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Volume VII

DEVELOPMENT AND VALIDATION OF METHODS FOR APPLYING PHARMACOKINETIC DATA IN RISK ASSESSMENT

VOLUME VII OF VII: PBPK_SIM

Clement International Corporation
K.S. Crump Division
1201 Gaines Street
Ruston, LA 71270

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HARRY G. ARMSTRONG AEROSPACE MEDICAL RESEARCH LABORATORY
HUMAN SYSTEMS DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO 45433-6573

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TECHNICAL REVIEW AND APPROVAL

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The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

JAMES N. McDOUGAL, Maj, USAF, BSC
Deputy Director, Toxic Hazards Division
Harry G. Armstrong Aerospace Medical Research Laboratory

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Operating instructions for the use of the software provided (PBPK_SIM) are outlined and explained. PBPK_SIM procedures for installation, data entry, execution and interpretation are described. Topics also discussed are command files and applicable statistical considerations.

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FOREWORD

This report has been prepared by Clement International Corporation, K.S. Crump Division, for the Department of the Air Force, Harry G. Armstrong Aerospace Medical Research Laboratory, Wright Paterson Air Force Base in response to a request to investigate the incorporation of pharmacokinetic modeling into quantitative risk assessment. This report contains the results of this multiyear effort and reflects the changes in direction and priorities as this project has evolved. The Project Director was Dr. Kenny Crump and the Principal Investigator for this project was Mr. Bruce Allen; other investigators who provided technical support and internal peer review were Drs. Crump and Annette Shipp. Mr. Allen was assisted in the pharmacokinetic modeling and analyses primarily by Mr. Christopher Rambin and by Ms. Robinan Gentry. The sensitivity analyses were conducted by Mr. David Farrar, Dr. Crump, Dr. Richard Howe, and Mr. Allen. The software was developed by Ms. Cynthia Van Landingham, Mr. William Fuller, Mr. Eric Brooks, Dr. Howe, and Mr. Allen. The authors wish to acknowledge the support provided by Dr. Jeffery Fisher and Lt. Col. Harvey Clewell, who are at the Harry G. Armstrong Aerospace Medical Research Laboratory, Wright Paterson Air Force Base, and Drs. Melvin Andersen and Michael Gargas, formerly with the Harry G. Armstrong Aerospace Medical Research Laboratory and now with CIIT.

PREFACE

This volume consists of the documentation (manual) written to accompany the software package, rBPK_SIM. PBPK_SIM was designed to implement the type of uncertainty analysis that was discussed in Volume VI of this report. PBPK_SIM also facilitates the linking of PBPK modeling with dose-response (multistage) modeling, a vital link for PBPK-assisted risk assessment.

Accompanying this manual are diskettes that contain the PBPK_SIM source code and an executable program (PBPK_SIM.EXE). On Disk 1 is a dependency file (PBPK_SIM.MAK) which can be used with MICROSOFT'S MAKE to compile and link the PBPK_SIM program. The additional libraries and object files needed are listed in this file.

The diskettes also contain examples of a CSL file (PHNEW.CSL), an executable ACSL program (PHNEW.EXE), and a data file for use with PHNEW (TEST926.WPS). Files containing default values for human and animal physiological parameters (HUMAN.HUM, RAT.ANM and MOUSE.ANM) are also provided.

PBPK_SIM

Program written by
Richard Howe, Ph. D.
Cynthia Van Landingham, M.S.
Chris Rambin, M.S.
William Fuller, B.S.
Eric Brooks, B.A.

Introduction

PBPK_SIM is a software package that combines physiologically based pharmacokinetic (PBPK) modeling and dose-response modeling capabilities in the context of Monte Carlo simulation and uncertainty analysis. The purpose of PBPK_SIM is to allow the user to investigate the effects on model output of uncertainty or variability in input parameter values.

PBPK_SIM is accessed through a user-friendly windowed environment that prompts the user for commands. PBPK_SIM is linked to PBPK models defined through the software package called ACSL. The link is through data files, created through PBPK_SIM, that in turn create command files (CMD files) recognized by ACSL. The data files also tell ACSL how many model simulations to run.

When more than one model simulation is run, the input parameters to the PBPK model are allowed to vary. That variation is also defined in the data files, through certain probability distributions.

Monte Carlo simulation techniques are used to sample parameter values from the probability distributions associated with each parameter. For each set of parameter values (one set for each simulation) the PBPK model is run and selected output variables are stored. This can be done for seven groups of animals, differing with respect to dose level or route of exposure (i.e., for up to seven dosed groups), and for one additional exposure scenario, usually associated with a human pattern of exposure.

The output variable values obtained as a result of the PBPK model simulations can be examined. Simple descriptive statistics, such as the mean, median, variance, and percentiles, can be calculated for the sample of output variable values obtained.

The output variable values can also be used further to obtain risk estimates. In that case, those values would be used as dose values input into a dose-response model (a version of the multistage model selected by the user). The user-supplied response rates associated with a control group and the one to seven dosed groups (used in the generation of output variable values) constitute the rest of the input. The response rates may also be allowed to vary, in order to examine the impact of response-rate uncertainty on risk estimation.

Any risk estimates generated in this manner can also be summarized via calculation of the descriptive statistics described above.

Thus, PBPK_SIM is a package that works with the ACSL system. It contains a user-friendly front end to aid the user in the definition of command files that ACSL can recognize. It automates the running of several PBPK model simulations, where the dose inputs or parameter values can change from simulation to simulation. It links the PBPK modeling results to dose-response modeling. Finally, PBPK_SIM calculates statistics to describe the variability (uncertainty) associated with any user-selected output variables, whether those are dose surrogates (variables from the PBPK model) or risk estimates.

Contents

Requirements and Installation

For the proper execution of the PBPK_SIM program, the following hardware and software are required:

IBM PC, PC/XT, PC AT, PS/2, or 100 % compatible with a numerical co-processor chip and one of the following minimal configurations of disk drives:

1) dual 720K, 1.44M, or 1.2M diskette drives or
2) a hard disk and one diskette drive (any type).

RAM requirements:	Depend largely on the number of simulations desired and the number of output variables selected. The minimum is approximately 500K.
Graphics adapter:	CGA, EGA, PGA, MCGA, VGA, or Hercules.
Software:	MS-DOS or PC DOS 3.0 or greater ACSL Version 8P1/MS

PBPK_SIM is designed to run on either a color or monochrome monitor system. When PBPK_SIM is loaded, a check is performed to determine the type of graphics card being used. If the computer is configured with a color graphics card and monochrome monitor, PBPK_SIM attempts to operate as if a color monitor is attached. The graphics card check may be over-ridden by invoking PBPK_SIM in the following way.

PBPK_SIM *flag*

where *flag* is c indicating a color monitor is to be used or m indicating a monochrome monitor is to be used.

PBPK_SIM's Design

PBPK_SIM was designed on an IBM PC AT, an IBM PS/2 Model 80, and an IBM PS/2 Model 70. The user interface is written in the C language using the Microsoft C Compiler (Version 6.0). Windowing and data-entry functions were provided by the Vitamin C package produced by Creative Programming. The programs used for determining the models fit are written in FORTRAN using Microsoft's FORTRAN Compiler (Version 5.0). Simulation is performed using ACSL Version 8P1/MS.

Installing PBPK_SIM

1) Place the PBPK_SIM Program diskette #1 in a floppy disk drive and make it the current drive by typing the following command at the DOS prompt.

n:

where n is the drive letter of the active diskette drive.

2) With the prompt now indicating the active floppy drive, type

n:\> **INSTALL m**

where m is the letter of the drive on which PBPK_SIM is to be installed.

The INSTALL program will create a subdirectory called PBPK_SIM on the m drive and copy all necessary files into that subdirectory.

3) Type the following command:

TYPE C:\CONFIG.SYS

If a line similar to the following

DEVICE=ANSI.SYS

is not displayed (there may be a drive and path specification between the equal sign and ANSI.SYS), you must edit your CONFIG.SYS file (with any editor that can load and save in ASCII format). A DEVICE=*drive:\path*\ANSI.SYS statement must be added to the CONFIG.SYS so that the Dos Shell option will function correctly. If ANSI.SYS is in the root directory of your boot disk, *drive* and *path* are not required. Otherwise, replace them with the actual drive and path for ANSI.SYS.

File structure

The following is a brief description of the files provided with or produced by the PBPK_SIM program.

PBPK_SIM.DFT	contains the default values that are used by the PBPK_SIM program.
PBPK_SIM.EXE	PBPK_SIM executable file.
*.CSL	ACSL CSL files.
*.EXE	compiled ACSL CSL files.
*.RR?	files created by the sImulation runs for use when creating Dose and Risk statistics. One file for each dosed group, will be created using the numerals 1-7 to replace the ?. An RRH file will be created for the human doses.
*.WPS	PBPK_SIM input data files.
*.HUM	files containing default values for human parameters used by the data files.
*.ANM	files containing default values for animal parameters used by the data files.
*.WCC	one file for each CSL and EXE combination. Controls which WPS files belong with which CSL files.

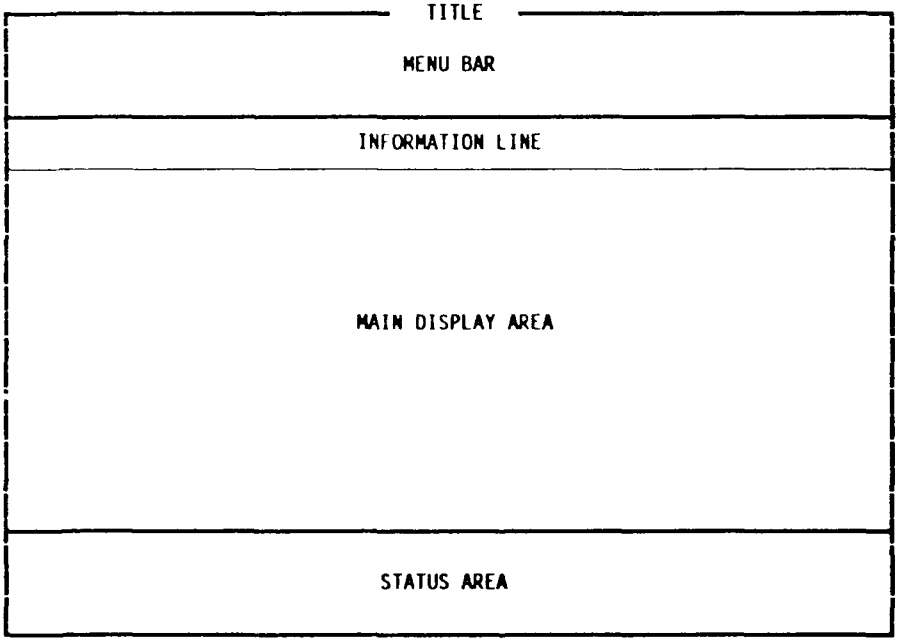
Windowing, Selection Sets, and Data Entry

Windowing

PBPK_SIM uses windows for accepting and displaying information. Commands available in a window are listed (either horizontally or vertically) and are invoked by positioning the cursor on the command and pressing ENTER or by typing the letter highlighted in the command name. Thus, the user is not required to remember or type the commands.

PBPK_SIM is designed to help the user avoid errors in data entry by not accepting invalid entries (letters in place of numbers, or vice-versa), numbers that are out of range, or a value that is logically inconsistent. When the program recognizes an error, a message is displayed in the STATUS AREA or in a pop-up window showing the error type and the cursor is not allowed to leave the field or the entry window will not close until the error is corrected.

All of the main windows in the PBPK_SIM program are based upon the window design seen here:



Title — shows the location of the program by supplying the name of the window being executed.

Menu Bar — displays the other windows or other functions that can be accessed from the current window. The following keys are active when the selection cursor is on the menu bar:

Enter	transfer control to window or function the selection cursor is highlighting.
Left Arrow	move selection cursor one name left. If the selection cursor is on the left-most selection, the cursor wraps around to the right-most selection.
PgDn	allows the user to access information in the main display area. If the window contains no information in this area, this key is not active.
Right Arrow	move selection cursor one name right. If the selection cursor is on the right most selection, the cursor wraps around to the left-most selection.
highlighted letters	transfer control to window or function whose name contains the letter.

Information line

This line is used to display the selected directory.

Status area

Display information about the status of certain commands, what keys are required for certain movement, and error messages that do not have a disastrous effect on the program.

Main display area

Information being used by the active window (procedure) is displayed in the main display area. The information changes depending upon which window is in use. To access this information press PgDn. The selection cursor is then moved to the top field in the main display area. For a discussion of the keys available for editing an individual field within the main display area, refer to page 7. The following keys are active from within the main display area for most of the menu windows:

Backtab	(Shift-Tab) moves the selection cursor left one field. If there is no field to the left, it moves the cursor up one field. This key is not active when on the first field. Not active in the Main Menu, Data, and sTatistics windows.
Down Arrow	moves the cursor down one field. This key is not active when on the bottom field.

Enter	moves the cursor down one field. If the key is pressed when on the bottom field, the cursor remains in that field.
Left Arrow	moves the cursor one field left. If the selection cursor is on the left-most selection, the cursor wraps around to the right most selection. Active in this manner only for the Main Menu, Data, and sTatistics windows.
Esc	aborts the editing of all the fields in the main display area and places the cursor back on the menu bar. Not active in the Main Menu, Data, and sTatistics windows.
F10	saves all changes and places the cursor on the menu bar. When this key is used while on a field which has a selection set window, the value saved is the value in the field, not the value on which the selection set cursor is positioned. Not active in the Main Menu, Data, and sTatistics windows.
Right arrow	moves the cursor one field to the right. If the selection cursor is on the right-most selection, the cursor wraps around to the left-most selection, but this key is active in this manner only for the Data, sTatistics, and Main Menu windows.
Tab	moves the cursor right one field. If there is no field to the right, it moves the cursor one field down. This key is not active when on the last field. Not active in the Main Menu, Data, and sTatistics windows.
Up Arrow	moves the cursor up one field. This key is not active when on the top field.

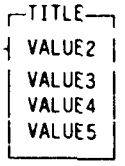
Selection Sets

WARNING: Use Up Arrow and Down Arrow to move through fields which contain selection sets. By using the arrow keys, the previously selected value for the selection set will be preserved. If Enter is used, the values for each field will be set to the first value in the selection set.

Selection sets are used by PBPK_SIM to list the values that are available to the user for the associated field. The user may also be allowed to enter an unlisted value with the keyboard. Since the selection set may not show all of its values on the screen, the user may be required to scroll down the list to view the additional values. This can be accomplished by using the keys described below.

If additional values are available, a hash mark will appear on the side of the window as illustrated below.

Notice the hash mark -------->



The user should be aware that when a selection set is used, pressing F10 (to save the information) will not change the value of the current field. This is important when the final field of an input screen uses a selection set. In this case the user must make his choice of the selection set value by pressing Enter and then F10.

The displaying of a selection set also uses a windowing effect. The selections will appear in a box at a predetermined location on the screen. A difference between selection sets and windows is that a selection set's box size grows and shrinks as selections are added or subtracted from the list. To select one of the values in the selection set, the user uses the following keys:

Ctrl-End	moves the selection cursor to the last field in the selection set.
Ctrl-Home	moves the selection cursor to the first field in the selection set.
End	moves the selection cursor down one field. If the selection cursor is on the bottom field, this key is not active.
Home	moves the selection cursor up one field. If the selection cursor is on the top field, this key is not active.
Enter	selects the value highlighted in the selection set and moves the cursor to the next field in the window unless already on the final field.

