmacokinetic parameter. The selection of Time, Volume and Amount units can be changed at any time without affecting calculated results. The last two cells in the Units group indicate the units of Concentration and Dose that the program will use, based on the selected entries. These options are used to standardize the units of all calculations since no conversion factors are employed in the equations. Convert your data to match the available unit options, if needed.

Dose Data Inputs

Enter the amount of the Dose, in the units shown. Note that the unit of Dose is the same as that selected for the Amount. The main parameters that use dose information are the volume of distribution and systemic clearance. These parameters will display a zero or give an error if dose data is not entered. The program uses a default value of 1 to avoid such error displays.

Enter the subject's dose weight in kilograms.

New Data Area

The entries in the Time and Concentration columns of the New Data section provide the source blood level profile data that is used by the program when the "Apply Curve Stripping to Data Set" calculation mode is selected. The data values must conform to the units indicated (see above). Data sets can be added by cutting and pasting, by manual entry, or by other means (e.g., linking to external sources or programming). The New Data Set button in the header initiates the analysis of the set of data

Data values placed in the New Data columns should always be arranged or sorted in ascending time point order, and should always begin with zero as the first time point. Additionally, the first concentration value (corresponding to time zero) should also be zero. *In summary, always start your data set with 0, 0.*

The program treats the first (zero time) data differently depending on the dose route selected and the calculated parameter. For any dose route, the program substitutes a

zero for any other initial concentration value. The program automatically deletes time zero when plotting intravenous dose curves but always starts oral (extra-vascular) dose plots at time zero. It always uses zero time as the starting point for calculating curve areas by the trapezoid rule. The zero time concentration value after intravenous dosing is a computed value ("$C_{initial}$"). The program automatically changes the first (zero time) concentration from a zero to a null to allow for semi-log plotting, and displays a user notification of this fact.

In order to perform semi-log plotting, the final concentration value cannot be zero. If necessary, replace a final zero concentration with a small value (e.g., something in the range of the analytical detection limit).

Imported Exponential Terms

The Imported Exponential Terms box provides an alternate source of data for computing PK parameters. When the calculation mode option is changed to "Use Imported Exponential Terms", the use of the Data Analysis sheet to perform curve stripping is bypassed and the intercept and rate values for each of the exponential terms (see section 4.2) that describe a blood level curve are read directly from the import box. Intercept and rate data for 1 to 3 terms should be entered in the appropriate columns. Unused cells can be blank (null) or zero. Make sure the correct units for the intercept and rate are selected. If the terms derive from an oral dose, the sign of the intercept for the absorption phase must be negative.

To insure that the program adjusts the worksheet displays for optimum viewing, always repeat the selection of the Dose Route and Calculation Mode radio buttons after making any changes to the disposition constants.

PK parameters that employ exponential terms in their formulas switch instantly between using the disposition constants in the imported terms box and those in the modules on the Data Analysis sheet, depending on the calculation mode selection. Thus, the Imported Exponential Terms are applied to the single and multiple dose PK parameter tables and graphs only when the "Use Imported Exponential Terms" option is selected. Otherwise, the results are derived from the disposition constants in the stripping modules on the Data Analysis sheet.

When the calculation mode is set to "Apply Curve Stripping to Data Set", the imported terms are disconnected from all parameter formulas and graphs and can be left in the import box without fear of interference. On the other hand, the imported terms can at any time be graphed using the Plot Imported Terms section located as the bottom view of the Curve Fit sheet.

Two tri-exponential data sets (representing an oral and an iv dose) are provided as test values for the imported terms box. A copy button accompanies each test set to facilitate copying the values to the imported terms area. Whenever a test set is copied, the program detects the current selection mode and dose route and offers to change them, if needed. You can also make these changes manually at any time.

Select Calculation Mode

The Select Calculation Mode gives two options to identify whether the source data for the graphs and calculations refers to the concentration-time values in the New Data

columns or to the disposition constants in the Imported Exponential Terms box. The selection should be made or refreshed after the data are placed and after the dose route is selected.

Selection of the "Use Imported Exponential Terms" is all that is required to instantly substitute the imported terms into their appropriate formulas and adjust the param-eter tables, graphs, and views to match the data and dose route. Similarly, selection of the "Apply Curve Stripping to Data Set" mode instantly redefines the data source and revises the formulas, parameter tables, graphs, and views accordingly. Once a data set has been analyzed, the calculation mode can be toggled to instantly compare results calculated using the imported terms with results calculated from the concentration-time data The New Data Set button initiates the analysis of time-concentration data sets which is done using the curve stripping modules in the Data Analysis sheet. The exponential constants for the concentration-time profile are retained in the Data Analysis sheet for substitution in the result formulas.

In summary, the first time a new set of concentration-time points is analyzed, set the calculation mode to "Apply Curve Stripping to Data Set", then select the New Data Set button and proceed with the curve stripping. This will define and fix the constants in the exponential terms related to the concentration-time profile. Once this is done, the calculation mode can be used as toggle, if desired, and the program will know where to look for the respective input data. See section 2.3 for additional information on how the calculation mode affects the flow of data in *PK Solutions*.

Select Dose Route

The Select Dose Route gives two options for identifying the route of administration used in obtaining the source data (oral or intravenous). The dose routes are assumed to be via bolus or gavage. The option buttons apply to both data sources (New Data set or Imported Exponential Terms). Always refresh or select the appropriate option during the setup of an analysis because this action notifies the program how to perform certain calculations and which views to arrange.

New Data Set Button

Selecting the New Data Set button initiates the procedure for curve stripping the concentration-time data switches you to the Data Analysis sheet. The button also triggers an extensive initialization of the entire program affecting all worksheets, and resets the Data Analysis sheet to default conditions. Therefore, use the button with caution because it serves as both a *"start"* and a *"start over"* button. A button with the same name and functions is located on the Data Analysis worksheet.

Scratch Pad Area

The indicated scratch pad area is unlocked and available for use to paste pending data sets, for notes, or other purposes.

2.3 Data Flow

Figure 2 illustrates which of the *PK Solutions* worksheets are involved in each of the two calculation modes, and how the data flows between them. Note that the Curve Fit sheet is isolated from the other sheets because the Curve Fit sheet provides curve fitting and plotting features that are independent of results or graphs appearing elsewhere.

When the calculation mode is set for curve stripping, selection of the New Data Set button results in the initialization of all worksheets to receive the new data set followed by copying of the concentration-time data from the Data Setup sheet to both the Curve Fit and Data Analysis sheets. The double-headed arrows indicate that the curve stripping information generated on the Data Analysis sheet is shared dynamically with other worksheets. Since the parameter tables and graphs on other sheets are dynamically connected to the Data Analysis sheet, the displayed results are meaningless until the curve stripping procedure is completed and the exponential terms and their respective disposition constants are defined. Note also that the concentration-time values located in the New Data columns on the Data Setup sheet are not dynamically connected to the rest of the program and can be updated at any time in anticipation of running a new data set.

Selection of the Use Imported Exponential Terms calculation mode immediately invokes a different arrangement. In this case, the imported terms are connected directly to the Single Dose and Multiple Dose sheets and dynamically update the appropriate PK parameters and graphs. Since the Data Analysis and Curve Area sheets contain data that is not relevant to the imported exponential calculation mode, they are excluded from the data path.

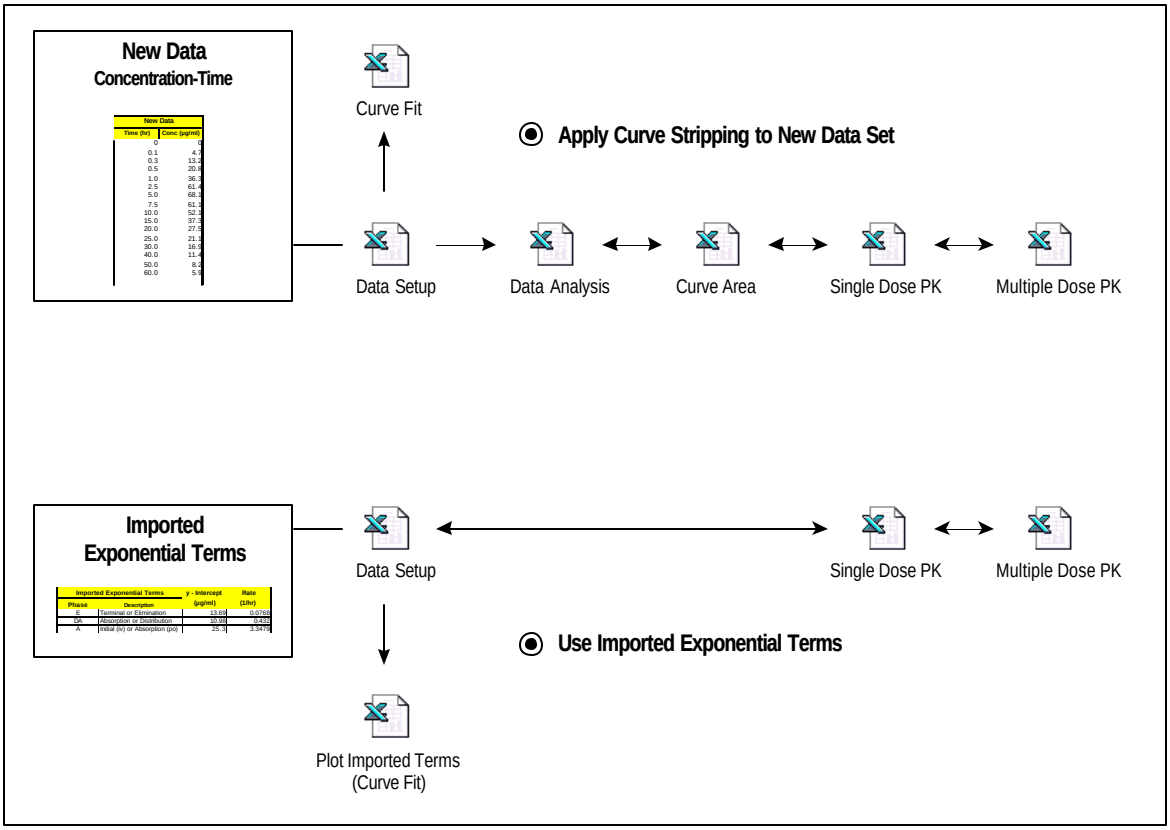


Figure 2. Effect of Calculation Mode on Data Flow and Worksheet Connections

3. Data Analysis Worksheet

3.1 Description

The Data Analysis worksheet is designed to help you apply the method of residuals (or curve stripping) to define the underlying exponential terms that best describe the current concentration-time data set. The main section of the sheet that is used for curve stripping is shown in Figure 3. Up to three exponential terms can be defined for data derived either from intravenous or extravascular (oral) routes of administration. The curve stripping procedure determines the basic pharmacokinetic parameters of half-life, rate and concentration intercept for each phase of the blood level curve. These results are used, along with curve area data, to compute, tabulate and graph model-independent PK results when the curve stripping calculation mode is selected.

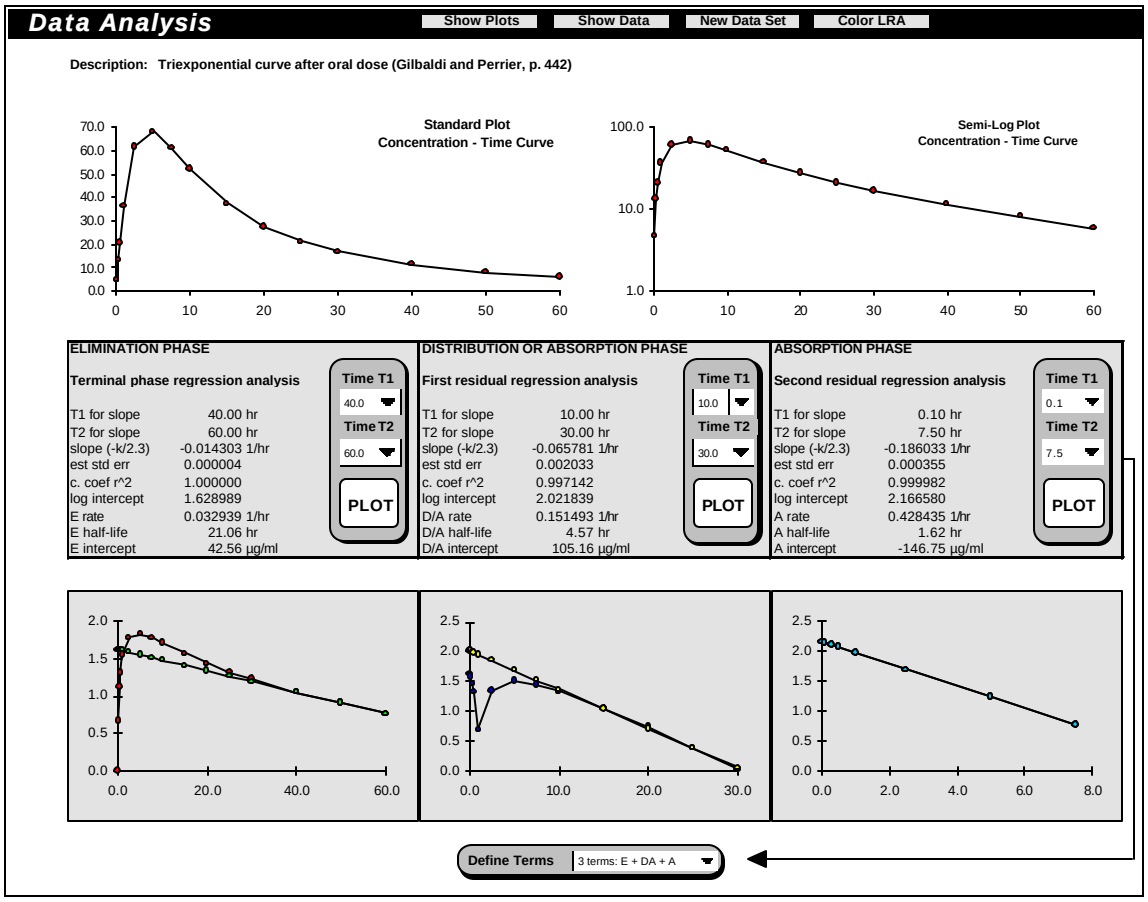


Figure 3. Main Curve Stripping Sections on the Data Analysis Worksheet

3.2 Stripping Modules

The working part of the sheet consists of three identical stripping modules. Each module allows you to work with a set of curves to identify and define the terminal linear portion of a semi-logarithmic plot. The modules work from left to right, stripping the curve term by term. This arrangement allows up to three exponential terms to be defined. The program can handle both oral (extravascular) and intravenous dose data using the same modules. The names of the modules come from the model on which they are built, which is explained as follows.