Input and editing keys

There are two types of input modes available in PBPK_SIM. These are overstrike mode and insert mode. When a character is typed while in insert mode, all characters to the right of the cursor are moved one space to the right and the character inserted. In overstrike mode, the character typed replaces the character at the cursor position. The user can toggle between the two modes by pressing Insert. If the mode is overstrike and the user presses Insert, the mode changes to insert. The mode (insert or overstrike) may have an effect upon the way characters are entered into the variables. If there is a difference between the use of a key in insert mode and overstrike mode, it will be described. The keys are described here:

Backspace	When in insert mode, erases the character to the left of the cursor and moves characters to the right one space left. When in overstrike mode merely erases the character to the left and moves the cursor one space left. The remaining characters are not affected.
Backtab	Moves the cursor left one field when the cursor is located in the main display area.
Ctrl-Home	Moves the cursor to the first field in a selection set window.
Ctrl-End	Moves the cursor to the last field in a selection set window.
Down Arrow	Moves the cursor down one field when the cursor is located in the main display area.
Left Arrow	Moves the cursor one field left when on the menu bar or in the main display area of the Main Menu, Data and sTatistics menus. Moves the cursor one character left without disturbing the character when in an input data field.
Right Arrow	Moves the cursor one field right when on the menu bar or in the main display area of the Main Menu, Data and sTatistics menus. Moves the cursor one character right without disturbing the character when in an input data field.
Up Arrow	Moves the cursor up one field when the cursor is located in the main display area of the window.
Delete	Deletes the character on which the cursor is positioned and moves the characters to the right one space to the left.
End	Moves the cursor down one field when the cursor is located in a selection set window.
Esc	Used to abort the editing or execution of a process and return the selection cursor to the menu bar of the last window viewed before pressing Esc.
F2	In the Data menu windows, closes the current page and opens the next page.
F3	In the Data menu windows, closes the current page and opens the previous page.

F10	When pressed in an input data screen, saves the information and places the selection cursor on the menu bar.
Home	Moves the cursor up one field when the cursor is located in a selection set window.
Insert	Toggles between insert and overstrike modes.
PgUp	Moves the cursor from the main display area of the window to the menu bar and selects the highlighted option. This key is active only in the Main Menu, Data, and sTatistics windows.
PgDn	Moves the cursor from the menu bar to the main display area of the window.
Enter	When on the menu bar of any window, the main display area of the Main Menu, Data, or sTatistics window, or a selection set window, Enter selects the highlighted option or file for execution. When in the main display area of other windows, it moves the cursor to the next field, except on the last field where it remains in that field.
Space Bar	When in insert mode, places a blank at the cursor location and moves characters to the right one space right. When in overstrike mode, replaces the character at the cursor with a blank and moves the cursor one space right.
Tab	Moves the cursor right one field when the cursor is located in the main display area of the window.
Other displayable characters	When in insert mode, places that character at the cursor location and moves characters to the right one space. When in overstrike mode, replaces the character at the cursor with the new character and moves the cursor one space right.

Executing PBPK_SIM

PBPK_SIM is executed by loading the file PBPK_SIM.EXE, which ordinarily is in the subdirectory PBPK_SIM on a hard disk or in the root directory on a diskette. If the current subdirectory is the one containing PBPK_SIM.EXE, simply type

PBPK_SIM

at the DOS prompt and the program is loaded and executed. If, however, the current subdirectory does not contain the executable file, DOS must be told where to find it.

There are two alternatives available for executing PBPK_SIM when its executable file is not in the current subdirectory. One is to put a batch file that executes PBPK_SIM in a subdirectory that is a part of DOS's search path. The current path setting can be determined by typing

PATH

at the DOS prompt. DOS will respond with something like

PATH=C:\DOS;C:\WORDPROC;C:\SPRDSHT;C:\UTILITY;

By adding the subdirectory PBPK_SIM which contains the PBPK_SIM.EXE file to the list of subdirectories the user may execute the program without having to specify the directory location. However, the *.DFT file which accompanies the program must be located in the directory where PBPK_SIM is invoked.

If no path is specified, one can be set up by editing the file AUTOEXEC.BAT (in the root directory of your boot drive or diskette) and inserting the statement

PATH=

followed *immediately* by the drive and subdirectory containing PBPK_SIM.EXE. When editing the AUTOEXEC.BAT file, save the file as ASCII text. If a word processor is used, do not save the file in its proprietary format. Note that the PATH= statement must appear in the AUTOEXEC.BAT file before any line that transfers control to another batch file (unless the CALL statement is used), and that changes to AUTOEXEC.BAT do not take effect until the machine is re-booted or AUTOEXEC is re-executed by typing AUTOEXEC at the DOS prompt.

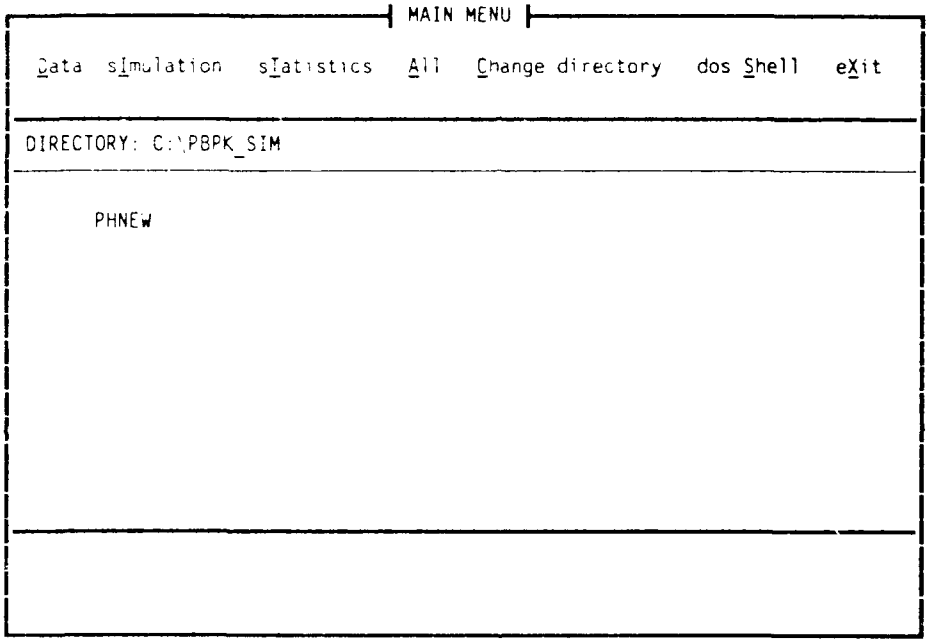
PBPK_SIM is designed to run on either a color or monochrome monitor system. When PBPK_SIM is loaded, a check is performed to determine the type of graphics card being used. If the computer is configured with a color graphics card and monochrome monitor, PBPK_SIM attempts to operate as if a color monitor is attached. The graphics card check may be over-ridden by invoking PBPK_SIM in the following way.

PBPK_SIM *flag*

where *flag* can be either C or M. C indicates a color monitor is to be used and M indicates a monochrome monitor is to be used. Once PBPK_SIM has been run using the c or m, the user is not required to use the c or m the next time the program is invoked. The program will always come up in the same manner it was last invoked.

Main Menu

After successfully invoking PBPK_SIM, the Main Menu appears. All ACSL CSL files in the current directory that have been compiled are listed in the main display area. If more than one CSL file exists, the first file will be highlighted and the cursor active in the main display area. However, if only one CSL file exists it will be selected (flashing) and the cursor will be highlighting the Data option on the menu bar. If no CSL files exist in the directory the cursor will also be highlighting the Data option.



The options available from the Main Menu are displayed on the menu bar at the top of the screen. An option is selected by pressing the highlighted letter within the option or by pressing Right Arrow or Left Arrow until the desired option is highlighted and then pressing Enter. Each option and its functions are briefly described below.

Data displays the data files available for use by the selected ACSL CSL file. Only those data files that were created using the selected CSL file will be displayed. Allows selection, creation, copying, and deletion of data files.

sImulation performs simulation runs using the values in the selected data file and the associated ACSL model.

sTatistics displays the available ACSL output files (files with extension RR? where the ? is a digit 1-7 or H) which have been calculated for any of the available data files associated with the selected CSL file. Allows the user to select and perform statistical calculations on a set of ACSL output files.

All	performs both the simulation run and statistical calculations on the selected set of data files. This operation is identical to running the sImulation on a data file and then running both the Dose statistics and the Risk statistics on the simulation output.
Change Directory	opens a window for entry of a different drive, directory, or both.
dos Shell	temporarily suspends the execution of PBPK_SIM and invokes DOS.
eXit	ends execution of PBPK_SIM and returns to the calling process (usually DOS).

File Selection (Main Display Area)

The PgUp, Enter, and PgDn keys are used to move between the menu bar and the main display area provided more than one CSL file was located. Left Arrow, Right Arrow, Up Arrow, and Down Arrow are used to move among the CSL files in the main display area. Once the desired file is highlighted, pressing Enter or PgUp selects the file. The selected file is indicated by flashing.

The Main Menu options above are discussed in more detail in the following sections.

Data

Allows the selection, creation, modification, deletion, or printing of a data file associated with the selected ACSL CSL file. The creation, modification, deletion, or printing of a Human or Animal species file is also possible through the Data window. See the Data section on page 16 for a complete description.

sImulation

Performs sImulation runs on the selected data file and ACSL CSL file. Using the data file, the program creates an ACSL CMD file for each dose group. This CMD file is then sent to ACSL for computation. The output variables selected in the data file are calculated by ACSL and stored in files with extensions of RR?. The '?' is replaced by the dose number when running for animal data and an 'H' when running for human data. Therefore if the selected data file contains 4 dose groups excluding the control dose group, the file RR1, RR2, RR3, RR4, and RRH will be created by the sImulation run. These files are then used by the sTatistic facility to compute statistics on the output variables.

An example of a CMD file is contained in the sImulation section of the manual (page 40).

All

The All option provides the user a way to perform both the sImulation and the sTatistics at once. Since the procedure can be very time consuming depending on the number of simulations selected, this would be a good selection for times the computer can be left unattended.

sTatistics

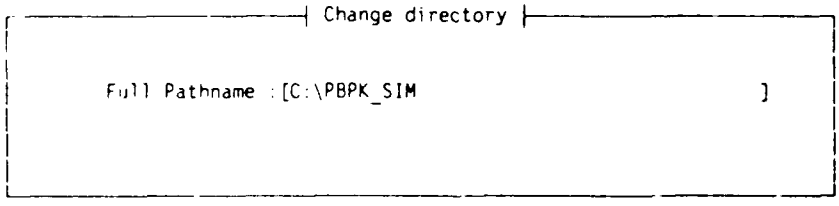
Enables the user to perform statistical calculations on data in the files that were produced by the sImulation runs. These files contain the values for the output variables selected in the data file. For a description of the functions available, see the sTatistics section of this manual (page 43).

Change directory

The Change directory option allows the user to change the current disk drive and/or directory so that a different set of ACSL files may be used. Since the PBPK_SIM program stores all of the files it creates in the current directory, this is an excellent way to manage the storage of the files.

The species files are accessible only if they are located in the current directory. Therefore the user may wish to go to the dos Shell and copy the species files from one directory to another.

The name of the drive and/or directory must be known in advance. Selection of the Change directory option will open a window with the cursor positioned on the field where the drive and/or directory are to be entered.



After the path name has been entered, pressing Enter will execute the function. If a valid name was entered, that is, if the path exists as specified, a message will appear in the Change directory pop-up stating that the change was successful. Pressing any key will close the Change directory window and return the cursor to the menu bar. However, if the path name was not valid, an error message will state that the path could not be found and a valid path name requested again. At any time, Esc may be pressed to cancel the function.

dos Shell

The dos Shell option will suspend the execution of PBPK_SIM and return the system to DOS. Any valid DOS command or application can be run if it fits into the amount of available memory. Typing the command EXIT and pressing Enter will reenter PBPK_SIM with all its settings unchanged.

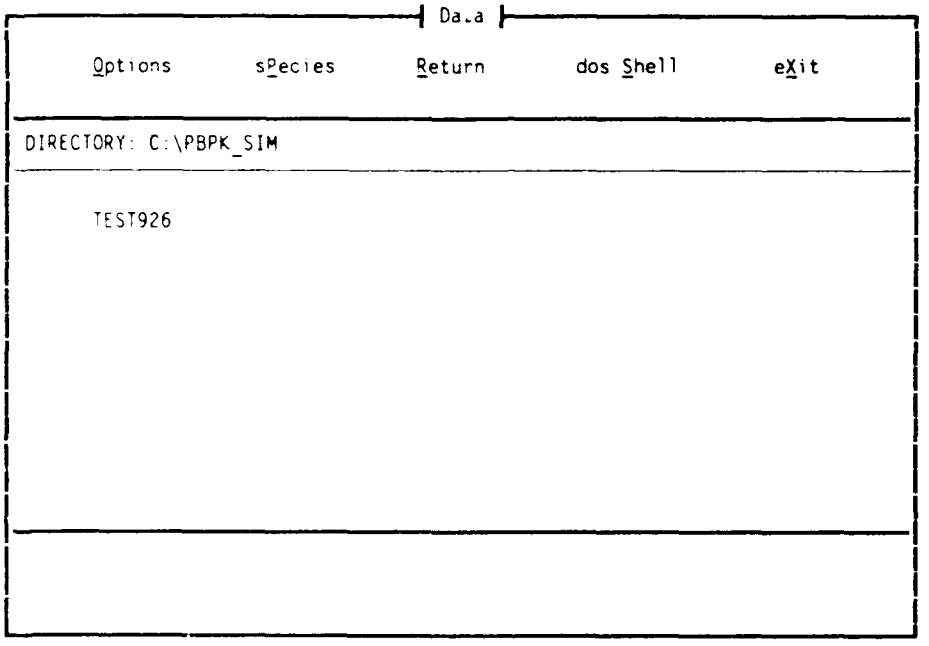
It is recommended that no files used by PBPK_SIM be deleted, copied or created while in the dos Shell, excluding the species files as mentioned above. PBPK_SIM keeps track of all files internally; therefore, external file creation, deletion, or modification could cause problems.

eXit

Terminates the execution of PBPK_SIM and returns to the calling process (usually DOS).

Data

When the Data menu is entered, the cursor highlights the first data file in the main display area. If there is only one data file, or no data file, the cursor will highlight the Options option of the menu bar and if a data file exists, it will be flashing to indicate it is the selected file.



The options on the menu bar may be selected using Left Arrow, Right Arrow, and Enter. Each option is listed, with its functions briefly described, as follows:

Options opens a pull-down window for the Edit, cReate, Copy, Delete, and Print data file functions.

sPecies opens pull-down window for the Edit, cReate, Copy, Delete, and Print species file functions.

Return returns control to the MAIN MENU window.

dos Shell temporarily suspends PBPK_SIM and invokes DOS.

eXit ends the execution of PBPK_SIM and returns to the calling process (usually DOS).

File Selection

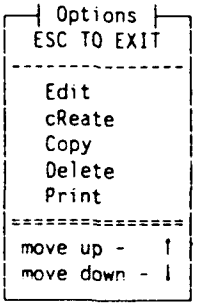
The PgUp, Enter, and PgDn keys are used to move between the menu bar and the main display area provided more than one data file exists for the selected ACSL CSL file. Left Arrow, Right Arrow, Up Arrow, and Down Arrow are used to move among the data files in the main display area. Once the desired file is highlighted, pressing Enter or PgUp will select the file. The selected file is indicated by flashing.