The general equation describing the disposition of drugs in plasma is given by the summation expression:

$$C = \sum C_n \exp(-l_n t)$$

where C_n and l_n are the zero-time intercepts and rate constants, respectively, for each exponential term. The corresponding triexponential function can be written as:

$$C = Ae^{-at} + De^{-bt} + Ee^{-gt}$$

In this expression, the intercepts are given letter designations indicating the three common drug disposition phases of *A*bsorption, *D*istribution, and *E*limination that are typically encountered after an oral dose.

The curve stripping technique involves mathematical subtraction of each term from the remaining expression in order to isolate and determine the individual constants associated with each phase. Accordingly, the Data Analysis sheet is divided into three color-coded sections or modules, each representing one of three possible exponential terms and its corresponding disposition phase. The modules are arranged from left to right in the order in which data is stripped, beginning with the so-called Elimination Phase, followed by the Disposition Phase and the Absorption Phase. Section 3.4 provides details on how to use the modules.

Since the modules must cover all situations, some allowance needs to be made for the names used to describe them. For example, if only two exponentials can be obtained by stripping oral data, then the second module will relate to the absorption phase rather than the disposition phase, and the third module will not be used at all. To accommodate this scenario, the second module is called the Distribution *or* Absorption Phase. Second, if the data comes from an intravenous dose, referring to the second or third obtainable exponential term as the Absorption Phase is not strictly accurate. In such cases, the last stripped term or module would more accurately be referred to as the *initial* phase.

Variables used in formulas are referred to by names rather than cell references to make the formulas more meaningful and traceable. The names of the variables derive from the titles of the modules in which they are defined. The convention is as follows:

E_named variables come from the Elimination Phase module.
DA_named variables come from the Distribution or Absorption Phase module.
A_named variables come from the Absorption Phase module.

Finally, to accommodate both oral and intravenous dose data under one setup, the program automatically assigns a negative value to the intercept of the last extractable term for oral data since this term represents drug absorption into the system.

3.3 Components

New Data Set Button

As mentioned earlier, the concentration-time data set in this sheet is the set most recently passed to the sheet the last time the New Data Set button (located both here and on the Data Setup sheet) was selected. The data on this sheet is dynamically connected to the results worksheets only when the curve stripping calculation mode is in effect. If the New Data Set button is selected, the program will wipe this sheet clean and start over with whatever data is in the New Data columns. The program always gives you a chance to cancel the action. The other buttons in the header are used to control the display, as described below.

Current Concentration-Time Plots

Two curves are displayed depicting both a standard and semi-logarithmic plot of the current concentration-time data. The Show Plots button toggles between hiding and showing these plots. One of the purposes of the plots is to give a quick indication as to the linearity of the semi-log data. If the semi-log plot is curvilinear and the standard plot appears linear, then the data probably represents a zero-order process and cannot be further analyzed by this program.

Residuals and Linear Regression Data Table

Immediately below the concentration-time curves, the sheet contains a table of calculations resulting from the operation of the stripping modules on the data set (Fig. 4). The underlying formulas also control whether the data in this region is displayed or blanked, and this, in turn, dynamically affects the display of the stripping (residuals) charts. The Show Data button toggles between hiding and showing this table.

The table, modules, and charts are color coded to clarify their relationships. The Color LRA button will apply the color code to the table to illustrate the data driving the stripping charts for each module. The colored ranges include the points you selected as defining the terminal linear region of each curve and are used in computing the slope and intercept of each exponential term. Remove the color code by selecting the Color LRA button again.

Time	Concentration		LRA of Concentration		First Residual		LRA of 1st Residual		Second Residual		LRA of 2nd Residual	
(hr)	(µg/ml)	log	log	(µg/ml)	(µg/ml)	log	log	(µg/ml)	(µg/ml)	log	log	(µg/ml)
0.0			1.629	42.56	-42.56	1.629	2.022	105.16	147.72	2.169	2.167	146.75
0.1	4.7	0.672	1.628	42.42	-37.72	1.577	2.015	103.58	141.30	2.150	2.148	140.60
0.3	13.2	1.121	1.625	42.14	-28.94	1.462	2.002	100.49	129.43	2.112	2.111	129.05
0.5	20.8	1.318	1.622	41.86	-21.06	1.324	1.989	97.49	118.55	2.074	2.074	118.46
1.0	36.3	1.560	1.615	41.18	-4.88	0.688	1.956	90.38	95.26	1.979	1.981	95.62
2.5	61.4	1.788	1.593	39.20	22.20	1.346	1.857	72.01	49.80	1.697	1.701	50.29
5.0	68.1	1.833	1.557	36.10	32.00	1.505	1.693	49.31	17.31	1.238	1.236	17.24
7.5	61.1	1.786	1.522	33.24	27.86	1.445	1.528	33.77	5.91	0.772	0.771	5.91
10.0	52.1	1.717	1.486	30.62	21.48	1.332	1.364	23.12				
15.0	37.3	1.572	1.414	25.97	11.33	1.054	1.035	10.84				
20.0	27.5	1.439	1.343	22.03	5.47	0.738	0.706	5.08				
25.0	21.1	1.324	1.271	18.68	2.42	0.383	0.377	2.38				
30.0	16.9	1.228	1.200	15.85	1.05	0.023	0.048	1.12				
40.0	11.4	1.057	1.057	11.40								
50.0	8.2	0.914	0.914	8.20								
60.0	5.9	0.771	0.771	5.90								

Figure 4. Residuals and Linear Regression Section of Data Analysis Worksheet

Stripping Modules

The stripping modules are the main user interaction area where the constants for each exponential term are calculated. The drop-down lists allow point-and-click selection of the first (T1) and last (T2) time points of the initial curve that are judged to bracket the terminal linear region. When the PLOT button is selected, the corresponding linear curve is redrawn. The slope and intercept of the linear curve provide the required pharmacokinetic disposition constants defining each exponential term. The linear extrapolations are color-coded to have the same symbol color as the background color of their respective stripping modules. The stripping procedure is des-cribed in the next section.

The area immediately below each module is used to display instruction messages to help the user select appropriate settings, if needed. The messages only appear when the current settings need to be changed.

Stripping Charts

Below each module are working plots showing the original concentration or residual curves superimposed on the corresponding user-defined, linear extrapolated curve. The charts are designed to provide optimum display characteristics without user intervention. Logarithms are used for the Y-axis since semi-logarithmic plots are prone to give display warnings whenever a fractional value is encountered. The program automatically excludes plotting of the zero time point for intravenous data, since this value is calculated as the sum of the y-intercepts ($C_{initial}$).

A concession that is required to allow a single program to handle all data types, is to always treat the 1st residual (i.e., the original concentration minus the linear extrapolation) as a positive number. This results in a somewhat unexpected display of the 1st residual curve for oral data, as illustrated by the sharp dip in the curve on the right panel in Figure 5 at the point where the residual changes sign. In practice, the region of interest is where the lines appear to converge. Thus, the dip and odd display for this case have no effect on the procedures or results.

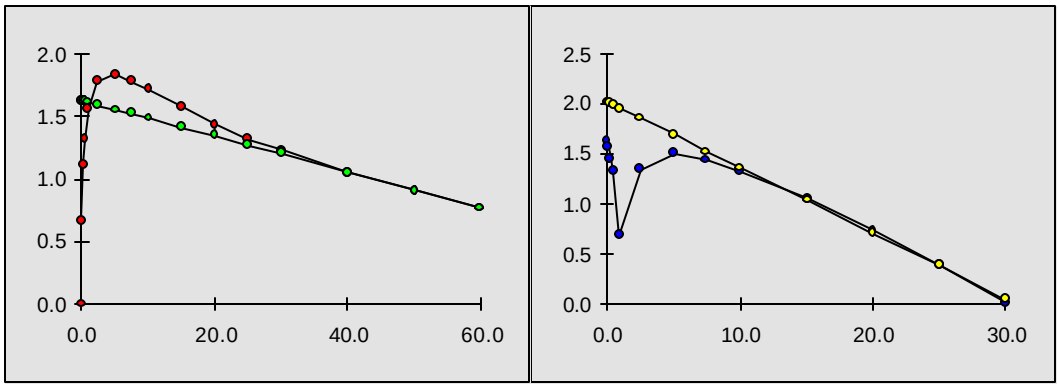


Figure 5. Oral Data Illustrating Consequence of Using the Absolute Value of the 1st Residual

Composite Plot

A plot of all curves is provided at the bottom of the sheet (Fig. 6). You can temp-orarily magnify a region for closer examination by selecting the chart and stretching it to a larger size. Be sure to select the Undo operation as your next action, if you want the chart to automatically return to its original size. Otherwise, you will have to resize the chart manually.

As with all charts (and most other elements) in *PK Solutions*, you can modify the chart in any way that suits your purposes and then cut and paste it into another document such as a report or presentation. Make sure you save your current working document prior to making these alterations so you can preserve the original layout.

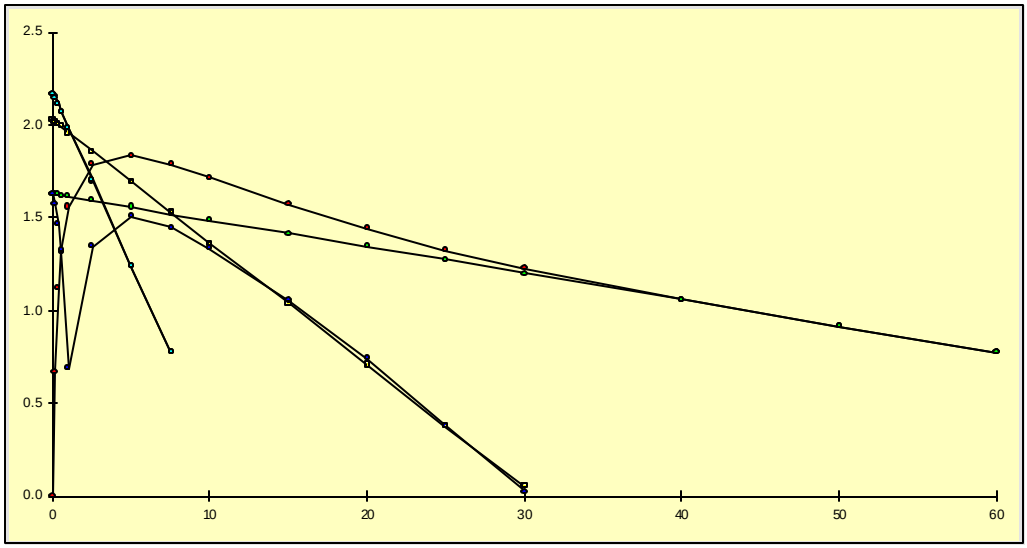


Figure 6. Composite Plot of Residuals and Linear Regression Results

3.4 Data Setup and Stripping Procedure

This section outlines the procedure to be used for setting up and stripping a new data set. Some indication of what the program does in response to your actions is also included. Data for use in learning and testing the stripping procedures can be copied from the Test Data sheet.

1. On the **Data Setup** sheet, enter a **Description** of the sample set. This will appear on all worksheets.

2. Use the drop-down list to select the appropriate **Units** for the sampling **Time**, **Volume**, and **Amount**. If needed, enter alternate unit descriptions on the line with the red indicator below the appropriate unit box, then select it from the drop-down list.

3. Enter the **Dose** amount and the **Weight** of the animal at dosing in the **Dosage Data** table.

4. Place the concentration-time data in the proper columns in the **New Data** area. If all or most of the concentration values are less than one, better displays are obtained by changing the data to larger numbers (e.g., multiply times 1000) and adjusting the units accordingly. Make sure the data set is sorted starting with zero time in the first row. A minimum of 4 data points is required. The zero time and concentration must both have the value of zero (0). If you encounter an intravenous data set that indicates the zero time concentration is >0, then use some small value (such as 0.01 min) as the time of this sample. The program will not accept zero as the final concentration since such a value cannot be plotted on a semi-log plot. If your data set includes zero as the final concentration, then replace the zero with a very small value (e.g., 0.01) or some value equivalent to the limit of detection. Insure that the last data point is followed by a blank row.

5. Click the appropriate button for the **Select Dose Route** options.

6. Select the Apply Curve Stripping to Data Set option button as the **Select Calculation Mode** option.

7. If you have not done so yet, this is a good place to choose the **File|Save As** menu option and proceed with naming and saving the current data set in a new workbook. You can continue to analyze and capture different data sets in any workbook.

8. Select the **New Data Set** button. The same button appears on both the Data Setup and Data Analysis sheets. If you okay the prompt to proceed, the program will reset and initialize all worksheets according the options selected and then switch the view to the Data Analysis sheet. There will be a short pause depending on the speed of your computer. The default setup in the Data Analysis stripping modules uses the last two points of each concentration or residual curve to define the linear regression line. This is intended only as a start and to give a recognizable display to the charts. The default setup gives meaningless results, so continue with the analysis of the data and the extraction of the exponential terms using the stripping modules as described in the next 3 steps.

9. The objective of the data analysis is to produce the most reliable linear extrapolation of the linear region, if any, of the terminal portion of the primary curve. Starting with the **Elimination Phase** module (where the primary curve is the red concentration-time curve), select the best T1 and T2 values that bracket the linear portion of the curve. Select **Plot** to see the results of applying linear regression analysis (LRA) to the identified range and plotting the resulting linear regression line in yellow. The regression coefficient is given in the module to help evaluate the fit of the line with the data. The program dynamically

subtracts the linear extrapolated curve from the primary curve to generate the data and chart display for the first residual. The first residual is plotted (using absolute values) below the D/A module (blue curve).

10. Repeat the previous step using the Plot button in the **Distribution or Absorption Phase** module to define the best linear extrapolation of the blue curve, which is plotted as the yellow line in the middle chart. As before, the difference between the two curves, if any, produces a second residual, which is depicted by the magenta curve in the chart beneath the Absorption Phase module. If this step ends with little or no second residual, then only two exponential terms can be extracted from the blood profile curve and you should proceed to Step 12.

11. If the second residual has sufficient magnitude, continue in the same manner to define its terminal linear region using the **Absorption Phase** module. For an oral dose, the intercept and rate constants obtained in this module represent the absorption phase. For an intravenous dose, the results calculated with the third module pertain to the initial distribution phase of the drug (i.e., first term in the triexponential equation).. In either case, completion of this module means that 3 terms have been extracted from the blood level data.

    Note: Changes can be made to the modules in any order. When the stripping is complete, the linear segments (T1 to T2) for each module should be contiguous from module to module and cover the time points progressively. Overlapping or repeating T1 or T2 values in different modules is not allowed. A warning message appears under the module when overlapping selections need to be corrected.

12. The final required step is to notify the program of the number of exponential terms extracted, that is, the number of modules used. Select the appropriate response from the drop-down list on the **Define Terms** button. A confirmation box will appear for you to okay. If the box does not appear, select the Define Terms button again.

13. That's all! Completion of the stripping procedures is all the user input required. The program automatically computes the AUC and AUMC values for the data set (see Curve Areas sheet) and uses these, in conjunction with the pharmacokinetic parameters displayed in the stripping modules, to produce the tables of parameters and graphs shown in the Single Dose PK and Multiple Dose PK sheets.

4. Curve Fit Worksheet

4.1 Description

The Curve Fit worksheet contains two working regions. The top work area is used to apply Excel's Solver add-in tool to perform a least squares curve fitting operation on the disposition constants residing in the Data Analysis sheet modules. The lower work area provides an automated procedure for reconstructing the curve described by the disposition constants residing in the Imported Exponential Terms table on the Data Setup sheet. The view that the worksheet displays when it is first opened depends on the calculation mode set in the Data Setup sheet. If the mode is set for applying curve stripping, then the sheet opens with the curve fitting work region in view. If the mode is set for imported exponential terms, then the sheet opens with the exponential plot region in view. Buttons are provided in the header of each region to toggle between the two views.

As previously discussed (section 2.3), although the Curve Fit sheet uses source data from the Data Setup and Data Analysis worksheets, it is isolated from the rest of the program so that any work done on this sheet has no effect on the contents of other worksheets.

4.2 Initial Setup

An important aspect of the program setup procedures is how it treats the first zero time and concentration data. The program will always read data expecting it to start with time zero. It will always substitute any other value and use zero as the initial (zero time) concentration for oral dose routes and will always use a calculated extrapolation ($C_{initial}$) as the zero time concentration for an intravenous dose. The value of $C_{initial}$ used for intravenous doses depends on the number of stripping terms selected on the Define Terms button at the end of the curve stripping process. The New Data Set button initiates the set up of the Curve Fit table making it ready for use with oral data. But it cannot be used for intravenous data until the stripping process is completed and the Define Terms button has informed the program what value to use for $C_{initial}$.

4.3 Curve Fit Procedures

Figures 8a and 8b illustrate the components of the top work area of the Curve Fit sheet. The purpose of this work area is to apply least squares curve fitting to the pharmacokinetic disposition constants (intercepts and rates) determined by curve stripping in order to graphically assess how well the stripped data conforms to an idealized curve. This goal should be distinguished from that of determining the disposition constants through mathematical curve fitting procedures using the raw concentration-time data.

The sheet is automatically set up with the current concentration-time data set when the New Data Set button is run, and is ready for use after completing the curve stripping on the Data Analysis sheet. The program places the curve stripping results in the Strip column of the Curve Comparison table for use as seed values. The Excel Solver tool is then used to apply the principle of least squares analysis to produce a new set of constants (Fit column) for the exponential

equation that best fits the data. This procedure can fit an exponential equation containing from one to three terms. The exponential equation that is fitted has the form (see section 3.2):

$$C = Ae^{-at} + De^{-bt} + Ee^{-gt}$$

Selecting the "Curve Fitting Procedures" object opens a stepwise list of instructions for manually running the curve fitting procedure. Click on the list to close it. Alternately, the curve fitting procedure can be run automatically in two steps by selecting the Apply Curve Fit button. The first step sets up the tables and the terms to be fitted in preparation for using the Solver. A message box then appears asking whether to continue with the Solver or stop. This step is necessary because the Solver acts smoother in some versions of Excel than in others.

Typically, the first time in an Excel session that the Solver is called programmatically (i.e., with the button), a "File Not Found" box will open waiting for you to locate it. If this occurs, cancel the box and select the Solver from the Tools menu. The Solver Parameters window will open and should appear as in Figure 7. You can then proceed by selecting the Solve button. This will inform Excel of the location of the Solver for the duration of the current session. If the curve fitting appears to be changing different cells in the fit column that expected, open the Solver Parameters window and make sure the range names are typed in as shown.

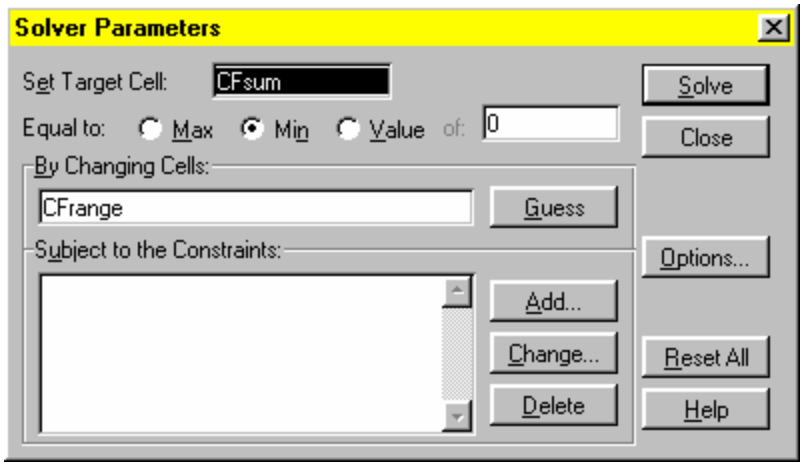


Figure 7. Solver Parameters Window with Predefined Settings

The Solver is an add-in tool that comes with Excel. If it is not present on the Tools menu, you will have to install and configure it. To install it, select the Tools| Add-Ins menu item. In the Add-Ins dialog box, place a check mark by the Solver Add-In and click Okay. To configure it, open the Solver by selecting it from the Tools menu, and fill in the data ranges as shown.

4.4 Curve Fit Results

Application of the curve fitting procedure updates the data table at the top of the worksheet. In turn, the table is used to update a plot of each fitted exponential term, as well as a composite curve describing the sum of the exponentials (Figure 8a). In addition, the program automatically updates a publishable graph that superimposes the observed concentration data (symbols) on a curve of the fitted results (Figure 8b). Like all charts in PK Solutions, these can be edited, printed, or copied and pasted elsewhere using standard Excel methods.

One application for the curve fitting results is noted on the sheet as follows:

> The curve stripping mode uses the constants in the Strip column for all PK parameter calculations. To use the curve fitting results for the PK calculations, copy the data in the Fit column to the Imported Exponential Terms box and select the Exponential Terms mode.

This note is a suggestion to let *PK Solutions* recalculate the PK parameters using the fitted results as though they were imported exponential terms. If you do as described and copy the fitted disposition constants from the Fit column into the Imported Exponential Terms box, then you can use the Select Calculation Mode options to toggle between viewing PK parameter results based on the original analytical data or on the fitted exponential terms.

Curve Fit

Apply Curve Fit | Show Terms Plot

Description: Triexponential curve after oral dose (Gilbaldi and Perrier, p. 442)

Time (hr)	Conc. (µg/ml)	E Phase (µg/ml)	DA Phase (µg/ml)	A Phase (µg/ml)	Conc=f(T) (µg/ml)	Square of Difference
0.0	0.0	35.2	89.4	-124.6	0.0	0.00001
0.1	4.7	35.1	88.4	-118.8	4.7	0.00162
0.3	13.2	34.9	86.3	-108.0	13.2	0.00030
0.5	20.8	34.7	84.2	-98.1	20.8	0.00110
1.0	36.3	34.2	79.3	-77.2	36.3	0.00044
2.5	61.4	32.7	66.3	-37.6	61.3	0.00257
5.0	68.1	30.3	49.1	-11.4	68.1	0.00011
7.5	61.1	28.2	36.4	-3.4	61.1	0.00173
10.0	52.1	26.1	27.0	-1.0	52.1	0.00020
15.0	37.3	22.5	14.8	-0.1	37.2	0.00346
20.0	27.5	19.4	8.1	0.0	27.5	0.00062
25.0	21.1	16.7	4.5	0.0	21.2	0.00553
30.0	16.9	14.4	2.5	0.0	16.8	0.00320
40.0	11.4	10.7	0.7	0.0	11.4	0.00024
50.0	8.2	7.9	0.2	0.0	8.1	0.00320
60.0	5.9	5.9	0.1	0.0	5.9	0.00192
					SoD sum:	0.02623

Curve Comparison		
Constant	**Strip**	**Fit**
E rate	0.0329	0.0298
E intercept	42.559	35.212
D/A rate	0.15149	0.11979
D/A intercept	105.157	89.429
A rate	0.42843	0.47920
A intercept	-146.751	-124.643

Curve Fitting Procedure

The cuve stripping mode uses the constants in the Strip column for all PK parameter calculations To use the curve fitting results for the PK calculations, copy the data in the Fit column to the Imported Exponential Terms box and select the Exponential Terms mode.

If the Find box appears when you first run the Apply Curve Fit button, open and close the Solver from the Tools menu to teach Excel its location.

Plots of each fitted exponential term, plus composite of all terms.