The files listed in the main display area are only those data files associated with the selected ACSL CSL file. For each ACSL CSL file that has had at least one data file created, there exists a file on the disk with the same name as the ACSL CSL file but with an extension of 'WCC'. This file contains the names of the data files that have been created for the ACSL CSL file. Since these 'WCC' files are only updated through the use of the Options selection from the menu bar, the user should refrain from copying, creating, or deleting any 'WPS' files from the DOS prompt. While the program will only display the valid 'WPS' files, the 'WCC' file may contain useless information.

The Data menu options above are discussed in more detail in the following sections.

Options

Choosing Options on the Data menu bar will open a pull down window that lists the functions available, as shown below

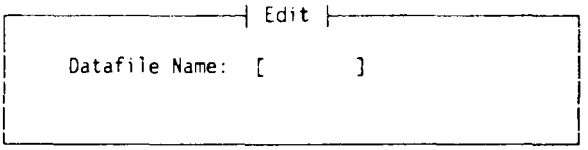


A function is chosen by pressing the highlighted letter within the function or by positioning the cursor on the desired function using Up Arrow and Down Arrow and pressing Enter. If the cursor is on the top selection and Up Arrow is pressed, the cursor is then positioned on the bottom selection. Similarly, if Down Arrow is pressed while the cursor is on the bottom selection, the cursor will be positioned on the top selection. The function highlighted by the cursor is executed by pressing Enter. Pressing Esc or Space Bar will close the window and return the cursor to the word Options on the menu bar. By invoking the appropriate function, an empty data file can be created, an existing data file edited, an existing data file printed, an existing data file can be copied into a new data file, or an existing data file can be deleted. Note that if a new data file is created, it will be the selected data file. If the selected data file is deleted, the first data file in the list will be the selected data file. To change the selected data file, press PgDn when the cursor is located on the menu bar.

When a function is selected, a window will be displayed requesting the information needed for the execution of the function. When entering the data file name that is requested, no extension should be given since PBPK_SIM appends the data file extension, 'WPS', to the data file that is entered. If an extension is provided, an error message will be displayed indicating that the data file entered was invalid or could not be found. The user can abort the function by pressing Esc whenever the program is waiting for input. The cursor will then be returned to the menu bar.

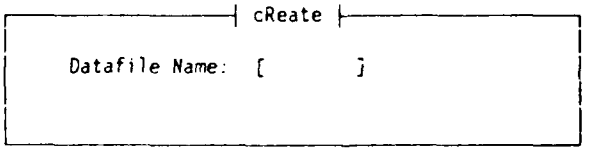
Edit

The Edit function allows the user to make changes to a data file in the current ACSL CSL file list. The name of the selected data file will appear when the window is opened but it can be overwritten. If the name entered is the name of a non-existent data file or an existing data file that does not belong to the selected ACSL CSL file list, an error message will be displayed and the name of the data file requested again. Once a valid name has been provided, the same edit window screen used with the cReate function is opened. The screens and the process for changing the data are described beginning on page 29. After all changes have been made to the data, the Edit window is closed and the selection cursor returned to the menu bar.



cReate

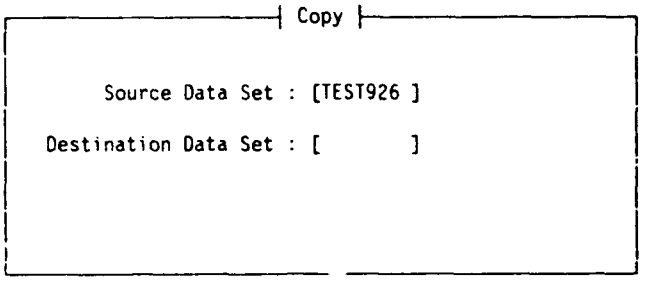
The cReate function opens an empty data file in the current ACSL CSL file list. The name of the data file to be created is entered in the field provided when the cReate window opens. If the name entered is the name of an existing data file in any ACSL CSL file list, an error message will be displayed and the name of the data file to be created requested again. Once a valid name has been provided, the selected CSL file is parsed (see page 67). After parsing, an edit window screen is opened to allow data entry. The screens and the process for entering the data are described on page 29. After the data have been entered, the edit window is closed and the selection cursor returned to the Options window highlighting the cReate option. The newly created data file is highlighted and blinking in the main display area to show that it is the selected data file.



Copy

When the Copy window first opens, the source data file name will be the current data file. This name can be changed to any of the data files in the current ACSL CSL list by typing over the existing name. Once the source data file name has been entered, the name for the destination data file must be provided. If the destination name exists in any ACSL CSL list or if the source name does not exist, a message will be displayed indicating the error and the cursor repositioned requesting new input and

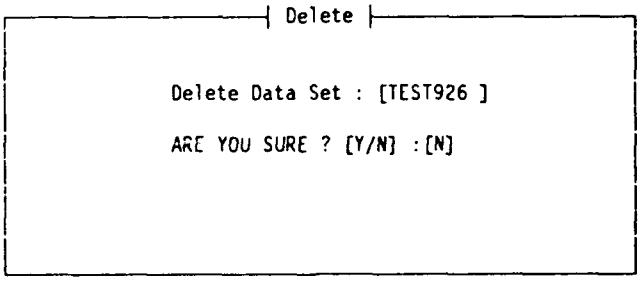
output files. When valid data files have been entered, completion of the Copy function will result in the source data file being copied into the destination data file.



Once the Copy function is completed, the cursor returns to the menu bar and the new data file is placed in the main display area.

Delete

To erase a data file from the list for the current ACSL CSL model, choose the Delete function in the Options pull down window. The delete window will appear with the selected data file appearing as the data file to be deleted. A different data file can be entered by typing over the name provided. Once the correct data file is entered, press Enter. A message will appear asking if the user is sure he wants to delete the data file. If so, enter a Y and press Enter; if not, enter an N and press Enter. If an N is entered, the delete function is exited and control returned to the menu bar. Once the data file has been deleted from the disk, a message will appear in the Delete window confirming a successful operation. Pressing any key will then close the Delete window and return the user to the menu bar.

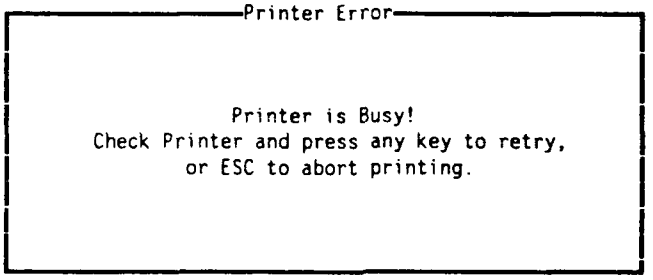


If the data file entered is not the name of a data file in the current ACSL CSL list, a message will appear in the delete window indicating that the data file does not exist and another name will be requested. If the data file deleted was the selected data file, then the data file in the upper-left corner of the main display area becomes the selected data file. The selected data file will be blinking.

Print

The Print function will send the specified data file to the printing device that has been specified by the operating system as LPT1: or to a user specified disk file. When the Print function is selected, a window appears with the two options, Diskfile or Printer. Using Up Arrow and Down Arrow, the user highlights the option desired

and selects it with Enter. Pressing ESC will close both windows and return the user to the command line. If the Diskfile option is selected, the user is then prompted for the file name. Once a correct data file is entered, press ENTER to print. If an error is detected with the printer (that is, the printer is not on line or is out of paper), then an error message is displayed (as shown below). Pressing ESC will cancel the printing operation. Pressing any other key indicates that the user wishes to try the print operation again.



Sample Printout: Data File Test926

Page 1

```
For Model --  PHNEW                      Last Modified -- 09/26/90   11:31
Experimental Species File  [MOUSE   ]    Human Parameter File  [HUMAN   ]
Simulations: Number  [35  ]              Length  [24  ]  hrs
Number of Integration Steps :  [10000   ]
Regenerate parameter vectors for each dose? [Y/N] [N]

SPECIAL CATEGORIES.
   CLOSED CHAMBER                                  [Y/N] Species [N]  Human [N]

HUMAN DOSE :
 Oral dose                          PDOSE  [0        ] mg/kg
 IV dose                            IVDOSE [0        ] mg/kg
 Length of IV infusion              TINF   [0.01     ] hrs
 Inhaled concentration              CONC   [50       ] ppm
 Length of inhalation exposure      TCHNG  [8        ] hrs
```

Page 2

```
PDOSE  Oral dose                                              mg/kg
IVDOSE IV dose                                                mg/kg
TINF   Length of IV infusion                    [0.01     ]   hrs
CONC   Inhaled concentration                                  ppm
TCHNG  Length of inhalation exposure            [6        ]   hrs
                    DOSE(S)                          RESPONDERS / # OF
                                                                ANIMALS
      PDOSE          IVDOSE          CONC
   [0         ]   [0         ]   [0         ]   [1         ] [48        ]
   [0         ]   [0         ]   [100       ]   [13        ] [50        ]
   [0         ]   [0         ]   [200       ]   [36        ] [50        ]
   [          ]   [          ]   [          ]   [          ] [          ]
   [          ]   [          ]   [          ]   [          ] [          ]
   [          ]   [          ]   [          ]   [          ] [          ]
   [          ]   [          ]   [          ]   [          ] [          ]
   [          ]   [          ]   [          ]   [          ] [          ]
```

Page 3

Output Variables

AF	AI	AIO	AL	AM
AO	AR	AS	√ AUCB	√ AUCL
AX	√ CA	CF	CI	CINT
CIZONE	CL	CP	CR	CS
CV	CVF	√ CVL	CVR	CVS
CX	CXPPM	DOSE	IALG	IV
IVR	IVZONE	KF	KL	MR
NSTP	PF	PL	PR	PS
QC	QF	QL	QP	QR
QS	RAF	RAI	RAL	RAM
RAO	RAR	RAS	RATS	RAX
RMR	TMASS	VCH	VF	VL
VMAX	VR	VS		

Page 4

Parameter categories	Distribution	Constrained
BLOOD FLOW RATES	[Normal]	[N]
KINETIC CONSTANTS	[Lognormal]	[N]
FRACTIONAL VOLUMES OF TISSUES	[Dirichlet]	[Y]
PARTITION COEFFICIENTS	[Lognormal]	[Y]
CHEMICAL SPECIFIC DATA	[Normal]	[N]

Page 5

Constrained Parameters
[Lognormal Distribution]

Parameter description	Parameter	Group	Rank Within Group
Fat/air partition coefficient	PFA	[1]	[1]
Liver/air partition coefficient	PLA	[1]	[2]
Slowly perfused tissue/air partition	PSA	[1]	[2]
Richly perfused tissue/air partition	PRA	[1]	[2]
Blood/air partition coefficient	PB	[1]	[2]

Page 6a

[Normal Distribution] for Unconstrained Variables

Parameter	Description	MOUSE Mean	MOUSE Variance	HUMAN Mean	HUMAN Variance
QPC	Alveolar ventilation	[20]	[0.0]	[14.7]	[0.0]
QCC	Cardiac output	[13.8]	[0.0]	[17.8]	[0.0]
QLC	Fractional blood flo	[0.25]	[0.0]	[0.26]	[0.0]
QFC	Fractional blood flo	[0.09]	[0.0]	[0.05]	[0.0]
QRC	Fractional blood flo	[0.51]	[0.0]	[0.44]	[0.0]
QSC	Fractional blood flo	[0.15]	[0.0]	[0.25]	[0.0]
MW	Molecular weight	[165.83]	[0.0]	[165.83]	[0.0]

Page 6a

[Lognormal Distribution] for Unconstrained Variables

Parameter	Description	MOUSE Mean	MOUSE Variance	HUMAN Mean	HUMAN Variance
BW	Body weight	[0.028]	[0.0]	[70.0]	[0.0]
KMAX	Maximum velocity of	[3.96]	[4.9]	[0.33]	[0.034]
KM	Michaelis-Menten con	[1.47]	[8.4]	[1.86]	[13.2]
KFC	First order metaboli	[0.0001]	[0.0]	[0.0001]	[0.0]
KA	Oral uptake rate	[5.0]	[0.0]	[5.0]	[0.0]

Page 6b

[Lognormal Distribution] for Constrained Variables
[Group 1]

ANIMAL Upper Bound [1917] Lower Bound [0.01]

HUMAN Upper Bound [1950] Lower Bound [0.01]

		MOUSE		HUMAN	
Parameter	Description	Mean	Variance	Mean	Variance
PFA	Fat/air partition co	[816]	[76000]	[1230]	[238672.0]
PLA	Liver/air partition	[50.9]	[316]	[60.6]	[7665.0]
PSA	Slowly perfused tiss	[43.8]	[234]	[31.9]	[2857.0]
PRA	Richly perfused tiss	[50.9]	[1252]	[60.6]	[3255]
PB	Blood/air partition	[16.9]	[35.0]	[12.0]	[17.5]

Page 6b

[Dirichlet Distribution] for Constrained Variables
[Group 1]

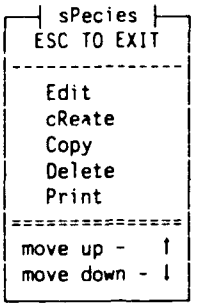
ANIMAL Theta [1456] HUMAN Theta [1621]

		MOUSE	HUMAN
Parameter	Description	Expected Proportion	Expected Proportion
VLC	Fraction liver tissu	[0.055]	[0.026]
VFC	Fraction fat tissue	[0.049]	[0.23]
VSC	Fraction slowly perf	[0.767]	[0.62]
VRC	Fraction richly perf	[0.049]	[0.05]
VCC	Fraction carcass rem	[0.08]	[0.074]

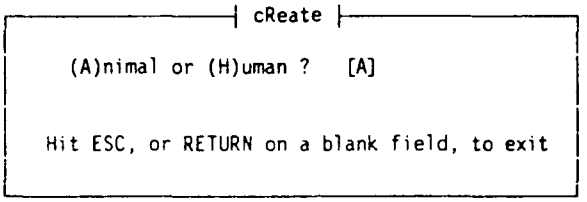
sPecies

The species files are used when creating or editing data files to set the preferred value for any variable in the data file which has the exact same name as a variable in the species file. The species file may contain variables that are not listed in the data file and the species file does not have to contain all the variables that are listed in the data file. The species files are provided to make it easy for the user to keep preferred values for physiological parameters for different species.

When the sPecies selection is chosen from the Data menu bar, a pull-down window is opened listing the function available. A function is chosen by pressing the highlighted letter within the function name or by positioning the cursor on the desired function using Up Arrow and Down Arrow. If the cursor is on the top selection and Up Arrow is pressed, the cursor is then positioned on the bottom selection. Similarly, if Down Arrow is pressed when the cursor is on the bottom selection, the cursor will be positioned on the to selection. The function highlighted by the cursor is executed by pressing Enter. Pressing Esc will close the window and return the cursor to the menu bar.



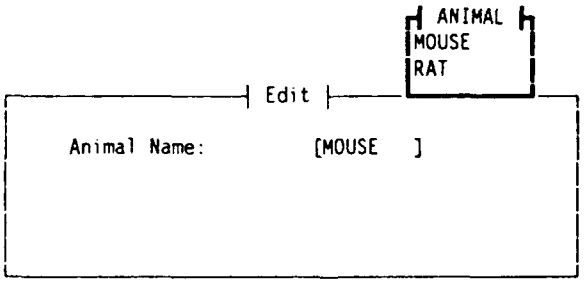
Selection of a function will open a window which requests whether an animal or human file is to be accessed.