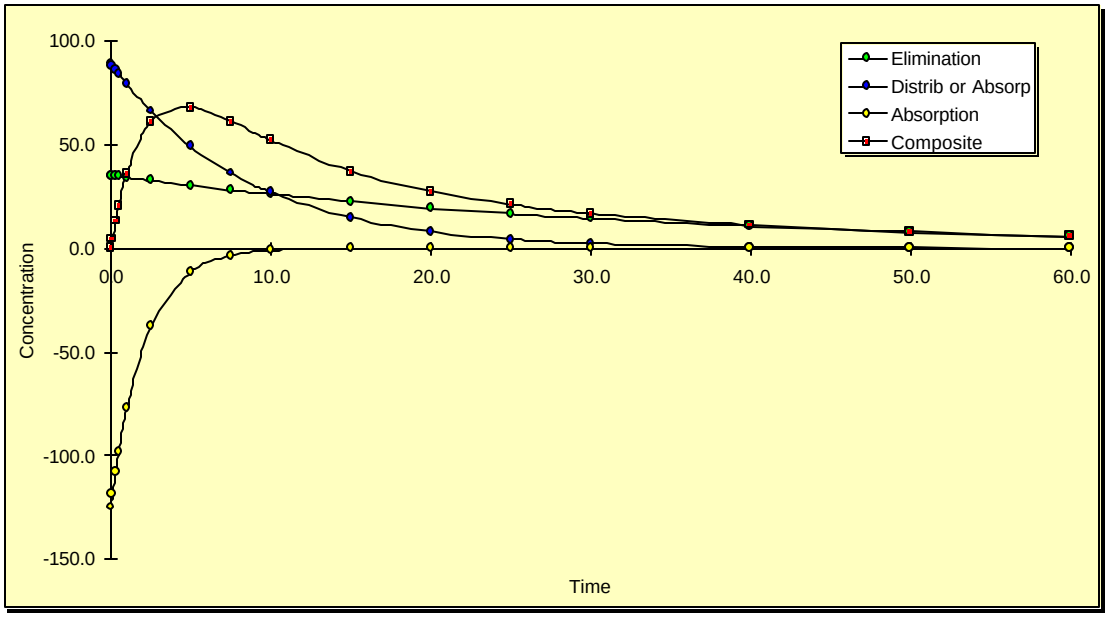


Figure 8a. Top Region of Curve Fit Worksheet Used for Curve Fitting Data Analysis Results

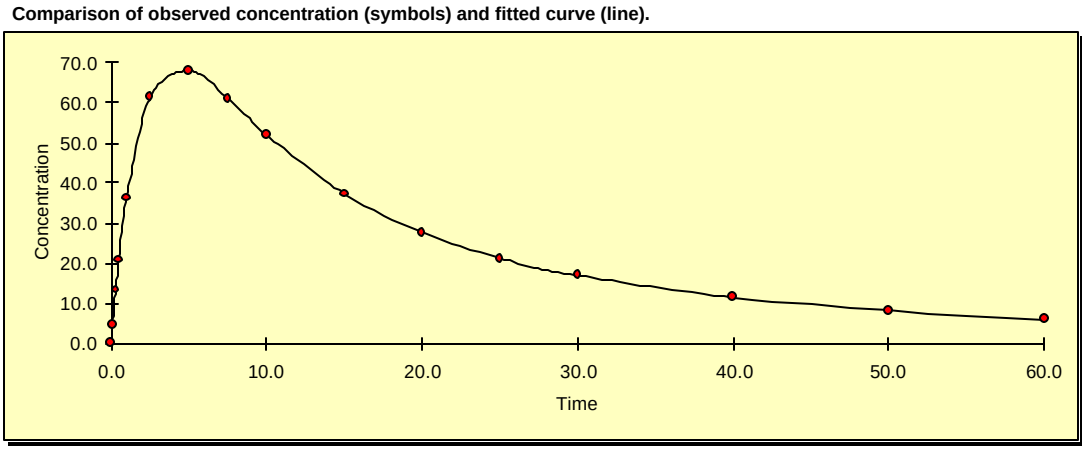


Figure 8b. Top Region of Curve Fit Worksheet. Overlay Graph of Fitted and Observed Values

4.5 Plot Imported Terms Work Area

The work area for plotting imported exponential terms is illustrated in Figure 9. The graph displays a plot of concentration versus time based on the standard exponential expression:

$$C = Ae^{-at} + De^{-bt} + Ee^{-gt}$$

where the disposition constants (intercept and rate) for each term are read from the Import or Input Exponential Terms table. As the name of the table implies, the disposition constants which provide the shape of the curve can either be copied from the Imported Exponential Terms table on the Data Setup sheet, or typed directly into the appropriate cell in the table. For convenience, the button labeled “Copy Terms from Data Setup” automatically fills the table with whatever values currently reside in the exponential terms table on the Data Setup sheet.

The axis automatically change to fit the range of data supplied. However, an input cell is provided which allows you to make adjustments to the scale of the time axis by entering the highest value to be displayed. This feature can be useful for expanding the initial area of the curve.

Interacting with the dynamic curve display is particularly useful as a teaching aid. By observing the effect of changing the sign and magnitude of the disposition constants on the reconstructed blood level curve a better understanding can be gained about the relationships involved. For oral dose results, always use a negative sign for the intercept in the last term.

The Plot Imported Terms table and curve can be used independently from the rest of the workbook. Changes in this area do not affect any other graph or results table. Select the Show Curve Fit button in the header to toggle up to the top portion of the spreadsheet.

Plot Imported Terms

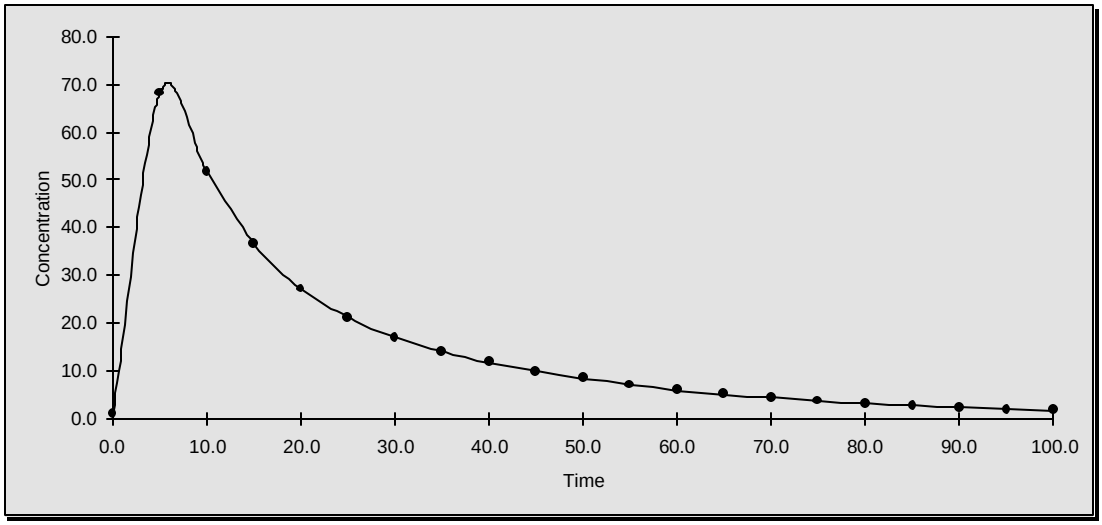
Copy Terms from Data Setup | Show Curve Fit

Description: Triexpoential after oral dose

Import or Input Exponential Terms		y - Intercept	Rate
Phase	Description	(µg/ml)	(1/hr)
E	Terminal or Elimination	42.56	0.0329
DA	Absorption or Distribution	105.16	0.1515
A	Initial (iv) or Absorption (po)	-146.95	0.4287

Time Axis
Max (hr)
100

Plot of imported exponential terms.



Time	E Phase	DA Phase	A Phase	Conc=f(T)
(hr)	(µg/ml)	(µg/ml)	(µg/ml)	(µg/ml)
0.0	42.6	105.2	-147.0	0.8
5.0	36.1	49.3	-17.2	68.2
10.0	30.6	23.1	-2.0	51.7
15.0	26.0	10.8	-0.2	36.6
20.0	22.0	5.1	0.0	27.1
25.0	18.7	2.4	0.0	21.1
30.0	15.9	1.1	0.0	17.0
35.0	13.5	0.5	0.0	14.0
40.0	11.4	0.2	0.0	11.7
45.0	9.7	0.1	0.0	9.8
50.0	8.2	0.1	0.0	8.3
55.0	7.0	0.0	0.0	7.0
60.0	5.9	0.0	0.0	5.9
65.0	5.0	0.0	0.0	5.0
70.0	4.3	0.0	0.0	4.3
75.0	3.6	0.0	0.0	3.6
80.0	3.1	0.0	0.0	3.1
85.0	2.6	0.0	0.0	2.6
90.0	2.2	0.0	0.0	2.2
95.0	1.9	0.0	0.0	1.9
100.0	1.6	0.0	0.0	1.6

Figure 9. Lower Region of Curve Fit Worksheet Used for Plotting Exponential Terms

5. Curve Areas Worksheet

5.1 Description

The Curve Areas worksheet (Fig. 10) uses the trapezoidal rule to calculate and plot areas under the standard concentration-time curve (AUC) and the first moment curve (AUMC). The sheet is automatically setup and completed each time a new data set is run by using the New Data Set button. Since no user interaction is required, sheet protection is applied after each update. Results include the area under the observed data points as well as extrapolation of the area to infinite time using the elimination rate constant. In addition, the observed values of C_{max} and T_{max} are tabulated. The Curve Areas sheet can be used independently from other program features to provide AUC calculations on any data set.

5.2 Connections

Whenever the New Data Set button is run, the program copies the concentration-time data present in the Data Set to the Curve Areas sheet and immediately computes and plots the trapezoidal area results. No links are maintained with the source concentration-time data on the Data Setup page. Total area computations (AUC_{∞} and $AUCM_{\infty}$) use the elimination rate in their formulas, and this is read directly from the elimination phase module on the Data Analysis sheet. Since this is a dynamic link, changes made in this module will update the calculations.

5.3 Calculations and Timing

A description of the formulas employed by the Curve Areas sheet are included in chapter 8. An important feature is that the program will always use zero as the initial (zero time) concentration for oral dose routes and will always substitute a calculated extrapolation ($C_{initial}$) as the zero time concentration for an intravenous dose. The value of $C_{initial}$ used for intravenous doses as well as the calculation of total areas for any dose route depends on the number of stripping terms used to describe data. This information is supplied using the Define Terms button at the end of the curve stripping process. Therefore, the curve area calculations will be accurate only *after* the Define Terms button is used. Changes on the Data Analysis sheet will not take effect until the Define Terms button is used.

5.4 Model-Independent Parameters

Data generated on the Curve Analysis sheet are read directly by “area”-based parameters in the single and multiple dose results tables. Parameters using AUC results, in part or in whole, are referred to as *model-independent* because they are computed without reference to or assumption about any specific compartmental model or kinetic behavior.

To quickly see which single and multiple dose parameters use area calculations, select the “Highlight model independent parameters” item from the drop-down listing at the top of the Single Dose PK sheet

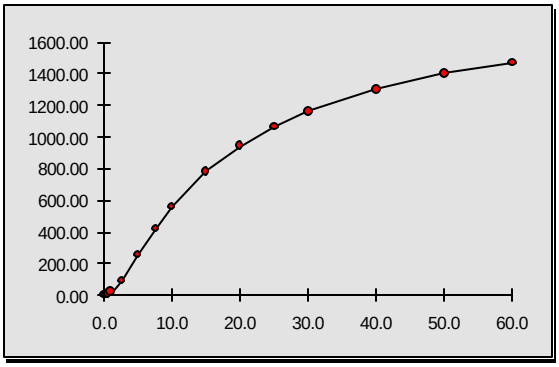
Curve Areas

Description: **Triexponential curve after oral dose (Gilbaldi and Perrier, p. 442)**

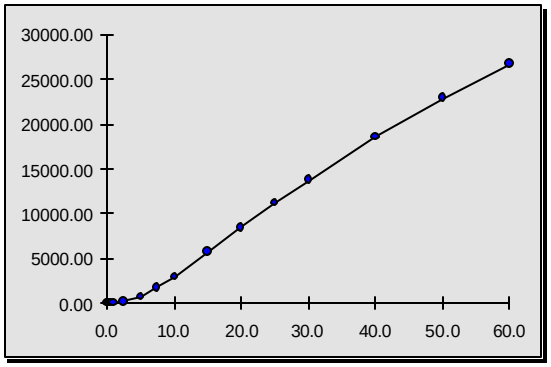
Trapezoid Calculation of AUC (µg-hr/ml)				Trapezoid Calculation of AUMC (µg-hr*hr/ml)			
Time (hr)	Conc (µg/ml)	Partial	Cumulative	Time (hr)	Time x Conc	Partial	Cumulative
0.0	0.0	0.00	0.00	0.0	0.0	0.00	0.00
0.1	4.7	0.24	0.24	0.1	0.5	0.02	0.02
0.3	13.2	1.79	2.03	0.3	4.0	0.44	0.47
0.5	20.8	3.40	5.43	0.5	10.4	1.44	1.90
1.0	36.3	14.28	19.70	1.0	36.3	11.68	13.58
2.5	61.4	73.28	92.98	2.5	153.5	142.35	155.93
5.0	68.1	161.88	254.85	5.0	340.5	617.50	773.43
7.5	61.1	161.50	416.35	7.5	458.3	998.44	1771.87
10.0	52.1	141.50	557.85	10.0	521.0	1224.06	2995.93
15.0	37.3	223.50	781.35	15.0	559.5	2701.25	5697.18
20.0	27.5	162.00	943.35	20.0	550.0	2773.75	8470.93
25.0	21.1	121.50	1064.85	25.0	527.5	2693.75	11164.68
30.0	16.9	95.00	1159.85	30.0	507.0	2586.25	13750.93
40.0	11.4	141.50	1301.35	40.0	456.0	4815.00	18565.93
50.0	8.2	98.00	1399.35	50.0	410.0	4330.00	22895.93
60.0	5.9	70.50	1469.85	60.0	354.0	3820.00	26715.93

AUC Summary		AUMC Summary	
Last conc. value	5.9 µg/ml	Last conc.-time value	354.0 µg-hr/ml
Last cum. AUC value	1470 µg-hr/ml	Last cum. AUMC value	26716 µg-hr*hr/ml
Remaining AUC(t-∞)	179 µg-hr/ml	Remaining AUMC(t-∞)	16185 µg-hr*hr/ml
AUC(0-∞)	1649 µg-hr/ml	AUMC(0-∞)	42901 µg-hr*hr/ml

Cumulative AUC



Cumulative AUMC



Curve Parameters:	Observed
Cmax	68.1 µg/ml
Tmax	5.0 hr

Figure 10. Curve Areas Worksheet Example

6. Single Dose PK Worksheet

6.1 Description

The Single Dose PK worksheet (Fig. 11) provides a table containing all the single dose pharmacokinetic parameters calculated by *PK Solutions*. The PK parameters are arranged in categories to facilitate comparison of results obtained using different computational methods for the same or similar parameters. Except for the last group, all parameters are calculated using model-independent methods. These fall into two different types, one based on graphical analysis using curve area measurements and the other based on simple linear pharmacokinetics assuming the form of a one to three term exponential expression (see section 3.2) For the sake of a limited comparison, a two-compartment model calculation is also included (last group). This a multi-functional worksheet capable of alternately displaying results obtained from curve fitting or from imported exponential terms with the click of a button. Just as easily, the sheet can switch between results for intravenous or oral dose routes. A drop-down box provides a number of display options to help you see which parameters are appropriate for various conditions. See chapter 8 for a detailed description of all formulas.