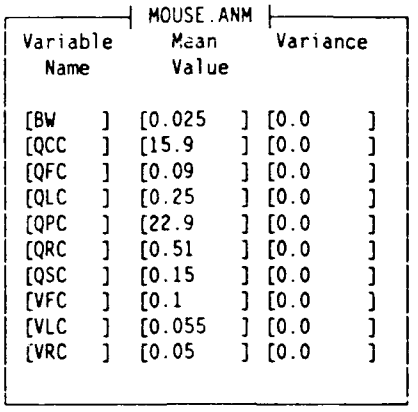
An animal file is chosen by pressing 'A' and Enter; a human file, 'H' and Enter. Selection of either 'A' or 'H' will open a window requesting the name of an animal or human file, as shown in the following function descriptions. For our discussion an animal file will be used but the same discussion can be made for a human file. The form of the window depends upon the function selected from the sPecies window.

Edit

The Edit function allows the user to make changes to an existing animal or human file. Having selected the 'A' option from the window described above opens the following window with a selection set containing all of the available animal files. The last animal file changed will appear in the data field.



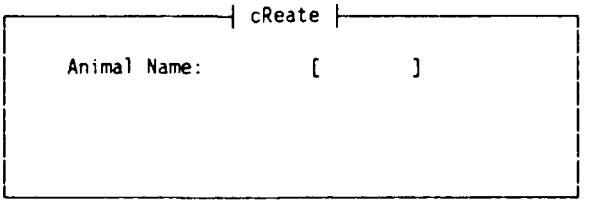
A new species file can be selected using Home and End to move through the selection set fields and pressing Enter to select the highlighted name. When a name is selected, a window opens displays all of the values that the file contains.



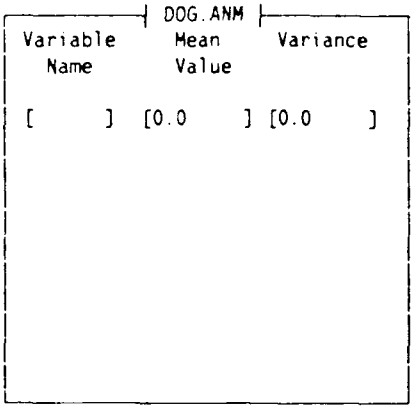
These values can be altered by using the Up Arrow, Down Arrow, Tab, and Shift-Tab to move to the appropriate field. Once the cursor is positioned on the desired field, any of the edit keys described in the Input/Edit Keys section of the window can be used to alter the data. There are two ways to effectively delete an input line from an animal or human file. The first is by deleting the values in all three column entries. The second and most efficient is by positioning the cursor on one of the fields within the input line and pressing Alt-D. When the Alt-D key is pressed an asterisk will appear at the front of the line indicating it is marked for deletion. Pressing the Alt-D key on a line marked for deletion will remove the asterisk and un-mark that line. Once all changes have been made, pressing F10 will save these changes. Pressing the Esc key at anytime will abort all changes that have been made.

cReate

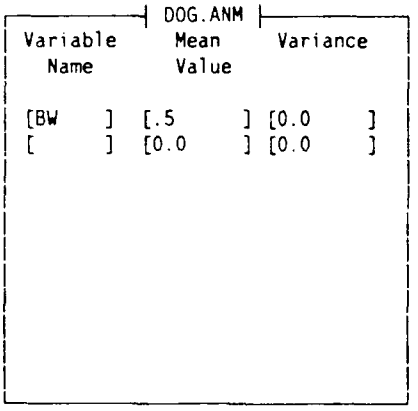
The cReate function allows the user to create an animal or human file, depending upon whether an 'A' or an 'H' was selected. When the function is selected a window is opened requesting the name of the animal/human file to be created.



If the user specifies the name of any existing animal/human file, an error message is returned stating this fact and a new name requested. Once a valid file name has been obtained a window is opened containing one input line.



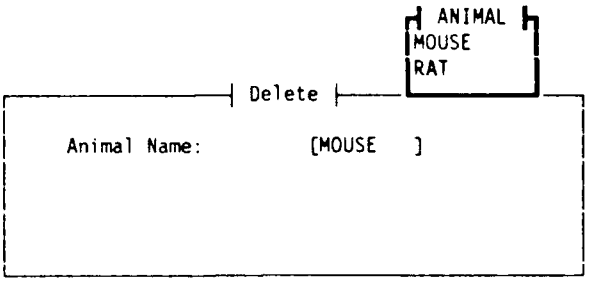
The user is required to enter the variable name and a mean value before a new line of input can be created. Once both the variable and mean value have been entered, the Down Arrow can be pressed and a new blank input line will appear.



The user may enter up to 100 variables in a species file. Once all the variables and their values have been entered, press F10 to save the file. Esc can be pressed at any time to abort the saving of the information.

Delete

When the Delete option is selected a window is opened requesting the name of the animal/human file to be deleted. The last file accessed is initially displayed in the data field. A selection set contains all of the files available for deletion.



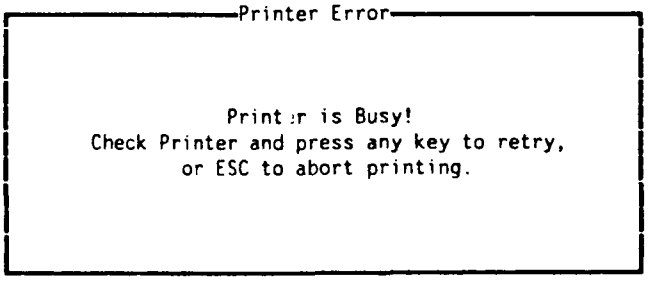
To select a file in the selection set, use Home and End until the desired file is highlighted and press Enter to delete the file. A confirmation of the deletion is then requested. Once a confirmation is received, the file is deleted. The delete function can be aborted at anytime by pressing Esc or by answering 'N' to the confirmation of deletion request.

Copy

When the Copy window first opens, the animal or human source file name will be the current name. This name can be changed to any of the animal/human files in the selection set by using Home and End to move the highlight and Enter to select the desired file. Once the source file name has been entered, the name for the destination file must be provided. If the destination name exists, a message will be displayed indicating the error and the cursor repositioned requesting new input and output files. When valid files have been entered, completion of the Copy function will result in the source file being copied into the destination file. Once the Copy function is completed, the cursor returns to the menu bar and the new data file is placed in the main display area.

Print

The Print function will send the specified animal/human file to the printing device that has been specified by the operating system as LPT1: or to a user specified disk file. When the Print function is selected, a window appears with the two options, Diskfile or Printer. Using Up Arrow and Down Arrow, the user highlights the option he desires and selects it with Enter. Pressing Esc will close both windows and return the user to the command line. If the Diskfile option is selected, the user is then prompted for the file name. Once a name is selected using Home and End, press Enter to print. If an error is detected with the printer (that is, the printer is not on line or is out of paper), then an error message is displayed (as shown below). Pressing Esc will cancel the printing operation. Pressing any other key indicates that the user wishes to try the print operation again.



The following is a sample printout for the animal species file RAT.

RAT.ANM

VARIABLE	MEAN	VARIANCE
BW	0.25	0.0
QCC	14.2	0.0
QFC	0.09	0.0
QLC	0.25	0.0
QPC	19.4	0.0
QRC	0.51	0.0
QSC	0.15	0.0
VFC	07	0.0
VLC	0.04	0.0
VRC	0.05	0.0
VSC	0.75	0.0
VCC	0.09	0.0

Return

The Return option closes the Data window and returns the user to the Main Menu window.

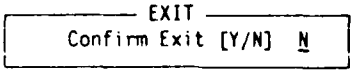
dos Shell

The dos Shell option will suspend the execution of PBPK_SIM and return the system to DOS. Any valid DOS command or application can be run. Typing the command EXIT and pressing Enter will reenter PBPK_SIM with all its settings unchanged.

In suspending the execution of PBPK_SIM, a period of time is required to swap the PBPK_SIM program to disk. The amount of time is approximately 2 seconds on an IBM AT. The same amount of time is also required in reloading PBPK_SIM after the EXIT command has been entered.

eXit

The eXit option opens a pop-up window (shown below) allowing confirmation before leaving PBPK_SIM. Typing Y and pressing Enter will end PBPK_SIM; N and Enter will close the confirmation window and return the cursor to the selection bar.



editing Data

For an explanation of how the CSL file is parsed, see page 67.

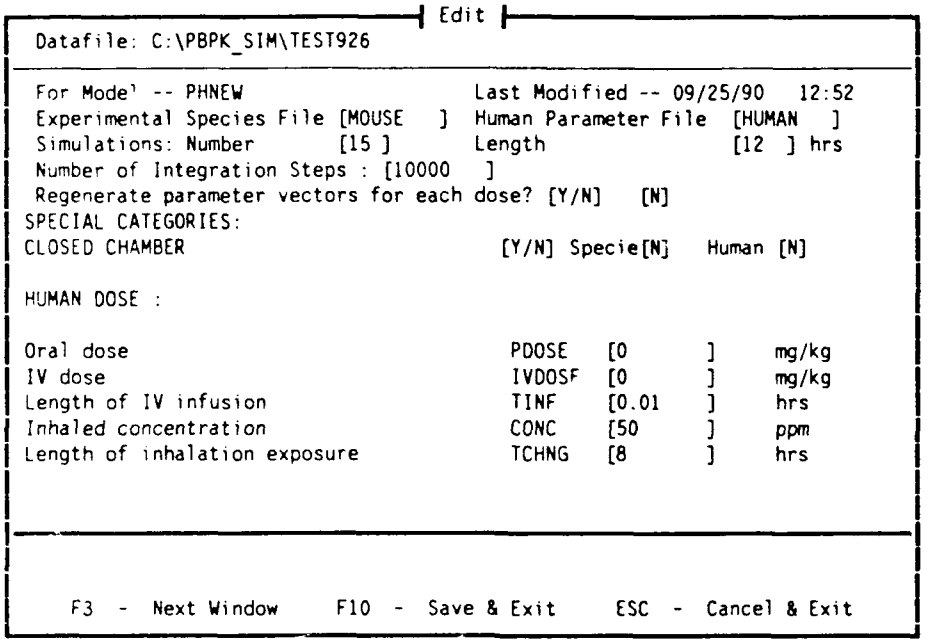
The editing of a data file is a lengthy process, therefore the user is allowed to save a data file before all information has been entered. However, the user must be certain that all the information has been entered before a simulation is run. The editing process can be divided into six pages. The F2 key is used to move to the previous page and the F3 key is used to move to the next page. Depending on the distribution types and constraints selected, PAGE4, PAGE5, and PAGE6 may contain more than one screen full of information. The F2 and F3 key are also used to move between these screens. This could cause a little confusion when moving back through the screens using F2. F2 will always move to the closest preceding first screen, not the last screen of the previous page. Thus, if the F2 key is pressed on any screen other than the first screen in a page, it will move to the previous screen within that page. To explain this further, let's take the following example which lists the number of screens per page.

PAGE1	1 screen
PAGE2	1 screen
PAGE3	1 screen
PAGE4	1 screen
PAGE5	3 screens
PAGE6	4 screens

When referring to a page and screen combination, the following terminology will be used: 5.2 refers to PAGE5, screen2, for example. Starting from PAGE1 and using F3 to move through the pages (and screens) will produce the following sequence: 1.1, 2.1, 3.1, 4.1, 5.1, 5.2, 5.3, 6.1, 6.2, 6.3, and 6.4. Screen 4 of PAGE6, the last screen, is being displayed. If F2 is used to move back through the pages the following sequence is produced: 6.4, 6.3, 6.2, 6.1, 5.1, 4 1, 3.1, 2.1, and 1.1. The user should note that when F2 is used within the depth of the window (6.4), one screen was traversed. However, once a new page is called, F2 will traverse pages instead of screens.

At any time the user can press F10 to save all the changes made or information entered. When F10 is pressed and no changes have been made to the data file, then the data file is exited and the user returned to the Data window. If F10 is pressed and changes have been made, then the user is asked if the data file should be saved and return to the Data window, or if the file should be saved and editing continued. For a more thorough discussion on saving and exiting a data file see the description at the end of this section. Pressing Esc will abort all the changes made to the entire data file since the last time the file was saved using F10, not just the page on which the user is currently editing. Once F10 or ESC is pressed and the appropriate answers provided (see Saving And Aborting A Data File), the user is return to the Data window. What follows is a discussion of each of these pages. This discussion includes an example of how the window will look and a discussion of each field within the page.

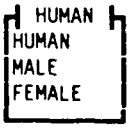
PAGE1



Page1 allows the user to enter general information about the data file and Human Dose information. The first line of the window indicates the location of the data file. The second line indicates which ACSL CSL file was used in the creation and the time and date of the creation.

Experimental Species File & Human Parameter File

These two fields allow the user to select default values that will be used by variables contained in both the animal/human file and the data file. A selection set is provided for each field listing the



available files. A file is selected by using Home and End to move the highlight cursor and pressing Enter to select the file. Once a file is selected using Enter, a window appears asking whether the current values for variables contained in both the animal/human file and data file should be updated to the values contained in the experimental species/human parameter file. If the user elects to update the values, PBPK_SIM runs through the list of variables replacing the values in the data file with those in the selected parameter file. To avoid getting the

update window each time the user moves through these two fields, Up Arrow and Down Arrow may be used. The only time the update window will be listed is when Enter is pressed in either of these two fields.

Simulations

Number — Specifies the number of simulations that should be run on the dose group being processed. The greater the number of simulations requested, the longer it takes to make the ACSL runs. Also the greater the number of simulations, the larger the memory requirement will be for calculation. The number of simulations that are possible will therefore be limited by the amount of RAM available.

Length — Length of time the model will simulate for each run.

# of Integration Steps

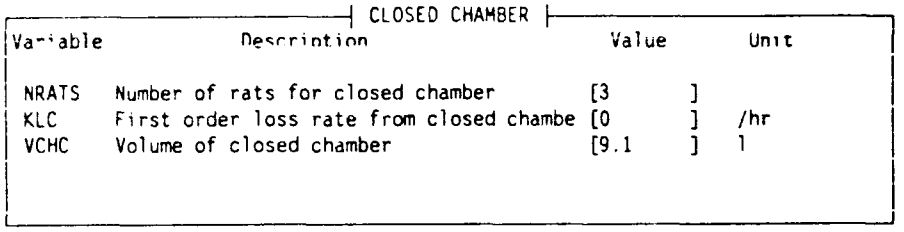
The number of integration steps per communication interval. Since there is only one communication interval per simulation when using PBPK_SIM, this variable controls the precision of the results. The larger the number, the more precise the integration will be, because of the fine step size and the longer time it will take ACSL to perform each simulation.

Regenerate parameter vectors for each dose? [Y/N]

Indicates whether the user wishes to regenerate the input parameter values each time the dose group is changed. Opting to regenerate for each dose group implies sampling from the probability distributions associated with the parameters n times for each dosed group, where n is the number of simulations requested. So, the total number of Monte Carlo samplings is n times the number of dosed groups. The alternative is to use the same set of sampled parameter values for each dose group. This implies sampling a total of n times.

Special Categories

The special categories section of the window lists those categories that were listed in the CSL file with an SCAT. The user must indicate whether the special category values will be used in the simulation runs. If a 'Y' is indicated for any of the Special Categories (SCAT) listed, a window similar to this one will appear enabling the user to specify



values for the variables contained within the Special Category. The name of the variable, its description, and units are all indicated and a value is requested. If an 'N' is pressed, no Special Category window will appear.

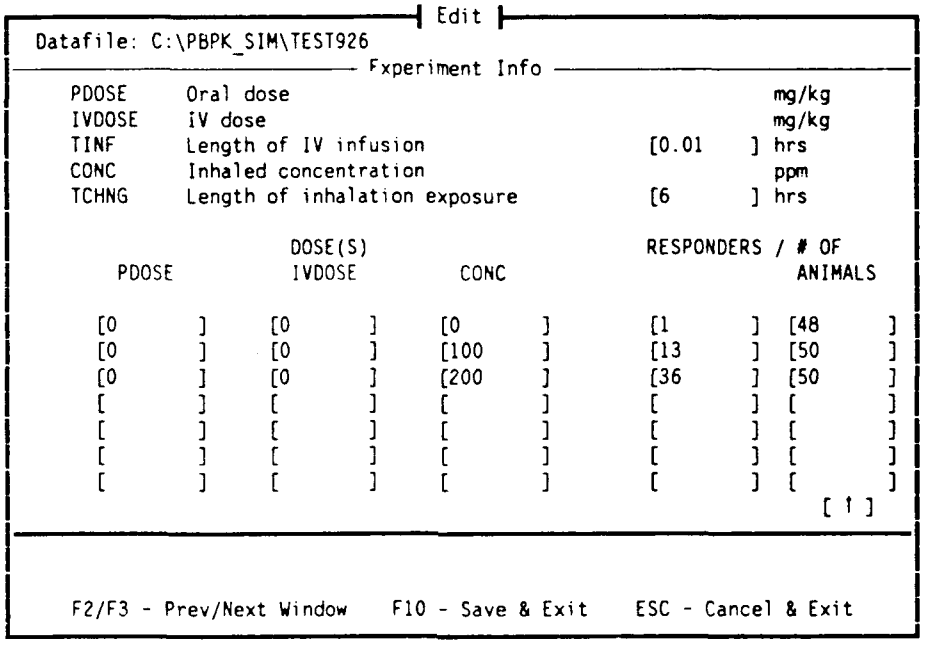
Although only enough space is provided to show two Special Categories, more can be listed. If the CSL file contains more than two SCATs, the message (More than 2 Special Categories Exist) is displayed. As the user moves through the list of special categories, the list will scroll to show others that may be hidden from view initially.

Human Dose

The Human Dose section of the window lists the length of dosing and dose level variables for each dose type (route) that was listed in the Dosing Information category in the CSL file. A maximum of five different types of doses and therefore five lengths may be provided. The variable name, its description, and units are listed and a value is requested. Although only enough space is provided to show six dose and length values, more can be listed. As the user moves through the dose and length values, the window will scroll to show the variables that are currently hidden from view. These are the Dose values which will be used in creating the RRH file for a simulation run.

PAGE2

Page 2 is used for entering the doses and lengths of dosing for an experiment.

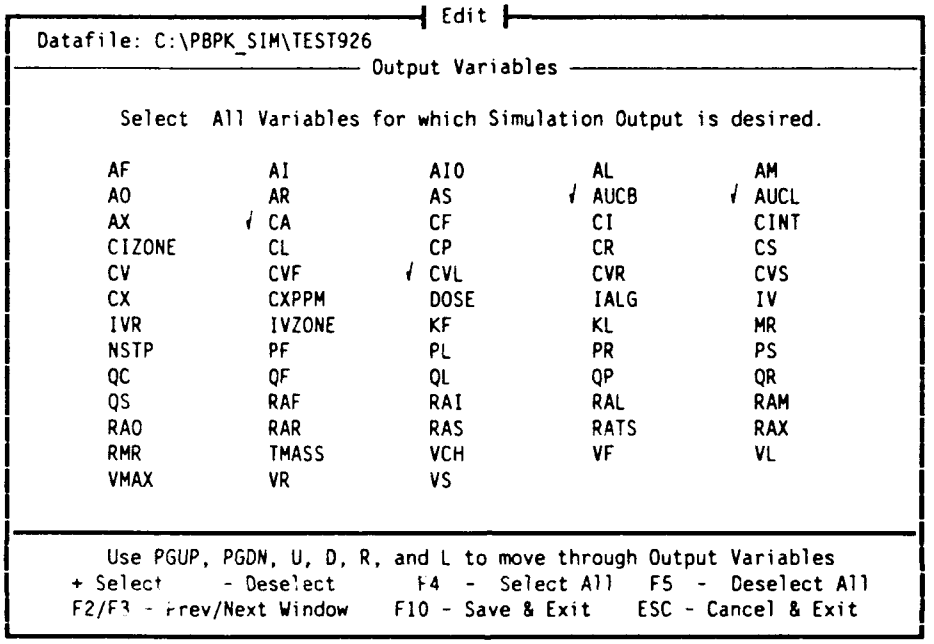


The upper section of the window lists the dose name, length name, descriptions, and units. Values are entered in the upper section for the lengths only. The lower section of the window allows values for up to eight dose groups to be specified. One of these dose groups must be a control group in which each dose is given a value of 0.0. This control group will not figure in the sImulation calculations, but is necessary for the performance of Risk statistics. The fields under Responders are for the number of animals that were determined to have the response of interest for the experiment. The fields under # Of Animals are used to hold the total number of animals that were at risk in the experiment (the number of animals exposed).

A value may or may not be listed for each of the dose types within each dose group. If a dose value is not given, it is assumed to be zero. The number of dosed groups is calculated by counting up how many of the possible 8 input lines contain values for at least one dose. These dose values are then used by sImulation in calculating the RR? files, where ? is substituted for the current dosed group number (1-7) being used. Dosed group numbering ranges from 1-7 instead of 1-8 because the control group is ignored during sImulations.

PAGE3

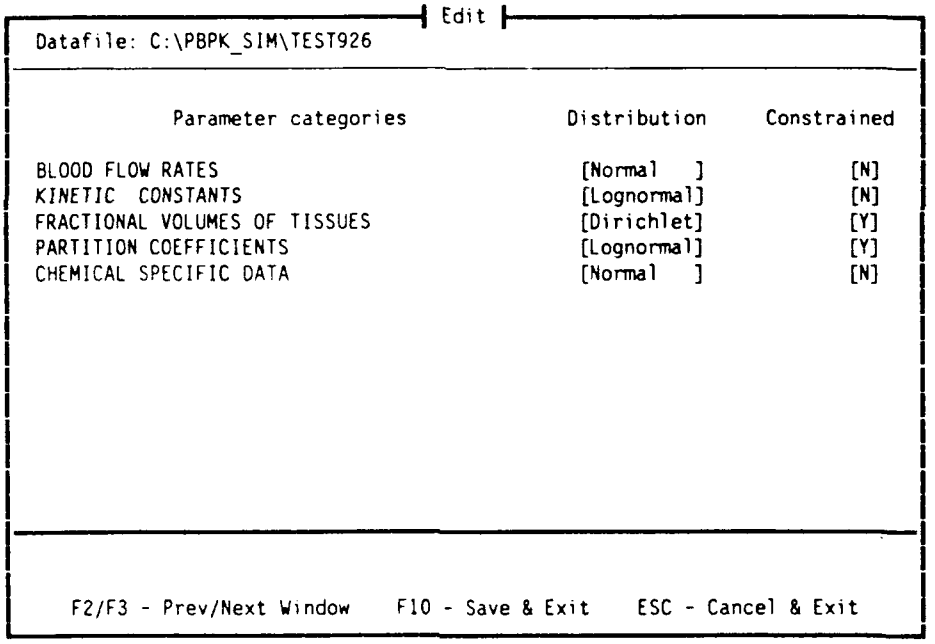
Page3 is where the output variables that will be listed in the RR? files from simulation runs are selected. The variables listed are taken from the CSL file. They include all variables used that are not set using the Constant declaration.



The Up Arrow, Down Arrow, Left Arrow, Right Arrow, PgUp, and PgDn keys are used to move through the listed variables. To select the highlighted variable, press the + (plus) key. When the + key is pressed a check (√) will appear beside the variable name. The variable can be deselected by pressing - (minus) and the check will disappear. F4 may be used to select all of the variables at once, and F5 to clear all of the selections.

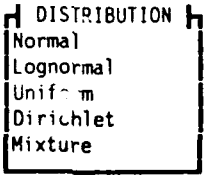
PAGE4

The regular categories, those described with the CSL file using CAT, are displayed on Page 4.



The descriptive name for each category is displayed and the user is allowed to selected the probability distribution type that will be associated with each variable within that category and whether the entire category is constrained or unconstrained.

The distribution types available are displayed in a selection set. Using Home and End, the user can position the highlight over the desired distribution and select that distribution by pressing Enter.



A description of what each distribution type indicates follows:

Normal — The parameter values are centered at the mean value, $\bar{x}$. A value of $\bar{x}+y$ is equally probable as a value of $\bar{x}-y$, i.e., the distribution is symmetric about the mean. All values are theoretically possible (including negative values) although some values may be extremely unlikely, depending on values of the mean and variance. The user must specify a mean and variance for each parameter with a normal distribution.

Lognormal — The log-transformed parameter values are normally distributed. A lognormal distribution is appropriate for parameters that can assume only positive values (i.e., the lognormal distribution is not defined for parameters that can take on the value zero or negative values. Depending on the mean and variance, a lognormal distribution can have a variety of shapes. The user must specify estimates of the mean and variance of the untransformed (observed) parameter values. PBPK_SIM will convert these values into the appropriate statistics for defining the corresponding lognormal distribution.

Uniform — The parameter values are known to fall between a lower limit and an upper limit, but all values between those limits are equally likely. Such a distribution may be useful when a parameter is bounded above and below (based on some considerations) but no information is available suggesting that certain values within the bounds are more probable. The user must specify an upper bound and a lower bound.

Dirichlet — The Dirichlet distribution is the continuous analog of the multinomial distribution. It describes the probability distribution of two or more parameters (proportions) whose sum equals 1. Thus, each parameter value must be less than 1 and when one parameter value changes, at least one other parameter value must change. The user must specify the preferred (mean) values of the parameters and a term called THETA which determines the variability in the parameter values. Larger values of THETA correspond to less variability. WARNING: Since the sum of the parameters must be 1, be sure to include "dummy" parameters if the parameters of interest are proportions but may not sum to 1. If, for example, the volumes of the compartments of interest do not constitute the total body weight, include a "carcass" compartment that can be considered to constitute the remainder of the body. Then the compartment volumes (expressed as proportions of the total body weight) will correctly sum to 1 and the sampling from the Dirichlet distribution will return values representative of the means and THETA provided by the user.

Answering Y to the Constrained field indicates that the variables should be constrained to fall within an upper bound (UB) and a lower bound (LB) and that the variables in the category can be ranked if so desired. So, for example, UB > V_1 > V_2 > V_3 > ... > V_N > LB or UB > V_1 > (V_2 or V_3 or ... V_N) > LB. Answering N indicates that no constraints are necessary.

Only the Normal and LogNormal distribution will be affected by the constraint field. The Dirichlet distribution is automatically constrained so that

$$\sum_{i=1}^{n} V_i = 1$$

If the Dirichlet distribution is selected as unconstrained, PBPK_SIM will automatically update it to constrained. Any variable with a Uniform distribution is by definition constrained within bounds, therefore any group with the Uniform distribution is always listed as unconstrained. If a Uniform distribution is selected to be constrained, PBPK_SIM automatically makes it unconstrained.

PAGE4b

Page4b is only seen if a mixture distribution was chosen for one or more categories in Page4. A window similar to the Page4 window is opened. The only difference between the PAGE4 and PAGE4b window is that the categories are listed in PAGE4 while the variables within the categories are listed in PAGE4b. Selection of distribution and constraints occurs as described in PAGE4 with the exception that a mixture distribution is no longer allowed.

PAGE5

Page5 may consist of multiple input screens depending upon the distribution types selected and the constraint values. If no categories, or variables, are indicated as constrained and no Dirichlet distribution is selected, then PAGE5 will not be shown.

Page5 lists the variables that are constrained for each distribution type so that they may be grouped and ranked. The Uniform distribution is omitted from Page5 since all variables are contained within one group.

Edit

Datafile: C:\PBPK_SIM\TEST926

Constrained Parameters
[Lognormal Distribution]

Parameter description	Parameter	Group	Rank Within Group
Fat/air partition coefficient	PFA	[1]	[1]
Liver/air partition coefficient	PLA	[1]	[2]
Slowly perfused tissue/air partition	PSA	[1]	[2]
Richly perfused tissue/air partition	PRA	[1]	[2]
Blood/air partition coefficient	PB	[1]	[2]

F2/F3 - Prev/Next Window F10 - Save & Exit ESC - Cancel & Exit

The group number can be any number between 0 and 99. The rank number can also be any number between 0 and 99. When the values have been entered in the fields for group and rank, if the user goes to the next screen and then returns, the variables will be sorted in their group and rank order. Either all ranks within a group must be different, or all ranks but one must be the same.

PAGE6

Page6 may also consist of multiple input screens depending upon the distribution types selected and the constraint values. One screen will be produced for each distribution type that is not constrained. In addition, one screen will be produced for each constrained group, within each distribution type. This could result in numerous screens.

The following is an example of a screen for unconstrained variables with a distribution type of Normal.

Edit

Datafile: C:\PBPK_SIM\TEST926

[Normal Distribution] for Unconstrained Variables

Parameter	Description	MOUSE Mean	MOUSE Variance	HUMAN Mean	HUMAN Variance
QPC	Alveolar ventilation r	[20]	[0.0]	[14.7]	[0.0]
QCC	Cardiac output	[13.8]	[0.0]	[17.8]	[0.0]
QLC	Fractional blood flow	[0.25]	[0.0]	[0.26]	[0.0]
QFC	Fractional blood flow	[0.09]	[0.0]	[0.05]	[0.0]
QRC	Fractional blood flow	[0.51]	[0.0]	[0.44]	[0.0]
QSC	Fractional blood flow	[0.15]	[0.0]	[0.25]	[0.0]
MW	Molecular weight	[165.83]	[0.0]	[165.83]	[0.0]

F2/F3 - Prev/Next Window F10 - Save & Exit ESC - Cancel & Exit

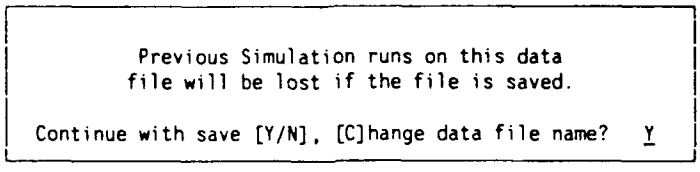
Each parameter that was indicated to be an unconstrained normally distributed variable is listed in the window along with its description. The user is required to enter values for the Mean and Variance for the experimental animal and the human. The only real restriction on the values are that they must be greater than or equal to zero and that if the Mean is zero, the Variance must also be zero.

In a Normal distribution constrained variables screen, the user must also supply upper and lower bounds which the values for the variables must fall between, in addition to the mean and variance. The lower bound must be less than the upper bound or an error condition will be raised.