Single Dose PK Parameters — Current Data Type = 3 Terms Stripped Oral Data Set

Description: Triexponential curve after oral dose (Gilbaldi and Perrier, p. 442)

Select Number of Terms to Use → 3 terms | 1 | 2 | 3 | Show all data

Routes	Pharmacokinetic Parameters		Total Curve	E Phase	D/A Phase	A Phase	Notes
							Hints
	General disposition parameters:						
iv, oral	Dose Amount	µg	1.0				
iv, oral	Dosage	µg/kg	1.0				
oral	Fraction dose absorbed (F)		1.00				For oral doses, enter fraction of dose absorbed, if known. F=1 for iv doses.
iv, oral	Intercept	µg/ml		42.6	105.2	-147.0	For oral doses, the sign of the last coefficient (absorption phase) should be negative.
iv, oral	Slope	1/hr		0.0	-0.1	-0.2	Obtained by linear regression analysis of selected region on graph.
iv, oral	Rate	1/hr		0.0	0.2	0.4	Rate = 2.303 x slope
iv, oral	Half-life	hr		21.0	4.6	1.6	Time for concentration to diminish by one-half. Remains constant for 1st order kinetics.
	Descriptive curve parameters:						
iv	C initial (iv)	µg/ml	0.8	*42.6*	*105.2*	*-147.0*	Sum of intercepts. Applies to bi- & triexponential iv data only.
oral	Cmax (obs)	µg/ml	68.1				Maximum observed concentration (from data).
oral	Tmax (obs)	hr	5.0				Time at maximum observed concentration.
oral	Cmax (calculated)	µg/ml	n/a				Only applies to biexponential oral data with no lag time. Corrected for fraction absorbed.
oral	Tmax (calculated)	hr	n/a				Only applies to biexponential oral data with no lag time. See Multiple Dose sheet.
oral	Lag time	hr	n/a				Estimated lag time. Only applies to biexponential (one-compartment) oral data.
	Curve area calculations:						
iv, oral	AUC(0-t) (obs area)	µg-hr/ml	1469.9				Cumulative area under curve for experimental time points only: AUC(0-t)
iv, oral	AUC∞ (area)	µg-hr/ml	1649.0				The AUC∞ is the most widely used parameter of the curve area. Trapezoid rule used.
iv, oral	AUC∞ (expo)	µg-hr/ml	1643.4	*1292.0*	*694.1*	*-342.8*	AUC∞ (expo) = Σ (intercept ÷ rate). Based on sum of exponential terms.
iv, oral	% of AUC ∞ (expo)	%	100.0	*78.6*	*42.2*	*-20.9*	The percentage each term contributes to the area under the curve.
	Statistical moment calculations:						
iv, oral	AUMC ∞ (area)	µg-hr*hr/ml	42901.0				Based on trapezoid calculations (see Curve Areas sheet).
iv, oral	AUMC ∞ (expo)	µg-hr*hr/ml	43007.9	*39225.5*	*4582.0*	*-799.6*	Calculated from exponential terms.
iv, oral	% of AUMC∞ (expo)	%	100.0	*91.2*	*10.7*	*-1.9*	The percentage each term contributes to the AUMC area.
iv, oral	MRT (area)	hr	26.0				Mean Residence Time (time for 63.2% of administered dose to be eliminated).
iv, oral	MRT (expo)	hr	39.3	*30.4*	*6.6*	*2.3*	MRT calculated from exponential terms.
	Volume of distribution calculations:						
iv	Vc (initial central comp)	ml	1.3	0.0	0.0	1.3	Calculates initial volume of central compartment (iv data only). Based on exponentials.
iv, oral	Vd (obs area)	ml	0.0				Based only on observed data. Use when final slope tends to overestimate AUC∞
iv, oral	Vd (area)	ml	0.0				Vd based on AUC. Widely used value, but reflects Vd only during elimination phase.
iv, oral	Vd (area) / kg	ml/kg	0.0				Above value divided by body weight in kg.
iv, oral	Vd (expo)	ml	0.0				Vd calculated using exponential terms.
iv	Vss (area)	ml	0.0				Steady state calculation based on trapezoid calculation of curve areas.
iv	Vss (expo)	ml	0.0	0.0	0.0	0.0	Steady state calculation based on exponential terms.
	Clearance calculations:						CL applies to iv doses. Use for oral only when absorption is rapid and complete.
iv, oral	CL (obs area)	ml/hr	0.001				Systemic clearance based on observed data points: AUC(0-t).
iv, oral	CL (area)	ml/hr	0.001				Based on AUC¥. Operative during elimination phase. Assumes F=1 (use with iv dose).
iv, oral	CL (area) / kg	ml/hr/kg	0.001				Above value divided by body weight in kg.
iv, oral	CL (expo)	ml/hr	0.001	0.001	0.001	0.001	Clearance calculated from exponential terms.
	Additional calculations:						
iv	Half-life from Vd and CL	hr	21.0				Applies only to iv bolus data. Uses trapezoid area calculations.
	2-Compartment Open Model:						Calculation of 2-comp. open model microconstants after iv dose using exponentials.
iv	k12	1/hr	n/a		0.043		Transfer rate of drug to peripheral from central compartment.
iv	k21	1/hr	n/a		0.067		Transfer rate of drug from peripheral to central compartment.
iv	k10	1/hr	n/a		0.074		Elimination rate (usually considered from central compartment).

Figure 11. Single Dose PK Parameters Worksheet Example

6.2 Layout

The Single Dose PK and Multiple Dose PK sheets have basically the same layout. The first column (not included on the Multiple Dose sheet) indicates the appropriate or allowable dose route for the corresponding PK parameter. The second column indicates the PK parameter and its selected units. A parenthetical note gives a quick indication whether the parameter calculation is based primarily on observed ("obs") data point only, on trapezoidal ("area") data, or strictly employs exponential disposition constants ("expo").

Four central columns are used to display calculated results. The colored Total Curve column contains the final result for any type of computation. Where the final result involves the summing of individual terms, the intermediate calculation for each term is supplied in its respective column. All intermediate results are italicized to indicate their contributory status. Several parameters do not involve summation, but do give different results depending on the number of exponential terms used. These are displayed without italics to emphasize their final status for the term associated with the column in which they reside. Refer to section 3.2 for a discussion of the designations used in the column titles. An exception to the layout is the general disposition constants (e.g., slope, intercept and their derivatives). These define the term in which they reside. For all other parameters, *the Total Curve contains the final result for all calculations based on the number of term indicated in the drop-down box*. All three columns contain formulas, but only those columns that match the number of terms in the drop-down box contribute data to the final computation.

The number of terms, thus, the number of columns displaying and contributing data is set either by the user when completing the stripping procedure (Define Terms button) or by the program when the calculation mode is switched to link to the imported exponentials. In the latter case, the program detects the number of terms supplied in the input box. If your data (imported or stripped) contains three terms, the Single Dose sheet will be set for three terms automatically. You may override (reduce) the number of terms using the drop-down box to see what effect this has on the final results. However, if your setup yields only two terms, selecting a higher number is like asking the program to read and include non-existent data. All you will get is #VALUE or !DIV/0 messages.

The column of short notes is included to provide some guidance on the use or limitation of each parameter or parameter set.

6.3 Important Connections

The descriptive title, units of measurement, Dose Amount and dose weight are read from the Data Setup sheet. These elements would normally be added as part of the setup process, but can be added or changed at any time. Descriptive data can be changed without affecting any program calculations. Information about the Dose is used, in conjunction with the Fraction Dose Absorbed (F), primarily by the Vd and CL calculations.

The sheet is directly affected by several actions. First, the sheet is automatically setup for such items as the calculation mode, the dose route, the number of exponential terms to read and use, and the optimum display to present when first viewed by the action of running a new curve stripping procedure. This all takes place between starting the procedure with the New Data Set button and notifying the program you are finished by selecting the appropriate number of stripped terms on the Define Data button (located below the stripping modules).

Second, after the stripping procedure is finished and the PK parameters are set up, toggling the calculation mode on the Data Setup sheet will instantly switch the link to the alternate data source thereby instantly changing all affected calculations. When the mode is set for curve fitting, the sheet continuously reads data from the Data Analysis sheet and the Curve Areas sheet to supply the appropriate formulas. When the calculation mode is set to use the imported exponential terms, those formulas that use exponential terms immediately switch to the import box for their source of data. You can use this feature to compare the effect of stripped and imported terms on the parameter results.

Another consequence of the dynamic linking of formulas to source data is that changes made in the source data will be immediately reflected on the parameter results sheets. But this is true only when the calculation mode is set to read the area where you are making changes. For example, modifying the setup of the stripping modules on the Data Analysis sheet will immediately update the formulas reading this sheet, but only during or at the time the calculation mode is set for curve stripping.

Finally, changing the Data Setup dose route selection between oral and intravenous routes modifies some of the conditions for several single dose calculations. The effect of this selection is more pronounced when it is performed as part of a new data run since it sets up the sheet "behind the scenes" ready to open displaying data optimized for the conditions you set. Always ensure that the proper dose selection is made when switching between calculation modes. That is why the selection pads are close together and painted red.

Changing the Dose Amount. If you wish to recalculate the suites of single and multiple dose PK parameters using the same input data while modifying only the dose amount, follow this procedure: Note T1 and T2 for each module in the Data Analysis sheet. Change the dose amount on the Data Setup sheet and run the Analyze New Data button. Dial in the T1 and T2 values in the stripping modules as they were before, then click on the Define Terms button to indicate the terms stripped (which should be the same as previously set). You can use the Report sheet to capture the results before and after the change in dose amount.

6.4 Display Options

As mentioned, the Single Dose and Multiple Dose worksheets are designed to efficiently handle a number of different options including data sources, dose routes, calculation forms, and variable number of exponential terms. When you first open one of these results pages, its display characteristics are fully optimized according to the information you previous supplied. As you use the sheets for broader purposes, such as comparing results from different calculation modes or viewing data from both dose routes, you may wish to have more control over the display. This control is provided with the drop-down selections available in the Display Options box above the Notes column. The selections you make here affect both the Single Dose PK and Multiple Dose PK sheets. Table 1 describes each option and its effect. Note that input cells (indicated by red text) are not affected by any of these options.

6.5 Current Conditions Indicator

The header on both results pages (Single and Multiple Dose worksheets) includes an indicator that describes the current setup for both pages. The description states:

- The number of exponential **Terms** currently selected and operating in the formulas and results.
- Whether the current data source is set for **Stripped** or **Imported** results.
- Which of the dose routes, **Oral** or **IV**, are operative for the calculations displayed.
- And, again, the data source as to whether it originates from the **Data Set** or the Imported **Exponentials** box on the Data Setup sheet.

Table 1. Description of Display Options for Single and Multiple Dose Sheets.

Show all data	Blackens grayed values so that all parameter results are visible.
Show oral dose data only	Dims results associated only with iv dose routes.
Show iv dose data only	Dims results associated only with oral dose routes.
Dim values based on trapezoid curve areas	Dims all results that use trapezoid area estimates (AUC, AUMC) in the formulas. Useful for differentiating between “area” and “expo” based data.
Color data connected to imported exponentials	Colors all values that are computed solely from disposition constants. This color-codes data from both the Imported Exponentials box and the curve fitting modules.
Highlight model-independent parameters	Highlights (bolds) the names of parameters that depend solely or in part on observed data or trapezoid area information for their results. This option can be active simultaneously with any other option. Repeating the selection toggles the highlight on and off.
Reset all	Removes all options (including the highlight) and displays all values.

7. Multiple Dose PK Worksheet

7.1 Description

The Multiple Dose PK worksheet operates as an extension to the Single Dose PK worksheet. It draws on the pharmacokinetic results calculated using single dose information and combines these with the user's input of a multiple dosing interval (*tau*) to compute and graph estimates of multiple dose pharmacokinetics parameters. The dynamic graphs provide instant feedback of the effects of the dose interval on blood level profiles and pharmacokinetics. In general, the ease of use, flexible analysis and dynamic nature of *PK Solutions* makes it an excellent tool to help grasp the principles underlying both single and multiple dose pharmacokinetics. All of the discussion about the Single Dose PK sheet in the previous chapter pertains as well to this sheet, since they were designed to be complimentary, and will not be repeated here.

7.2 Assumptions

The multiple dose calculations make some rather simplistic assumptions that are still quite tenable for most drug situations. The main limitation for the current version of *PK Solutions* is that the dose interval must be regular and equal. The calculations do not support unequal or mixed dose regimens. Other assumptions are that the drug behavior is characterized by linear pharmacokinetics, the repeated doses are administered during the post-distributive or elimination phase, and that each dose act independently in an identical fashion without altering absorption rates or systemic clearance.

7.3 Layout and Features

The initial configuration and display of the Multiple Dose sheet are preset according to the information supplied in your earlier interaction with the program. When you first open the sheet it will present a view similar to Figure 12, if the oral dose route is selected, or similar to Figure 13, if the iv route is in effect. Control over displays of the data values and the columns of terms are controlled by options available on the Single Dose sheet and effect both sheets simultaneously.

Unique to the Multiple Dose sheet is the set of configuration buttons located in the upper right side of the Notes column. This header region is common to both dose route options and stays in view allowing easy access for making changes to the dose interval input cell. The configuration buttons control whether the oral or iv results table is attached to the header, and which graph is placed near the top for convenient access when viewing the effect of changing the dose interval. If you need to read data behind a graph, click the "Move graphs" and they will be repositioned to the side. Alternately, graphs can be moved by selecting and dragging them.

The Ad Hoc Intravenous Calculations section provides a work area for performing a number of calculations. Starting a New Data Set resets the load dose to zero and presets *tau* to the half-life. An error message will warn if the dose interval is set below Tmax for oral doses.

Multiple Dose PK Parameters

Current Data Type = 3 Terms Stripped Oral Data Set

Description: Triexponential curve after oral dose (Gilbaldi and Perrier, p. 442)

Multiple Dosing Parameters		Total Curve	E Phase	D/A Phase	A Phase	Notes
						Red type indicates cells for manual data entry.
Dose interval (tau)	hr	**21.06**				Enter interval between doses.
Elimination half-life	hr	21.04				
Ratio of dose interval to half-life		1.00				Show iv data. Show oral data.
Dose Amount	µg	1.00				Get iv graph. Get oral graph. Move graphs.
Multiple Oral Dose Calculations						
First dose concentration values:						
C1(max)	µg/ml	68.1				Maximum concentration reached after first dose (Cmax from data)
C1(min)	µg/ml	25.6	*21.3*	*4.3*	*0.0*	Minimum concentration reached after first dose (calculated).
C1(ave)	µg/ml	46.0	*30.7*	*31.6*	*-16.3*	Based on average of the calculated AUC∞ (expo); not geometric average.
Prediction of steady state parameters:						Estimates based on constant dose intervals during elimination phase.
Css(max)	µg/ml	105.4				Maximum concentration during dosing interval at steady state.
Css(min)	µg/ml	46.9				Minimum concentration during dosing interval at steady state.
Css(max)-Css(min)	µg/ml	58.6				Peak to trough concentration difference at steady state.
Css(ave)	µg/ml	78.0	*61.3*	*33.0*	*-16.3*	Average concentration at steady state based on exponentials.
Css(ave) (area)	µg/ml	78.3				Based on dose interval and AUC∞ (area). Independent of absorption rate.
Accumulation factors:						
R based on Css(min)/C1(min)		2.0				Accumulation factor based on elimination rate constant.
R based on Css(ave)/C1(ave)		1.7				Accumulation ratio based on individual calculations.
Additional oral dose calculations:						
Tmax (1st dose, observed)	hr	5.0				Observed Tmax from data set (if applicable).
Tmax (1st dose, calculated)	hr	6.5			6.5	Calculated Tmax at for first dose.
Tmax(ss)	hr	4.7		7.4	4.7	Calculated Tmax at steady state. Cmax peaks earlier at steady state.

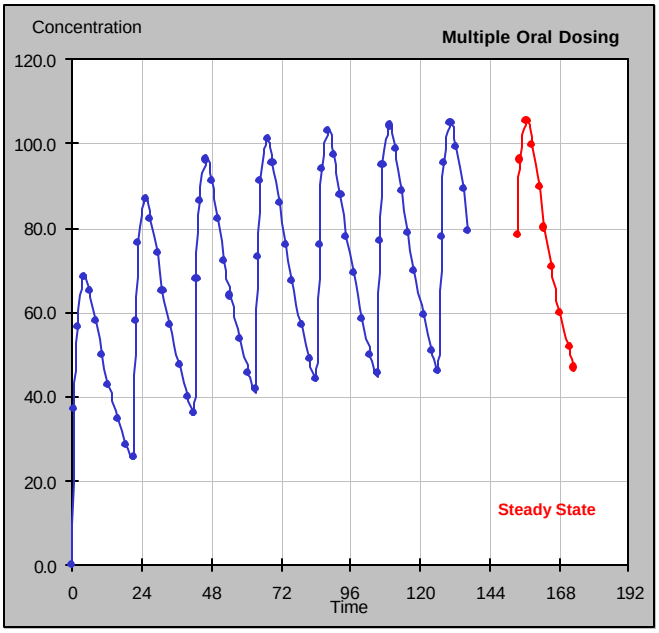


Figure 12. Multiple Dose PK Worksheet Illustrating Oral Dose Display Mode

Multiple Dose PK Parameters

Current Data Type = Imported IV Exponentials

Description: Triexponential curve after intravenous dose (Notari, p. 56)

Multiple Dosing Parameters		Total Curve	E Phase	D/A Phase	A Phase	Notes
						Red type indicates cells for manual data entry.
Dose interval (tau)	hr	**3.00**				Enter interval between doses.
Elimination half-life	hr	9.02				
Ratio of dose interval to half-life		0.33				
Dose Amount	µg	1.00				

Show iv data. | Show oral data.

Get iv graph. | Get oral graph. | Move graphs.

Multiple IV Dose Calculations						
						All parameters based on exponentials except where noted by "(area)".
First dose concentration values:						
C1(max)	µg/ml	50.0	*13.7*	*11.0*	*25.3*	Maximum concentration reached after first dose. Same as $C_{initial}$.
C1(min)	µg/ml	13.9	*10.9*	*3.0*	*0.0*	Minimum concentration reached after first dose.
C1(ave)	µg/ml	20.9	*12.2*	*6.2*	*2.5*	Based on average of the calculated AUC∞ (expo); not geometric average.
Prediction of steady state parameters:						Steady state is when rate of supply equals rate of loss.
Css(max)	µg/ml	106.9	*66.5*	*15.1*	*25.3*	Maximum concentration during dosing interval at steady state.
Css(min)	µg/ml	57.0	*52.8*	*4.1*	*0.0*	Minimum concentration during dosing interval at steady state.
Css(max)-Css(min)	µg/ml	50.0	*13.7*	*11.0*	*25.3*	Peak to trough concentration difference at steady state.
Css(ave)	µg/ml	70.4	*59.4*	*8.5*	*2.5*	Average concentration at steady state based on exponentials.
Css(ave) (area)	µg/ml	549.7				Css(ave) based only on dose interval and AUC∞ (area).
Vss	ml	0.1	0.1	0.1	0.1	Volume of distribution at steady state calculated from exponential terms.
Vss (area)	ml	0.0				Volume of distribution at steady state calculated using curve area.
Accumulation factors:						Three common expressions for estimating accumulation in the body.
R based on Css(max)/C1(max)		2.1				R is referred to as the "accumulation factor".
R based on Css(min)/C1(min)		4.1				R is defined in terms of the ratio of various steady state concentrations
R based on Css(ave)/C1(ave)		3.4				to various first dose concentrations, as indicated.
Time to reach % of steady state conc.:						Estimates based on data exhibiting monoexponential behavior.
To reach 95% Css(ave)	hr	39.0				The time required depends only on the half-life and is
To reach 99% Css(ave)	hr	59.9				independent of the dose frequency.
Ad Hoc Intravenous Calculations						
Loading dose to achieve Css(min):						Calculation assumes single dose follows monoexponential behavior
Calculated loading dose	µg	5				or that all doses are given in the postdistributive phase.
Input loading dose for graph	µg	**0**				Input the calculated or any loading dose here to see its effect on the graph.
Dose-based calculations:						
Number of doses (N) given		**5.0**				
Total time through Nth dose	hr	15.0				Total time from beginning of first dose to administration of Nth dose.
C(ave) during Nth dose	µg/ml	51.6	*40.6*	*8.5*	*2.5*	Average concentration during the Nth dosing interval.
Fraction of Css(ave) after N doses		0.7	0.7	0.7	0.7	Fraction of the average steady state concentration reached after N doses.
Postadministration decline:						
Time after last dose	hr	**1.0**				Enter time elapsed after last dose.
Css at t after ss dose	µg/ml	72.3	*61.6*	*9.8*	*0.9*	Concentration at any time (t) during any dosing interval at steady state.
Conc. at any time and dose	µg/ml	52.8	*42.1*	*9.8*	*0.9*	Calculates concentration at specified time (t) after any specified dose (N).

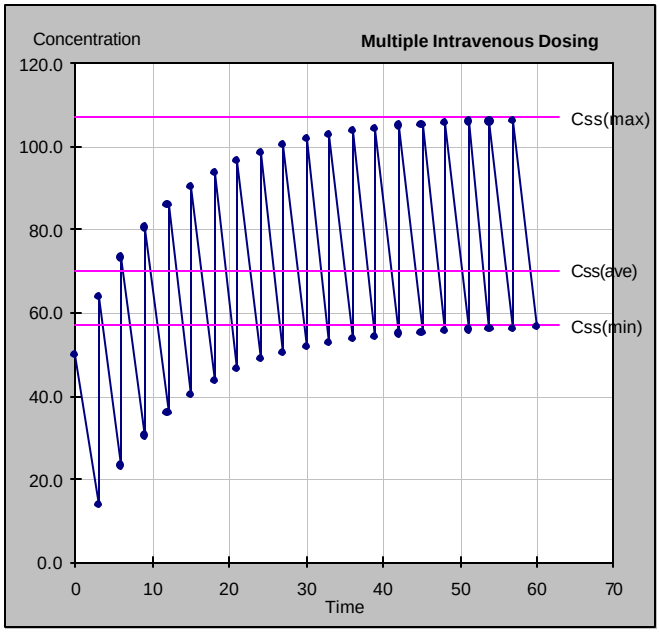


Figure 13. Multiple Dose PK Worksheet Illustrating Intravenous Dose Display Mode

8. Formulas

Single Dose Pharmacokinetics

General Disposition Parameters and Constants — Single Dose Pharmacokinetics

Dose Amount

D

Fraction of dose absorbed.
Used to correct dose amount for some oral dose calculations.

F

Exponential expression for 1st order kinetics.
(N49, eq. 1)*

$C = \sum C_n e^{-\lambda_n t}$
for *n* exponential terms

Y-Intercept. Coefficient of each exponential term. Note: the sign of the absorption coefficient is negative.

C_n

Slope

$s = \frac{-\lambda_n}{2.303}$

Rate constant

$\lambda_n = -2.303s$

Elimination rate constant

λ_z

Half-life

$t_{1/2} = \frac{0.693}{\lambda_n}$

* References are provided for most equations. The reference notation consists of the last initial of the first author (from the Bibliography), followed by the page number and the equation number, if supplied. For consistency, we have tried to use Gibaldi and Perrier wherever possible for formula references.

Descriptive Curve Parameters **Single Dose Pharmacokinetics**

$C_{initial}$
Initial concentration extrapolated to time zero for i.v. dose.
(G51, eq. 2.12)

$$C_0 = \sum C_n$$

Cmax (obs)
Applies to oral doses only.

$$C_{max} = \textit{maximum observed conc}$$

Tmax (obs)
Applies to oral doses only.

$$T_{max} = \textit{time point at } C_{max}$$

Cmax (calculated)
For biexponential oral data only.
(G38, eq. 1.110)

$$C_{max} = \frac{FD}{V} e^{-l_z t_{max}}$$

where V is Vd (area).