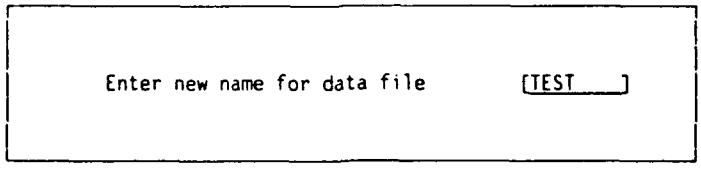
A Dirichlet distribution differs from the other distributions in that upper and lower bounds are not required but rather a THETA is required and the expected proportions must sum to 1. The values chosen for THETA express the uncertainty regarding the joint distribution of those expected proportions and determine the variance of each proportion.

Saving And Aborting a Data File

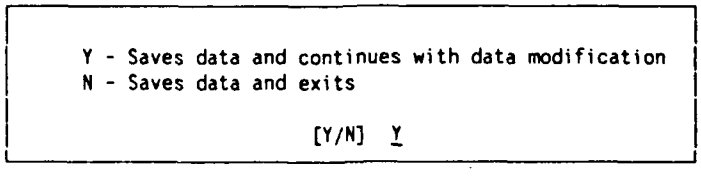
F10 is used to indicate that the selected file should be saved. When a file is to be saved, PBPK_SIM checks to see if any changes have been made to the file. If no changes were made, then the user is merely returned to the Data window. If changes were made and RR? files exist for the selected data file, then they must be deleted when the file is saved to prevent inaccurate information from being retained. The user will be prompted about this condition and asked if the file should be saved thus deleting the RR? files, if saving of the file should be aborted, or if a new file name should be entered. 'Y' indicates the file should be saved as is; 'N', abort the saving; and 'C', change the data file's name.



If the user elects to enter a new file name, a prompt will appear in which to enter the new name. A check is made to ensure that the new name is not an existing data file in any CSL file list.



If the user selected 'Y' from the first window, if the data file name was changed successfully, or if no RR? files existed for the original data file, the user will be shown the following window.



This window prompts the user to indicate either the data file should be saved and return to the current page of the data file to continue editing, or the data file should be saved and the user returned to the Data window.

Simulation

When the user requests that a simulation be run for a PBPK model using values for the parameters provided by a data file, a variation of Latin Hypercube sampling is used. Values for the parameters being varied among simulations are derived by making a random selection of the possible values for the parameter as defined by the distributions specified by the user in the data file. The following algorithm is used.

FOR: k = the number of parameters whose values are to be varied
n = the number of simulations requested
x_i = the ith constant being varied

1. Order the k variables by distribution, constrained group, and rank within the group.

2. For each x_i, i = 1, ..., k, define an n-dimensional vector (vector i) as follows
   a. Generate n p-values within uniform slices of the [0,1] interval each 1/n wide
   b. Randomly assign these p_values to the n elements of vector i.

   Step 2 forms a matrix which is k rows by n columns.

3. The correct inverse distribution is then applied to each element in a row. Since the variables were sorted in step 1, the constraints are applied as the values of the inverse distribution are computed so that the variables in a group are ordered and fall within the specified bounds. Each simulated value in a constrained group is forced to be greater than the lower bound (LB) by adding the p_value of the lower bound to the p_value generated in step 2. The resulting value is then scaled, by multiplying by the range of the p_values associated with the upper and lower bounds, before the inverse distribution is applied. If, for example, the constrained group is as follows:

   $$UB > V_1 > (V_2 \text{ or } V_3 \text{ or } \ldots \text{ or } V_N) > LB$$

   then the UB specified by the user is used to scale the first ranked element of the group and the value generated for the first ranked element is used to scale all the remaining elements of the group. If the ordering is as follows:

   $$UB > V_1 > V_2 > V_3 > \ldots > V_N > LB$$

   then the upper bound supplied by the user is used to scale the first element of the group and each of the remaining elements is scaled using the value generated for the preceding element as the upper bound.

4. Each column of the matrix contains values for the constants for one simulation.

A CMD file is then created with all the Prepar and Set statements necessary to run the number of simulations specified by the user plus one additional simulation where the constants are all assigned the preferred values (as entered by the user) of the parameters. The results from the

"preferred values" simulation are used in the risk statistics when the user chooses to regenerate the number of responders from a binomial distribution. The probabilities from a model fit to the dose surrogates obtained from the "preferred values" simulation are used as parameters of the binomial distribution which generates the new numbers of responders for each set of simulated dose values.

A sample CMD file is listed below. Descriptions of each group of variables are shown as comments. However, to conserve space, these comments are not listed in the actual CMD files created on disk.

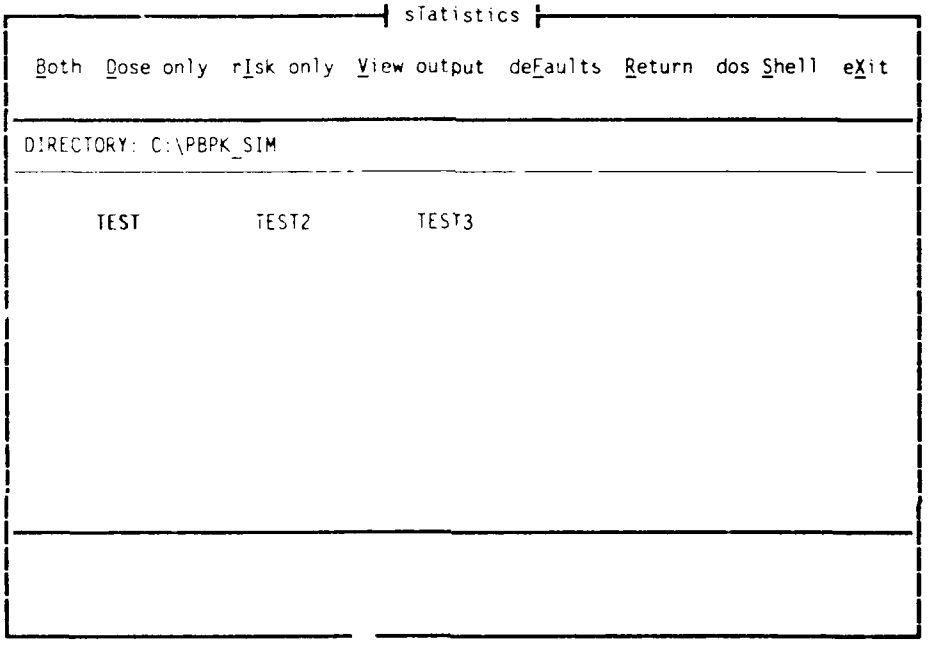
Sample CMD File

```
"Data created from file TEST on 09/26/90   11:31"

------setting up ACSL defaults ------------

S WESITG=.F.,NRWITG=.F.

------setting up prepar statements --------

PREPAR  'CLEAR',AUCB,AUCL,CA,CVL

------setting up simulation length control --------

S TSTOP=24,POINTS=1.0,H=10000

------setting up SCAT2 variables  --------

S VLCOP=0.055,VFCOP=0.1,VSCOP=0.7,VRCOP=0.05,VCCOP=0.095

------setting up variables that do not change --------

S QPC=20,QCC=13.8,QLC=0.25,QFC=0.09,QRC=0.51,QSC=0.15,BW=0.028
S KMAX=3.96,KFC=0.0001,KA=5.0,MW=165.83

------setting up special categories --------

S CC=.FALSE.

------setting up dose values --------

S PDOSE=0       ,IVDOSE=0       ,CONC=100

------setting up preferred values --------

S KM=1.97,PFA=8168,PLA=50.9,PSA=43.8,PRA=50.9,PB=16.9,VFC=0.1,VSC=0.7
S VRC=0.05,VLC=0.055,VCC=0.095
START

------setting up values that do change --------

S KM=7.0922,PFA=958.20,PLA=27.231,PSA=23.388,PRA=13.400,PB=18.130
S VLC=0.0692,VFC=0.0918,VSC=0.6870,VRC=0.0539,VCC=0.0978
START
S KM=4.1672,PFA=834.45,PLA=78.504,PSA=41.877,PRA=98.284,PB=11.068
S VLC=0.0529,VFC=0.1032,VSC=0.7117,VRC=0.0454,VCC=0.0865
START
S KM=1.5699,PFA=1191.9,PLA=57.013,PSA=76.975,PRA=21.958,PB=17.086
S VLC=0.0548,VFC=0.1010,VSC=0.6962,VRC=0.0479,VCC=0.0998
```

```
START
S KM=1.0939,PFA=671.27,PLA=46.410,PSA=64.056,PRA=28.623,PB=12.357
S VLC=0.0636,VFC=0.1037,VSC=0.6929,VRC=0.0473,VCC=0.0923
START
S KM=0.8346,PFA=1075.2,PLA=29.624,PSA=38.081,PRA=42.120,PB=9.0352
S VLC=0.0476,VFC=0.0952,VSC=0.7112,VRC=0.0526,VCC=0.0933
START
S KM=3.5335,PFA=361.98,PLA=61.758,PSA=24.901,PRA=56.51 ,PB=22.799
S VLC=0.0542,VFC=0.0991,VSC=0.7066,VRC=0.0527,VCC=0.0872
START
S KM=2.6661,PFA=768.51,PLA=39.313,PSA=43.509,PRA=18.965,PB=13.185
S VLC=0.0497,VFC=0.1090,VSC=0.7026,VRC=0.0557,VCC=0.0827
START
S KM=1.3050,PFA=748.10,PLA=69.399,PSA=36.428,PRA=47.281,PB=25.258
S VLC=0.0521,VFC=0.0956,VSC=0.7000,VRC=0.0534,VCC=0.0986
START
S KM=1.9095,PFA=879.94,PLA=38.094,PSA=30.537,PRA=67.725,PB=20.100
S VLC=0.0614,VFC=0.0993,VSC=0.6937,VRC=0.0471,VCC=0.0983
START
S KM=0.9133,PFA=611.98,PLA=49.024,PSA=57.506,PRA=77.79 ,PB=15.443
S VLC=0.0562,VFC=0.1267,VSC=0.6597,VRC=0.0489,VCC=0.1082
START
S KM=0.6894,PFA=936.01,PLA=33.682,PSA=35.445,PRA=35.993,PB=16.107
S VLC=0.0568,VFC=0.09  ,VSC=0.6960,VRC=0.0613,VCC=0.0958
START
S KM=0.5602,PFA=566.48,PLA=97.114,PSA=49.321,PRA=34.653,PB=14.477
S VLC=0.0419,VFC=0.1105,VSC=0.7175,VRC=0.0429,VCC=0.0869
START
S KM=0.3865,PFA=479.43,PLA=42.887,PSA=46.159,PRA=117.98,PB=30.311
S VLC=0.0588,VFC=0.1047,VSC=0.7012,VRC=0.0414,VCC=0.0937
START
S KM=0.2742,PFA=630.31,PLA=50.418,PSA=51.101,PRA=24.748,PB=19.271
S VLC=0.0508,VFC=0.0972,VSC=0.7010,VRC=0.0557,VCC=0.0950
START
S KM=0.2163,PFA=1266.7,PLA=54.029,PSA=32.573,PRA=51.592,PB=10.543
S VLC=0.0585,VFC=0.0825,VSC=0.7120,VRC=0.0412,VCC=0.1056
START
STOP
```

sTatistics

When the sTatistics menu is entered, the cursor highlights the first file on which statistics can be performed in the main display area. If there is only one file, or no file, the cursor will highlight the Both option of the menu bar and the selected file (if one exists). The files are created by running sImulations on the data file produced from the ACSL CSL files.



The options on the menu bar may be selected using Left Arrow, Right Arrow, and Enter. Each option is listed, with its functions briefly described, as follows:

Both equivalent to calling the Dose only and Risk only options one after the other.

Dose only performs statistics on the values of the variables selected for output from the associated data file and generated by ACSL (in a simulation run). Creates a table for each animal dosed group and one for the human dose.

rIsk only uses the values of the output variables (described above in Dose only) as input doses for fitting a dose-response model ($1 \le i \le 6$ stage, or multistage). Uses the animal dose-response data to compute maximum likelihood estimates and upper bounds on risk corresponding to the (simulated) human values of the same output variable.

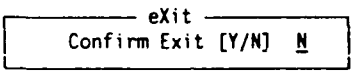
View output allows the user to browse an ASCII file.

deFaults For a complete description of the deFault option see the deFault section of this manual.

Return Closes the sTatistic window and returns the user to the Main Menu window.

dos Shell Suspends the execution of PBPK_SIM and returns the system to DOS. Any valid DOS command or application can be run. Typing the command EXIT and pressing Enter will reenter PBPK_SIM with all its settings unchanged.

eXit Opens a pop-up window (shown below) allowing confirmation before leaving PBPK_SIM. Typing Y and pressing Enter will end PBPK_SIM; N and Enter will close the confirmation window and return the cursor to the selection bar.



Main Display Area

The PgUp, Enter, and PgDn keys are used to move between the menu bar and the main display area provided more than one file exists for the selected ACSL CSL file. Left Arrow, Right Arrow, Up Arrow, and Down Arrow are used to move among the data files in the main display area. Once the desired file is highlighted, pressing Enter or PgUp will select the file. The selected file is indicated by flashing.

The files listed in the main display area are only those statistical files that have been created by running sImulations on the data files and are associated with the selected CSL file. For each CSL file that has had at least one data file created, there exists a file on the disk with the same name as the CSL file but with an extension of 'WCC'. This file contains the names of the data files that have been created for the CSL file. These names are then compared to see if a sImulation has been performed on that data file. If it has been simulated, it is listed as a valid selection.

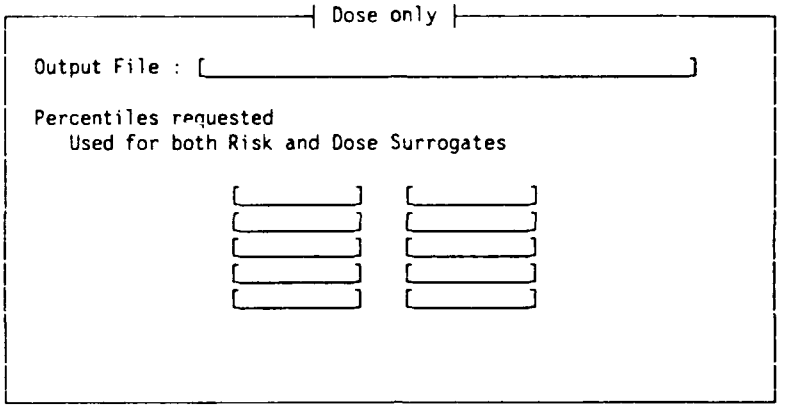
The sTatistical menu options above are discussed in more detail in the following sections.

Both

Choosing the Both option is identical to choosing the Dose only option and then choosing the rIsk only options. The Both option merely allows the user to run both Dose and rIsk options at once. See the descriptions of the rIsk an Dose options for a discussion of the windows and variables that are used in the Both option.

Dose only

Having selected the Dose only option, a window will appear requesting an output file name and the percentiles to be used in the statistical calculations. All information computed during the statistical calculations will be stored in the name provided in the Output File field.



If the output name given exists, the user will be asked to indicate whether the file should be [O]verwritten or whether the name should be [R]e-entered.