Tmax (calculated)
For biexponential oral data only.
(G38, eq. 1.110) (W171, eq. 11.19)

$$t_{max} = \frac{2.303}{l_a - l_z} \log \frac{l_a}{l_z}$$

where l_a and l_z are the apparent absorption and elimination rate constants, respectively.

Lag time
For biexponential oral data only.
(G36)

$$t_{lag} = \frac{\log C_a - \log C_z}{\left(\frac{l_a}{2.303} - \frac{l_z}{2.303} \right)}$$

where l_a and l_z are the apparent absorption and elimination rate constants, respectively.

Curve Area Calculations — Single Dose Pharmacokinetics

AUC(0-t) (obs area)
Trapezoid calculation of AUC using observed data points only (not extrapolated to infinity). Useful when final concentration values tend to exaggerate calculated AUC_∞
(G447, eq. D.7)

$$AUC_{(0-t)} = \sum_{i=0}^{n-1} \frac{t_{i+1} - t_i}{2}(C_i + C_{i+1})$$

where n is the number of data points.

AUC¥ (area)
Total AUC computed by combining AUC(0-t) with an extrapolated value.
(G448)

$$AUC_\infty = AUC_{(0-t)} + \frac{C_n}{l_z}$$

where C_n is the last concentration.

AUC¥ (expo)
Total AUC computed using exponential terms.
(N67, eq.21)

$$AUC_\infty = \sum \frac{C_n}{l_n}$$

% of AUC¥ (expo)
Percent each exponential term contributes to the total AUC.

$$\%\,AUC_\infty = 100\frac{(C_n / l_n)}{AUC_\infty}$$

Statistical Moment Calculations — Single Dose Pharmacokinetics

AUMC¥ (area)
Calculation of total area under the first-moment curve (plot of $C \bullet t$ vs t) by combining trapezoid calculation of $AUMC_{(0\text{-}t)}$ and extrapolated area.
(R482)

$$AUMC_{(0-t)} = \sum_{i=0}^{n-1} \frac{t_{i+1} - t_i}{2}(C_i t_i + C_{i+1} t_{i+1})$$

$$+ \frac{C_{last} \cdot t_{last}}{l_z} + \frac{C_{last}}{l_z^2}$$

AUMC¥ (expo)
Total AUMC computed using exponential terms.

$$AUMC_\infty = \sum \frac{C_n}{l_n^2}$$

MRT (area)
Mean Residence Time calculated using trapezoid area calculations extrapolated to infinity.
(G410)

$$MRT = \frac{AUMC_{\infty}}{AUC_{\infty}}$$

where both area terms use trapezoidal calculations.

MRT (expo)
Mean Residence Time calculated using exponential terms.

$$MRT = \sum \frac{1}{l_n}$$

Volume of Distribution Calculations — Single Dose Pharmacokinetics

Vc (initial central compartment)
Apparent volume of the central compartment for i.v. doses only.
(G52, eq. 2.15) (G214, eq 5.69)

$$V_c = \frac{D}{\sum C_n}$$

Vd (obs area)
Apparent volume of distribution based on $AUC_{(0\text{-}t)}$ trapezoid calculation and elimination rate. Use when AUC_{∞} (area) is exaggerated due to high last concentration.
(G36, eq. 1.101) (G212, eq. 5.62)

$$V = \frac{FD}{AUC_{(0-t)}\, l_z}$$

Vd (area)
Apparent volume of distribution based on trapezoid AUC_{∞} (area) and elimination rate. Applies mainly to i.v., but also to oral if complete absorption (F=1) is assumed.
(G36, eq. 1.101)(G212, eq. 5.62)

$$V = \frac{FD}{AUC_{\infty}\, l_z}$$

Vd (area) / kg
Apparent volume of distribution normalized by animal weight. Uses same formula as Vd (area).

$$V_{normalized} = \frac{V}{Body\,Weight\ (kg)}$$

Vd (expo)
Apparent volume of distribution calculated from exponential terms.

$$V = \frac{FD}{l_z \sum \frac{C_n}{l_n}}$$

where l_z is the elimination rate

Vss (area)
Apparent volume of distribution at steady state estimated graphically from trapezoidal total area measurements. Applies to iv dose. (G215, eq. 5.72) (N76, eq. 39)

$$V_{ss} = \frac{D \cdot [AUMC_\infty]}{[AUC_\infty]^2}$$

Vss (expo)
Apparent volume of distribution at steady state estimated from exponential terms. Applies only after iv and assumes elimination from central compartment.
(G215, eq. 5.70) (N76, eq, 36)

$$V_{ss} = D \cdot \frac{\sum \frac{C_n}{l_n^2}}{\left(\sum \frac{C_n}{l_n}\right)^2}$$

Systemic Clearance Calculations — Single Dose Pharmacokinetics

CL(sys) (obs area)
Systemic clearance based on $AUC_{(0\text{-}t)}$ trapezoid calculation. Use when total AUC_∞ (area) is exaggerated due to high last concentration.
(G36, eq. 1.100) (G212, eq. 5.62)

$$CL = \frac{FD}{AUC_{(0-t)}}$$

CL (area)
Systemic clearance based on trapezoid AUC_∞ (area). Applies mainly to i.v. data. Limited to oral data only if complete absorption (F=1) is assumed.
(G36, eq. 1.100)(N79, eq. 50)

$$CL = \frac{FD}{AUC_\infty}$$

CL (area) / kg
Systemic clearance normalized by animal weight. Uses same formula as CL (area).

$$CL_{normalized} = \frac{CL}{BodyWeight\ (kg)}$$

CL (expo)
Systemic clearance calculated using exponential terms.
(R304)

$$CL = \frac{FD}{\sum \frac{C_n}{l_n}}$$

Half-life based on Vd and CL
Alternate calculation of half-life using V (area) and CL (area). For i.v. data only.

$$t_{½} = \frac{0.693 \cdot V}{CL}$$

Two-compartment Open Model Microconstants — Single Dose Pharmacokinetics

k12
Microconstant calculated using exponentials. Applies to 2 term i.v. dose data only (DA and E modules).
(N115, eq.122)

$$k_{12} = l_1 + l_z - k_{21} - k_{10}$$

k21
Microconstant calculated using exponentials. Applies to 2 term i.v. dose data only.
(N115, eq.120)

$$k_{21} = \frac{C_1 l_z + C_z l_1}{C_1 + C_z}$$

k10
Microconstant calculated using exponentials. Applies to 2 term i.v. dose data only.
(N115, eq.121)

$$k_{10} = \frac{l_1 l_z}{k_{21}}$$

Multiple Intravenous Dose Pharmacokinetics

General — Multiple IV Dose Pharmacokinetics

Dose Interval (tau)
Time span between dosing intervals. Distinguish from time after dose (*t*).

t Constant dose interval
.

First Dose Concentration Calculations — Multiple IV Dose Pharmacokinetics

C1(max)
Maximum concentration after first dose interval (*t*). Equal to $C_{initial}$
(G114 eq 3.1)

$$C_{1(\max)} = \sum C_n$$

C1(min)
Minimum concentration at end of first dose interval (*t*).
(G114, eq. 3.2)

$$C_{1(\min)} = \sum C_n e^{-l_n t}$$

C1(ave)
Average concentration during first dose interval (*t*).
(G122, eq. 3.37)

$$C_{1(ave)} = \sum \frac{C_n (1 - e^{-l_n t})}{l_n t}$$

Prediction of Steady State Parameters — Multiple IV Dose Pharmacokinetics

Css(max)
Maximum concentration during any dosing interval at steady state. Included on graph. (G117, eq.3.20)

$$C_{ss(\max)} = \sum \frac{C_n}{1 - e^{-l_n t}}$$

Css(min)
Minimum concentration during any dosing interval at steady state. Included on graph. (G117, eq.3.21)

$$C_{ss(\min)} = \sum \frac{C_n e^{-l_n t}}{1 - e^{-l_n t}}$$

Css(max) - Css(min)
Difference between peak and trough concentration during steady state.

$$\Delta_{\overline{C}_{ss}} = C_{ss(\max)} - C_{ss(\min)}$$

Css(ave)
Average concentration at steady state. Included on graph.
(G122, eq. 3.38)

$$\overline{C}_{ss} = \sum \frac{C_n}{l_n t}$$

Css(ave) (area)
Average concentration at steady state calculated from trapezoidal AUC data for a single dose
(G134)

$$\overline{C}_{ss} = \frac{AUC_{\infty}}{t}$$

Accumulation Factors — Multiple IV Dose Pharmacokinetics

R based on Css(max)/C1(max)
Accumulation ratio based on maximum concentrations after first dose and at steady state.
(G121, eq. 3.34)

$$R = \frac{C_{ss(\max)}}{C_{1(\max)}}$$

R based on Css(min)/C1(min)
Accumulation ratio based on minimum concentrations after first dose and at steady state.
(G121, eq. 3.31)

$$R = \frac{C_{ss(\min)}}{C_{1(\min)}}$$

R based on Css(ave)/C1(ave)
Accumulation ratio based on average concentrations after first dose and at steady state.
(G122, eq. 3.40)

$$R = \frac{\overline{C}_{ss}}{C_{1(ave)}}$$

Time to Reach Percent of Steady State — Multiple IV Dose Pharmacokinetics

To reach 95% Css(ave)
Time required to reach 95% of average steady state concentration. Assumes one-compartment characteristics apply. (G124, eq.3.48)

$$t_{0.95\overline{C}_{ss}} = -3.32 \cdot t_{1/2} \cdot \log(1 - f_{ss})$$

where f_{ss} is the fraction of the steady state concentration.

To reach 99% Css(ave)
Time required to reach 95% of average steady state concentration. Assumes one-compartment characteristics apply. (G124, eq.3.48)

$$t_{0.99\bar{C}_{ss}} = -3.32 \cdot t_{½} \cdot \log(1 - f_{ss})$$

where f_{ss} is the fraction of the steady state concentration.

Ad Hoc Calculations — Multiple IV Dose Pharmacokinetics

Calculated loading dose
Loading dose required to produce an immediate steady state minimum concentration, Css(min).
(G128, eq. 3.57)

$$D_{load} = \frac{D}{1 - e^{-l_n t}}$$

Total time through Nth dose
Total time elapsed between first dose (t=0) and specified dose (N).

$$t_N = Nt$$

C(ave) during Nth dose
Average concentration during any dose interval (N). Becomes Css(ave) when steady state reached.
(G122, eq. 3.37)

$$\bar{C}_N = \sum \frac{C_n(1 - e^{-Nl_n t})}{l_n t}$$

Fraction of Css(ave) after N doses
Fraction of the ultimate average steady state concentration reached after N doses.
(G123, eq. 3.43)

$$f_{ss} = \frac{\sum C_n(1 - e^{-Nl_n t})/l_n}{\sum C_n / l_n}$$

where f_{ss} is the fraction of the steady state concentration.

Css at t after ss dose
Steady state concentration at any time (*t*) during a dosing interval at steady state.
(G117, eq. 3.19)

$$C_{ss} = \sum \frac{C_n e^{-l_n t}}{1 - e^{-l_n t}}$$

Conc. at any time and dose
Computes the concentration at any time during a dosing interval. Enter both time (t) and dose interval (N).
(G116, eq. 3.18)

$$C_N = \sum \frac{C_n(1 - e^{-Nl_n t})}{1 - e^{-l_n t}} \cdot e^{-l_n t}$$

Multiple Oral (Extravascular) Dose Pharmacokinetics

General and Graphing Function — Multiple Oral Dose Pharmacokinetics

Dose Interval (tau)
Constant time span between dosing intervals. Distinguish from time after dose (*t*).

t Assume equal dose intervals

Graphing Function
The graphing function is based on a mathematical generalization of the graphical superimposition principle. It involves the addition of a decay function (C_N) to the initial concentration (C_1) at repeated time points for a progressive series of doses (N). Assumes constant dose intervals given during the postdistribution phase.
(G457, eq. E.5)

$$C_{(N,t)} = C_{1(t)} + C_{N(t)}$$

where

$$C_{1(t)} = \sum C_n e^{-\lambda_n t}$$

and

$$C_{N(t)} = \frac{C_z e^{-\lambda_z t}(1 - e^{-(N-1)\lambda_z t})e^{-\lambda_z t}}{1 - e^{-\lambda_z t}}$$

First Dose Concentration Values — Multiple Oral Dose Pharmacokinetics

C1(max)
Observed maximum concentration taken from data set.

$$C_{\max}$$

C1(min)
Minimum concentration at end of first dose interval (*t*).
(G114, eq. 3.2)

$$C_{1(\min)} = \sum C_n e^{-\lambda_n t}$$

C1(ave)
Average concentration during first dose interval (*t*).
(G122, eq. 3.37)

$$C_{1(ave)} = \sum \frac{C_n (1 - e^{-\lambda_n t})}{\lambda_n t}$$

Prediction of Steady State Parameters — Multiple Oral Dose Pharmacokinetics

Css(max)
Computed from a simplification of the graphing function to a steady state form as shown. The Css(max) is evaluated as the maximum concentration during the steady state dosing interval.
(G457, eq. E.6)

$$C_{ss(t)} = C_{1(t)} + \frac{C_z \cdot e^{-l_z t} \cdot e^{-l_z t}}{1 - e^{-l_z t}}$$

where

$$C_{1(t)} = \sum C_n e^{-l_n t}$$

Css(min)
Computed using same steady state equation as Css(max) and evaluating the minimum concentration during a steady state dose interval.
(G457, eq. E.6)

Same as above.