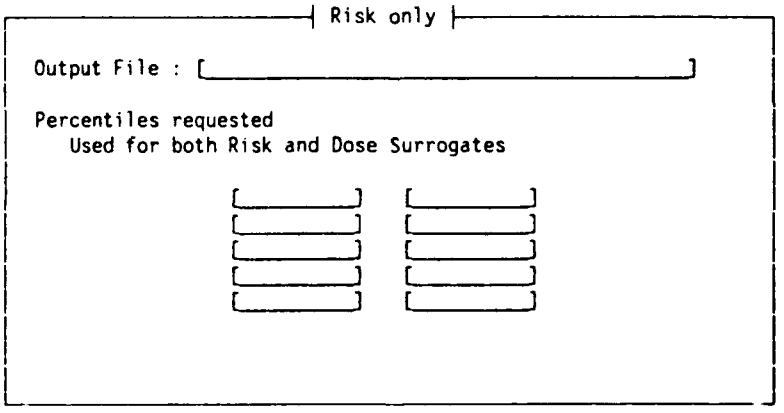
File exists, Overwrite, or Re-enter[O/R] ?[_]

While space for ten percentile values is provided, all ten fields do not have to be filled. The user may fill only as many as needed. The only restriction on the percentile fields is that their value be between 0 and 100, non-inclusive.

Once all necessary information has been provided, pressing F10 will begin the calculation of the statistical output. At any time during data entry, the user can press Esc to abort Dose only execution.

rIsk only

Having selected the Risk only option, a window will appear requesting an output file name and the percentiles to be used in the statistical calculations. All information computed during the statistical calculations will be stored in the name provided in the Output File field.

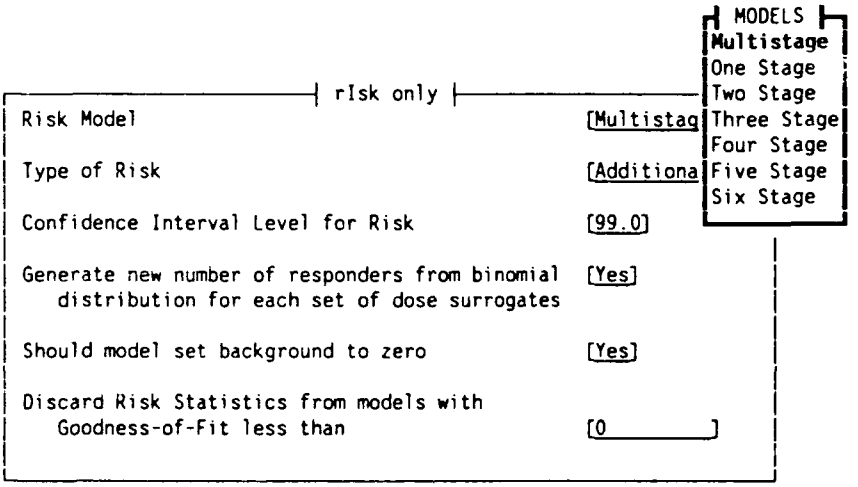


If the output name given exists, the user will be asked to indicate whether the file should be [O]verwritten or whether a the name should be [R]e-entered.

File exists, Overwrite, or Re-enter[O/R] ?[_]

While space for ten percentile values is provided, all ten fields do not have to be filled. The user may fill only as many as needed. The only restriction on the percentile fields is that their value be between 0 and 100, non-inclusive.

Once all necessary information has been provided, pressing F10 will bring up the next input window. At any time during the data entry the user can press ESC to abort Risk only execution.

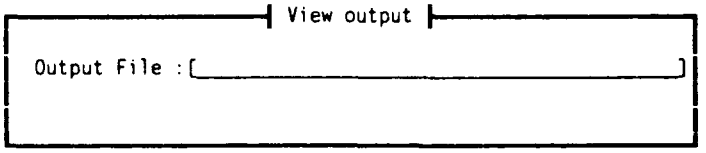


Explanation of these fields is in the Default section of this manual. Any values given here will apply only to this session of PBPK_SIM. In order to change these values for all sessions of PBPK_SIM, change the values on the Default screen and save the changes to disk.

View output

The View output option allows the user to view any ASCII file without exiting to a dos Shell. This is a browse-only window in which no editing is allowed and only the PgUp and PgDn keys are available for movement through the file. The line length of the window is 76 characters, therefore any line longer than 76 characters will wrap to the next line.

Selecting the View output option opens a window so that the user may specify the name of the file to be viewed.



A file in a directory other than the current directory may be viewed by specifying the file's entire path name. An example of the view window on the PHYSIM.CSL file follows.

```
                                PHYSIM.CSL
CONSTANT   TCHNG = 6.    $"#Length of inhalation exposure (hrs)"
"#ENDCAT"

"#CAT- BLOOD FLOW RATES"
CONSTANT   QPC = 14.     $"#Alveolar ventilation rate (l/hr)"
CONSTANT   QCC = 14.     $"#Cardiac output  (l/hr)"
CONSTANT   QLC = 0.25    $"#Fractional blood flow to liver"
CONSTANT   QFC = 0.09    $"#Fractional blood flow to fat"
CONSTANT   QRC = 0.45
"#Fractional blood flow to richly perfused tissue"
CONSTANT   QSC = 0.21
"#Fractional blood flow to slowly perfused tissue"
"#ENDCAT"

"#CAT- KINETIC  CONSTANTS"
CONSTANT   BW = 0.22     $"#Body weight (kg)"
CONSTANT   KMAX = 8.36 $"#Maximum velocity of metabolism (mg/hr-1kg)"
CONSTANT   KM = 0.36     $"#Michaelis-Menten constant (mg/l)"
    Page 2/11                                    more info - PgUp | PgDn
```

The bottom line indicates the number of pages in the file and the current page being displayed. The PgUp and PgDn keys will move the user through the document one page at a time. To return to the menu bar of the sTatistics window, press Esc.

Statistics provided

Statistics provided include the maximum, minimum, range, inner quartile range, arithmetic mean, and variance. Their definitions follow.

maximum	observation with the largest magnitude
minimum	observation with the smallest magnitude
range	difference between the maximum and minimum

inter-quartile range

difference between the 3rd (75%) and 1st (25%) quartiles (Q3-Q1). The program computes quartiles using this formula:

$$(1-g)x_j + gx_{j+1}$$

where

x	is the ordered vector of observations,
n	is the number of observations,
p	is the fractional quartile,
j	is the integer part of (n + 1) p,
g	is the fractional part of (n + 1) p, and
x_{n+1}	is treated as x_n.

arithmetic mean

$$\frac{\sum_{i=1}^{n} x_i}{n}$$

where

x_i	is the ith element of the ordered vector of observations and
n	is the number of valid observations.

variance

$$\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1}$$

where

x_i	is the ith element of the ordered vector of observations,
n	is the number of observations,
$\bar{x}$	is the arithmetic mean.

Sample Output

```
                Model:PHNEW
              Datafile:TEST926
  Experimental species:MOUSE
                  Dose:Level 1

  Variable                  Description
------------                ------------
  AUCB          Area under curve of concentration in blo
  AUCL          Area under curve of concentration in liv
  CA            Concentration in arterial blood
  CVL           Leaving liver venous blood conc
```

Variables	AUCB	AUCL	CA	CVL
Observations	35	35	35	35
Maximum	103.74	275.33	2.42296E-04	3.37041E-04
Minimum	21.472	39.720	-9.52870E-04	-2.35071E-03
Mean	50.172	136.97	-3.10243E-05	-9.46337E-05
Range	82.268	235.61	1.19517E-03	2.68775E-03
Q3 - Q1	28.191	64.484	1.34226E-04	1.41153E-04
Variance	412.50	2818.8	5.38001E-08	2.22102E-07
30th pctl	37.512	111.46	-1.55523E-06	-3.55727E-05
45th pctl	41.986	123.63	3.78032E-06	2.47388E-06
58th pctl	50.610	134.63	2.46156E-05	8.64228E-06
80th pctl	64.707	162.04	1.08080E-04	1.10870E-04

Model:PHNEW
Datafile:TEST926
Experimental species:MOUSE
Dose:Level 2

Variable	Description
AUCB	Area under curve of concentration in blo
AUCL	Area under curve of concentration in liv
CA	Concentration in arterial blood
CVL	Leaving liver venous blood conc

Variables	AUCB	AUCL	CA	CVL
Observations	35	35	35	35
Maximum	227.99	554.03	2.20054E-03	1.54464E-03
Minimum	45.658	148.29	-5.82603E-04	-8.12065E-04
Mean	113.91	319.88	8.89110E-05	6.18220E-05
Range	182.33	405.74	2.78314E-03	2.35671E-03
Q3 - Q1	48.661	163.67	1.67667E-04	1.77849E-04
Variance	1957.3	10001.	1.93588E-07	1.97606E-07
30th pctl	83.457	249.15	-6.89367E-06	-5.45642E-06
45th pctl	94.455	291.99	8.10409E-08	7.47653E-09
58th pctl	113.07	343.21	7.77064E-06	2.77616E-06
80th pctl	151.77	398.11	2.11007E-04	2.62692E-04

Model:PHNEW
Datafile:TEST926
Target species:HUMAN
Dose:Human

Variable	Description
AUCB	Area under curve of concentration in blo
AUCL	Area under curve of concentration in liv
CA	Concentration in arterial blood
CVL	Leaving liver venous blood conc

Variables	AUCB	AUCL	CA	CVL
Observations	35	35	35	35
Maximum	30.425	492.77	.21480	.21060
Minimum	9.4535	4.5547	1.36504E-02	1.34819E-02
Mean	17.300	84.458	7.24299E-02	7.14483E-02
Range	20.972	488.21	.20115	.19712
Q3 - Q1	7.8379	78.010	6.96199E-02	7.10677E-02
Variance	25.567	10177.	2.83177E-03	2.80405E-03
30th pctl	13.150	23.395	3.15982E-02	3.06227E-02
45th pctl	16.304	43.071	4.74657E-02	4.61972E-02
58th pctl	17.237	61.278	6.77605E-02	6.68784E-02
80th pctl	21.177	110.90	.10450	.10372

Model:PHNEW
Datafile:TEST926
Experimental species:MOUSE

Variable	Description
AUCB	Area under curve of concentration in blo

Degree (k)	Risk Type	Confidence Interval Size
1	Extra	95%

Model form

P (d) = 1 - exp (- q0 - q1 * d)

	P-value	MLE On Risk	Upper Bound On Risk
Observations	35	35	35
Maximum	.13570	.18208	.22574
Minimum	2.08162E-02	.11901	.14896
Mean	5.31821E-02	.14565	.18161
Range	.11488	6.30720E-02	7.67825E-02
Q3 - Q1	1.88218E-02	1.85800E-02	2.26570E-02
30th pctl	4.03427E-02	.13889	.17340
45th pctl	4.61950E-02	.14374	.17932
58th pctl	5.08864E-02	.14566	.18166
80th pctl	6.28918E-02	.15592	.19412

Model:PHNEW
Datafile:TEST926
Experimental species:MOUSE

Variable	Description
AUCL	Area under curve of concentration in liv

Degree (k)	Risk Type	Confidence Interval Size
1	Extra	95%

Model form

P (d) = 1 - exp (- q0 - q1 * d)

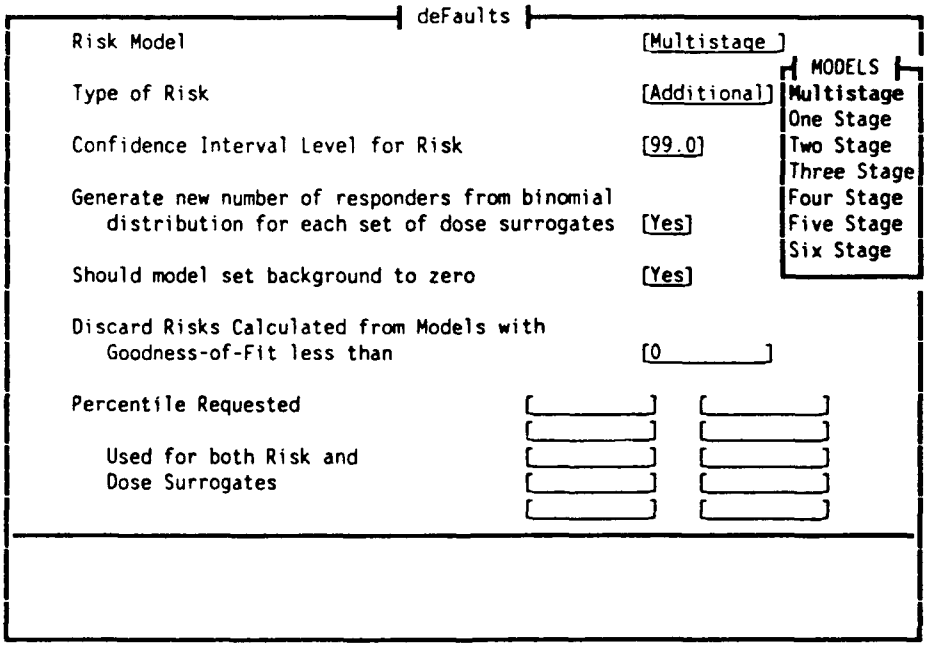
	P-value	MLE On Risk	Upper Bound On Risk
Observations	35	35	35
Maximum	.60746	.56948	.65782
Minimum	1.17975E-02	3.59328E-02	4.57446E-02
Mean	8.59227E-02	.18201	.22274
Range	.59566	.53355	.61207
Q3 - Q1	5.67988E-02	.14197	.17250
30th pctl	4.66672E-02	9.22211E-02	.11592
45th pctl	6.79199E-02	.13898	.17354
58th pctl	7.26558E-02	.17030	.21175
80th pctl	.10351	.24955	.30635

deFault window

All of the default values for running sImulations or sTatistics are set in the deFault window. The changes made from this window may be stored on disk in the PBPK_SIM.DFT file so that they become the default values loaded at the time PBPK_SIM is invoked. If the changes made are not saved to disk, then they are active only for the current execution of PBPK_SIM (until eXit is used). Default values changed anywhere other than this window are local to the window where the changes were made and apply only to the current session of PBPK_SIM. The default variables are:

risk model,
type of risk,
confidence interval level,
whether new responders should be generated from binomial distribution for each set of dose surrogates,
whether the background for the model should be set to zero,
the goodness-of-fit value for which models producing a fit less than that value should be discarded,
and up to ten requested percentiles.

The deFault window is arranged to show all the variables and their current values. Most of these values are set by selection from a pre-configured group of values that appear in selection sets. Invoking the deFault window produces the following display:



There is no menu bar for the deFault window. When all changes have been made to the window, the F10 can be used to save the changes. If F10 is pressed and no changes have been made,

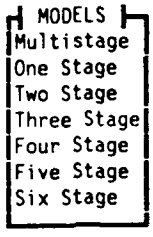
or anytime the Esc key is pressed, the window will be closed and control returned to the menu bar of the sTatistics window. If F10 is pressed and changes have occurred, the user will be prompted to indicate whether the changes should be saved to disk. A 'Y' indicates to save the changes to disk, thus making them the initial default values when PBPK_SIM is invoked. An 'N' indicates the changes are not to be saved to disk, therefore they will only be in effect during the current execution of PBPK_SIM.

Main Display Area

The cursor is initially positioned on the first field, Risk Model, of the deFault window. When the cursor is moved to any of the variables, except the Discard Risks Calculated from Models with Goodness-of-Fit less than and the Percentile Requested fields, a selection set will open listing the choices available for that variable. Home and End will move the cursor up and down through the possible values in the selection sets. A value is chosen by positioning the highlight cursor on the option and pressing Enter. Up Arrow and Down Arrow will move the cursor among the fields in the deFault window without changing the values. Pressing Esc at any time while editing the fields in the main display area aborts the changes and returns the user to the menu bar of the sTatistics window. Changes made to the fields can be saved by pressing F10.