Css(max) - Css(min)
Difference between peak and trough concentration during steady state.

$$\Delta_{\overline{C}_{ss}} = C_{ss(\max)} - C_{ss(\min)}$$

Css(ave)
Average concentration at steady state.
(G122, eq. 3.38)

$$\overline{C}_{ss} = \sum \frac{C_n}{l_n t}$$

Css(ave) (area)
Average concentration at steady state calculated from trapezoidal AUC data for a single dose
(G134)

$$\overline{C}_{ss} = \frac{AUC_{\infty}}{t}$$

Accumulation Factors — Multiple Oral Dose Pharmacokinetics

R based on Css(min)/C1(min)
Accumulation factor based on elimination rate constant.
(G387, eq. 10.1)

$$R = \sum \frac{1}{1 - e^{-l_z t}}$$

R based on Css(ave)/C1(ave)
Accumulation ratio based on average concentrations after first dose and at steady state. (G122, eq. 3.40)

$$R = \frac{\overline{C}_{ss}}{C_{1(ave)}}$$

Tmax (1st dose, observed)
Observed time of largest concentration value from data set.

$$t_{\max}$$

Tmax (1st dose, calculated)
Calculation of time at which maximum concentration occurs after a single dose. Applies to one-compartment characteristics, but calculated also to illustrate magnitude for 2-compartments.
(G142 eq. 1.106)

$$t_{\max_{calculated}} = \frac{2.303}{l_a - l_z} \log \frac{l_a}{l_z}$$

where l_a is the absorption rate and l_z is the elimination rate.

Tmax(ss)
Calculation of time at which maximum concentration occurs after dosing during steady state. Applies to one-compartment characteristics, but calculated also to illustrate magnitude for 2-compartments.
(G142, eq. 3.92)

$$t_{\max_{ss}} = \frac{2.303}{l_a - l_z} \log \frac{l_a(1-e^{-l_z t})}{l_z(1-e^{-l_a t})}$$

where l_a is the absorption rate and l_z is the elimination rate.

9. Report Worksheet

9.1 Description

The Report worksheet is located at the end of the several worksheets containing tables of the calculated pharmacokinetic results for a given data setup configuration. The purpose of the Report sheet is to provide an easy means for capturing, comparing, and arranging pharmacokinetic results prior to printing or exporting. Figure 14 provides a partial view of the layout of an example Report worksheet, including the three button options used to control the sheet contents and display. The Report sheet is very flexible providing user control over the number of result sets captured, their titles and arrangement in columns on the worksheet, and the specific parameters or groups of parameters to be displayed. The Report sheet can also be used to arrange results and then apply Excel's built-in statistics to individual parameters (rows). The Report sheet can be copied or exported to other workbooks or applications for more advanced statistical analysis, curve drawing, presentations or other purposes. In a teaching environment, the report sheet can serve as an assignment answer sheet.

Report [Select View] [Capture Data] [Rows & Colums]

Description:		Test Set of Oral Dose	Exponential Terms from Oral Data	Dose Interval 0.5 x half-life	Dose Interval 2 x half-life
Data Setup & Analysis					
Dose Route		Oral Dose	Oral Dose	Oral Dose	Oral Dose
Calculation Mode		Data Set	Imported	Imported	Imported
Terms Used		3 Terms	3 Terms	3 Terms	3 Terms
Multiple Oral Dose Parameters					
Dose interval (tau)	hr	**21.06**	**21.06**	**10.50**	**44.00**
Elimination half-life	hr	21.06	21.06	21.06	21.06
Ratio of dose interval to half-life		0.50	0.50	0.50	0.50
Dose Amount	µg	1.00	1.00	1.00	1.00
First dose concentration values:					
C1(max)	µg/ml	68.1	n/a	n/a	n/a
C1(min)	µg/ml	25.6	25.6	44.9	10.1
C1(ave)	µg/ml	46.1	46.0	55.2	30.5
Prediction of steady state parameters:					
Css(max)	µg/ml	105.5	105.5	145.5	79.9
Css(min)	µg/ml	46.9	46.9	104.2	13.2
Css(max)-Css(min)	µg/ml	58.6	58.6	41.3	66.6
Css(ave)	µg/ml	78.1	78.1	137.1	37.4
Css(ave) (area)	µg/ml	78.4	78.3	137.4	37.5
Accumulation factors:					
R based on Css(min)/C1(min)		2.0	2.0	3.1	1.3
R based on Css(ave)/C1(ave)		1.7	1.7	2.5	1.2
Additional oral dose calculations:					
Tmax (1st dose, observed)	hr	5.0	n/a	n/a	n/a
Tmax (1st dose, calculated)	hr	6.5	6.5	6.5	6.5
Tmax(ss)	hr	4.7	4.7	3.7	5.8

Figure 14. Partial View of an Example Report Worksheet

9.2 Control Options

Selection of the "Capture Data" button captures the current data setup configuration and calculated pharmacokinetic results and places these in the next available column. The results captured include all 87 calculations and parameters provided in the tables on the Single and Multiple Dose PK worksheets. Up to 253 result columns can be captured on one Report worksheet. The results that are captured are those that are current in the active PK Solutions workbook at the time the button is selected. The description of the captured data set (originally entered on the Data Setup sheet) is included at the top of each column. You can manually change this description to give some other title to any result column, if desired.

Of course, not all of the pharmacokinetic parameters tabulated are applicable to the current data set. For example, the intravenous dose parameters are not applicable when the oral dose route is selected. Two methods are available to control how the Report sheet displays captured results and distinguishes those which are appropriate. First, the captured results includes the format currently selected by the Display Options drop-down box on the Single Dose PK worksheet. As described earlier, the Display Options automatically gray out inappropriate results each time a data set is run. Thereafter, the display format can be changed to another Display Option selection at any time. When the "Capture Data" button is selected, the column of captured results retain the Display Options format currently selected.

The second method for controlling the parameter results displayed on the Report worksheet is through the menu options dialog box provided when the "Select View" button is clicked (Fig. 15). This menu allows you to select or deselect sections of results to tailor the types of results displayed. Deselecting a section of results does not remove them, it merely hides them temporarily from view. The Select View menu options can be changed at any time.

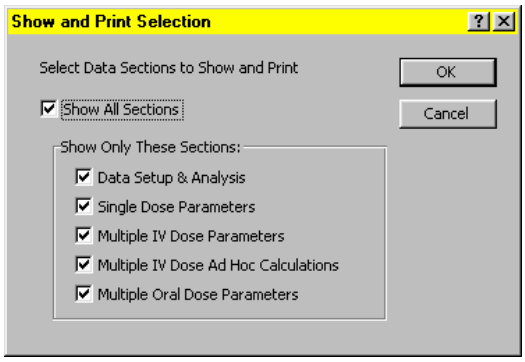


Figure 15. Select View Options for Report Worksheet

The "Row & Columns" button toggles on or off the display of rows and columns. Displaying the rows and columns allows you to easily modify the layout of the Report sheet prior to saving, copying, printing, or exporting. With the rows and column headers on, you can use Excel's built-in features to show or hide, insert or delete, or rearrange data rows and columns to suit your purposes.

Note: Sheet protection is not applied to the Report sheet. If you accidentally delete a row or column, use the Edit/Undo option immediately. Creating a *new* PK Solutions workbook from the original program template always refreshes the Report sheet to its initial condition with no captured data columns.

10. Test Data Worksheet

10.1 Description

A validation worksheet (Fig. 16) is provided that illustrates the results of applying *PK Solutions*' curve stripping and calculation procedures to seven oral (extravascular) and iv data sets taken from the literature See Chapter 11 for bibliography). Literature results are included (highlighted with red text) for comparison. The test results can be used to validate the reproducibility of *PK Solutions*, to corroborate the results with those taken from the literature, and to provide source data for training on the use of the stripping modules on the Data Analysis sheet.

A copy button accompanies each data set to automate copying the data to the Data Setup sheet. Upon confirming the copy command, the view is changed to the Data Setup sheet ready for you to continue with selection of the Dose Route and Calculation Mode.

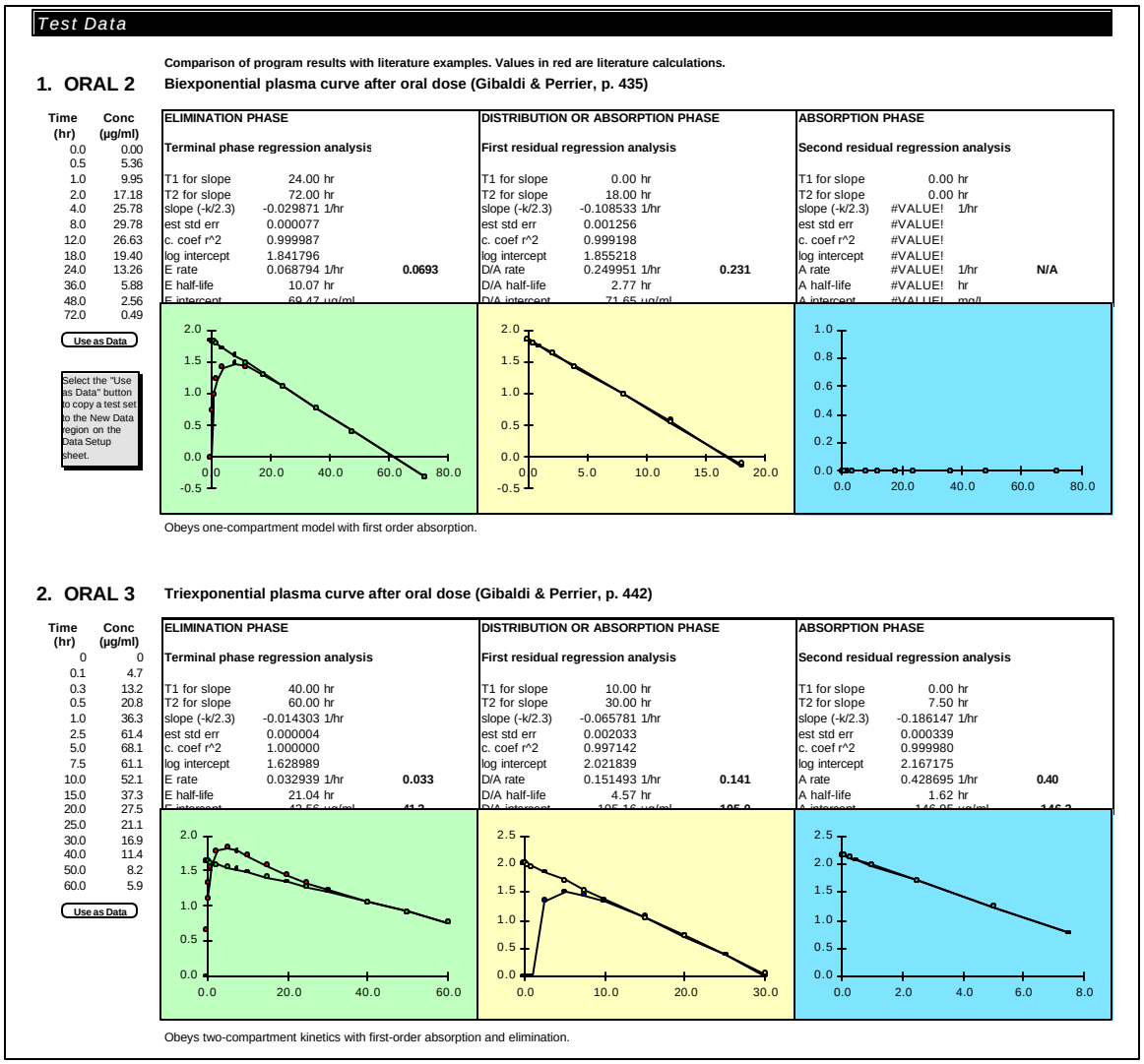


Figure 16. Illustration of Two of the Seven Test Sets Included on the Test Data Worksheet

10.2 Uses

The Test Data page serves three important functions:

Validation

Concentration-time values were taken from the literature reference cited. PK Solutions was run to compute the results indicated. The results can be compared with those presented in the literature, which are highlighted in bold red type. In all cases, the literature results validate the results obtained by PK Solutions within reasonable error.

Verification

The record of results obtained with prior runs can be used to verify the continuing reliability and accuracy of subsequent runs. This verification record is useful as a quality control measure to test PK Solutions on a maintenance schedule, as well as for performance between computers, operating systems, and versions of Microsoft Excel.

Training

The 7 data sets encompass and illustrate the most the most common variations in plasma level curves likely to be encountered. Below each data set is a button that moves the data set to the Data Setup sheet. Users can thus run through each or any of the seven test sets, comparing their Data Analysis sheet settings and final outcomes with those of the test data.

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