Risk Model

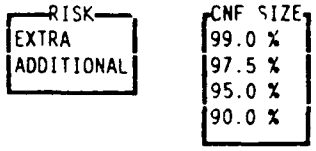
The Risk Model field allows the user to select the default model to be used from the following selection set.



The description of each model can be found in the Dose-Response Models section of the manual (page 69).

Type of Risk and Confidence Interval Level for Risk

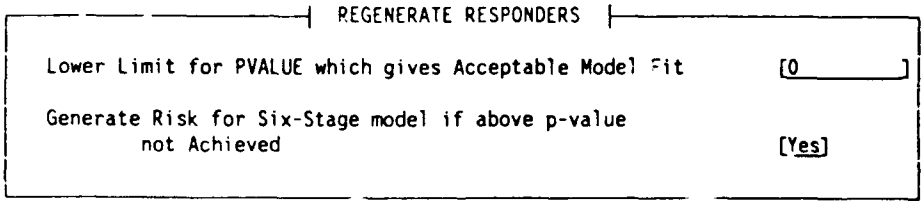
The Type of Risk and Confidence Interval Level for Risk input fields list the standard values used in risk assessment. The values are chosen from the windows shown below. These are used to calculate the maximum likelihood estimates and upper bounds on risk associated with a specific dose. The mathematical formulas for Extra and Additional Risk are defined in the DOSE-RESPONSE MODELS section of the manual along with explanation of how the type of risk and confidence interval size are used.



Generate new number of responders from binomial distribution for each set of dose surrogates

A response of No indicates that the number of responders (animals in each dose group with the response of interest) is taken from the input data file and is the same for each fitting of the dose-response model to the simulation data. A response of Yes indicates for each model fit to a set of simulation values a binomial distribution will be used to randomly generate a new number of responders for each dose group based on the probability of response for each dose group, based on the fit of the model (using preferred parameter values) to the original data.

When the user chooses to generate new numbers of responders for the fitting of the model to each set of simulation data, two additional parameters must be set. These are the lower limit on the goodness-of-fit p_value which will signify an acceptable fit for the preferred value data. If the model specified by the user does not give a fit that is greater than or equal to the value set here, the program will iteratively attempt to fit a model with one more parameter to the data, until an acceptable fit is achieved or the six-stage model has been fit and found lacking. If an acceptable fit is not achieved, the second variable will indicate whether risks are to be generated or the risk process is to be aborted for that simulation variable.



Should model background be set to zero ($q_0 = 0$)

If Yes sets parameter q_0 to 0.
No program estimates a value for q_0 during the model fitting stage.

Discard Risks Calculated fror. Models with Goodness-of-Fit less than

If the goodness-of-fit value returned for a model fit to one set of simulation data is less than the value provided in this field, then the risk statistics are not computed for that model and, therefore, are not available when creating the risk statistics output.

Percentiles Requested

The user may specify up to ten percentile values. While ten blanks are available the user may enter less than ten values. The only restriction on the fields are that the values fall between 0 and 100, non-inclusive. Before being used by PBPK_SIM, these values are divided by 100.

dos Shell

NOTE: Changing the directory in the dos Shell will have no effect on PBPK_SIM. Prior to executing the dos Shell option, the current directory is saved and then restored when EXIT is entered.

NOTE: The user should refrain from deleting or creating any files associated with the PBPK_SIM program while in the dos Shell. PBPK_SIM internally tracks the creation and deletion of many of its files. Therefore, if a file is created or deleted externally, PBPK_SIM will be unaware of this and will contain invalid data.

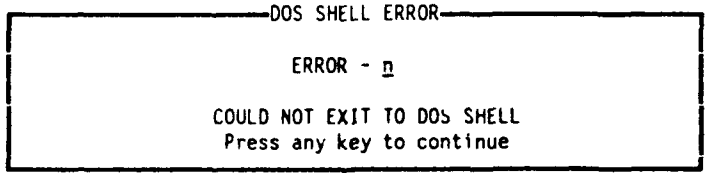
When tne dos Shell option is selected from one of the menus, the user is placed into a DOS command processing shell. Here the user may enter valid DOS commands, and they will be passed to the operating system and executed. Additional programs may also be executed while in the dos Shell provided there is enough RAM memory to carry out the execution.

When the user enters the dos Shell and after each command is executed, the following message will appear:

Type 'EXIT' to return to PBPK_SIM
prompt >

where *prompt* indicates the current directory.

If the system does not have enough RAM memory to execute the dos Shell option, the following message will be displayed:



where *n* is a number indicating the error condition. If a number not specified below appears, please contact the developers of PBPK_SIM. If the user is prompted to insert the disk with the temp file on it, see error code 2.

ERROR CODE	CONDITION
1	an error occurred when reading the temp file back into memory. This indicates that the file in which PBPK_SIM was stored has been corrupted. The only appropriate action is to reboot the machine.

2 the file in which PBPK_SIM was stored has been deleted. The only appropriate action is to reboot the machine.

9 an error occurred when attempting to read the temp file from EMS memory. The only appropriate action is to reboot the machine.

4 too many files have been opened. The dos Shell could not open a temp file in which to store PBPK_SIM. The solution is to increase the number in the FILES= statement of the CONFIG.SYS file.

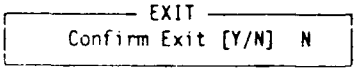
7 memory control blocks have been damaged. If this error occurs, contact us immediately.

10 not enough disk space. PBPK_SIM could not be saved because there was not enough disk space to create the temp file. The appropriate action is to remove files which are no longer needed from your hard disk.

If the dos Shell does not execute the commands given it, this could be because it does not know the location of COMMAND.COM or because enough RAM is not available. Check the PATH= setting in the AUTOEXEC.BAT file and the SHELL= statement in CONFIG.SYS to be sure that DOS can find COMMAND.COM. See your DOS manual for a discussion of the PATH and SHELL statements. If the error is because of insufficient RAM, removal of memory-resident utilities may be necessary before the dos Shell will work.

eXiting PBPK_SIM

To exit from PBPK_SIM, select the eXit option from any menu. When the cursor is on the menu bar, the eXit option can be selected through cursor movement with Left Arrow or Right Arrow, or by pressing the highlighted letter. After eXit has been selected, a pop-up confirmation window appears as below. Pressing Y and then Enter at this point will end execution of PBPK_SIM and return to the calling process (usually the operating system). Any other response will close the confirmation window and return the cursor to the menu bar.



CSL File Rules

A variation on the PHYSIM.CSL file (called PHNEW.CSL) has been provided to illustrate the way in which a .CSL file will have to be structured in order to use it in conjunction with PBPK_SIM. The rules for the form of the CSL files are listed below.

1. All comments must begin with the special character # immediately inside the opening quote mark. Comments are picked up by the parser in PBPK_SIM and used as descriptions for the constants, variables (dose surrogates and other output variables), and the categories (see rule number 2). Since ACSL does not allow any input past column 72, comments which cannot fit on the same line with the definition of the constant or variable must be placed on the line immediately after that definition. Only the first 40 characters of any comment (excluding any special characters such as # or #CAT-) are used as descriptions. In addition to a description of the constant, units can also be specified in a comment. The units are taken from within the comment and must be enclosed in parenthesis but do not need to be placed in the first 40 characters of the comment to be read properly. Up to 10 characters can be used to specify the units. The CONSTANT statement for BW below is an example of a comment which contains the units for the constant.

   CONSTANT BW = 0.22 $"#Body weight (kg)"

2. A category is a grouping of constants. Each category is defined by a set of comment statements bracketing the CONSTANT statements defining the constants which belong to the category. The comment statements which form the brackets for the category must be in the following form:

   "#CAT- *descriptive name for category*"

   "#ENDCAT"

   All constants must belong to a category. Categories are used to facilitate the selection of the distribution to be used when calculating values for the constants in the PBPK model and to set up special blocks of constants to be handled in specific ways by PBPK_SIM. Two of these categories must always be included. These are the SIMULATION LENGTH CONTROL and the DOSING INFORMATION categories. The constants which are defined in the example SIMULATION CONTROL LENGTH category in the provided PHNEW.CSL file must always appear with the names specified here but the comments, units and values can be varied to suit the user. The constant H is used in the definition of NSTP, the number of steps per integration performed by ACSL, in the INITIAL section. Similarly, POINTS and TSTOP are used to set the communication interval, CINT. Therefore, the two equations for CINT and NSTP listed below must also be included in the INITIAL section of the CSL file.

The setting of CINT is necessary for PBPK_SIM to be able to correctly read the information stored in the graphics files (files with the extension .RR?). The length of the simulation, controlled by TSTOP, can be set by the user within PBPK_SIM and the constant H can be adjusted in PBPK_SIM to either increase the speed (H small) or increase the precision of the integration (H large).

```
"#CAT- SIMULATION LENGTH CONTROL"
CONSTANT   TSTOP = 24.  $"#Length of experiment (hrs)"
CONSTANT   POINTS = 1.  $"#Number of points in plot"
CONSTANT   H = 10000
"#Used to set number of iterations per integration"
"#ENDCAT"

CINT  = TSTOP/POINTS     $"#Communication interval"
NSTP = CINT * H + 1      $"#number of integration steps"
```

The DOSING INFORMATION category contains both the doses and the lengths of dosing. The only restrictions are that there can be no more than five different types of doses (*i.e.*, oral, iv, air, etc.), the constants which define the length of dosing must begin with the letter T and must be defined immediately after the CONSTANT statement defining the dose to which they correspond. Since the lengths **must** begin with the letter T, the dose constants **cannot** begin with the letter T.

```
"#CAT- DOSING INFORMATION"
CONSTANT   PDOSE = 0.   $"#Oral dose (mg/kg)"
CONSTANT   IVDOSE = 0.  $"#IV dose (mg/kg)"
CONSTANT   TINF = .01   $"#Length of IV infusion (hrs)"
CONSTANT   CONC = 1000. $"#Inhaled concentration (ppm)"
CONSTANT   TCHNG = 6.   $"#Length of inhalation exposure (hrs)"
"#ENDCAT"
```

There are two special types of categories. The first type is for a group of constants which will be used by the PBPK model only under certain circumstances. An example of this type of category is the CLOSED CHAMBER special category shown below.

```
"#SCAT- CLOSED CHAMBER"
CONSTANT   CC = .FALSE.  $"#Default to open chamber"
CONSTANT   NRATS = 3. $"#Number of rats for closed chamber"
CONSTANT   KLC = 0.  $"#First order loss rate from closed chamber (/hr)"
CONSTANT   VCHC = 9.1 $"#Volume of closed chamber (l)"
"#ENDCAT"
```

Note that instead of **CAT-** the category comment begins with **SCAT-**. Each special category contains a logical variable as the first constant. If this constant has the value .TRUE. then the rest of the constants in the category will be used by the PBPK model. If its value is .FALSE., the model will not use these constants. PBPK_SIM

will request a value for the logical variable and only if the user specifies .TRUE. will values be requested for the other constants in the category.

The second type of special category, **SCAT2-**, sets up constants which are directly related in both name and value to the constants in a regular category. For example, the constants in **SCAT2- PREFERRED VALUES** are directly related to the constants in **CAT- FRACTIONAL VOLUMES OF TISSUES**. Note that the names of the constants in the special category are the same as the ones in the regular category with the addition of **0P** to each name. Since the zero (0) is used as a special indicator in the PREFERRED VALUES constant to show the name of the associated constant, the name of the associated constant should not contain a zero and must be 4 or less characters in length. When PBPK_SIM is calculating values to be used for the constants in the FRACTIONAL VOLUMES OF TISSUES category, the preferred value (mean) given for each of those constants by the user in a data file will be given to the associated constant in the PREFERRED VALUES category.

```
"#CAT- FRACTIONAL VOLUMES OF TISSUES"
CONSTANT  VLC = 0.04  $"#Fraction liver tissue"
CONSTANT  VFC = 0.07  $"#Fraction fat tissue"
CONSTANT  VSC = 0.75  $"#Fraction slowly perfused tissue"
CONSTANT  VRC = 0.05  $"#Fraction richly perfused tissue"
CONSTANT  VCC = 0.09  $"#Fraction carcass remaining"
"#ENDCAT"

"#SCAT2- PREFERRED VALUES"
CONSTANT  VLC0P = 0.04 $"#Fraction liver tissue or expected proportion"
CONSTANT  VFC0P = 0.07 $"#Fraction fat tissue or expected proportion"
CONSTANT  VSC0P = 0.75
"#Fraction slowly perfused tissue or expected proportion"
CONSTANT  VRC0P = 0.05
"#Fraction richly perfused tissue or expected proportion"
CONSTANT  VCC0P = 0.09 $"#Fraction carcass or expected proportion
"#ENDCAT"
```

Sample CSL File

```
PROGRAM: PHYSIOLOGICAL PHARMACOKINETIC MODEL (PHNEW)

INITIAL

LOGICAL    CC  $"#Flag set to .TRUE. for closed chamber runs"

"#CAT- SIMULATION LENGTH CONTROL"
CONSTANT   TSTOP = 24.  $"#Length of experiment (hrs)"
CONSTANT   POINTS = 1.  $"#Number of points in plot"
CONSTANT   H = 10000.0    $"#Number of integration steps"
"#Used to set number of iterations for integration"
"#ENDCAT"

"#CAT- DOSING INFORMATION"
CONSTANT   PDOSE = 0.   $"#Oral dose (mg/kg)"
CONSTANT   IVDOSE = 0.  $"#IV dose (mg/kg)"
CONSTANT   TINF = .01   $"#Length of IV infusion (hrs)"
CONSTANT   CONC = 1000. $"#Inhaled concentration (ppm)"
CONSTANT   TCHNG = 6.   $"#Length of inhalation exposure (hrs)"
"#ENDCAT"

"#CAT- BLOOD FLOW RATES"
CONSTANT   QPC = 14.    $"#Alveolar ventilation rate (l/hr)"
CONSTANT   QCC = 14.    $"#Cardiac output (l/hr)"
CONSTANT   QLC = 0.25   $"#Fractional blood flow to liver"
CONSTANT   QFC = 0.09   $"#Fractional blood flow to fat"
CONSTANT   QRC = 0.45
"#Fractional blood flow to richly perfused tissue"
CONSTANT   QSC = 0.21
"#Fractional blood flow to slowly perfused tissue"
"#ENDCAT"

"#CAT- KINETIC  CONSTANTS"
CONSTANT   BW = 0.22    $"#Body weight (kg)"
CONSTANT   KMAX = 8.36 $"#Maximum velocity of metabolism (mg/hr-1kg)"
CONSTANT   KM = 0.36    $"#Michaelis-Menten constant (mg/l)"
CONSTANT   KFC = 0.   $"#First order metabolism rate constant (/hr-1kg)"
CONSTANT   KA = 5.0     $"#Oral uptake rate (/hr)"
"#ENDCAT"

"#CAT- FRACTIONAL VOLUMES OF TISSUES"
CONSTANT   VLC = 0.04  $"#Fraction liver tissue"
CONSTANT   VFC = 0.07  $"#Fraction fat tissue"
CONSTANT   VSC = 0.75  $"#Fraction slowly perfused tissue"
CONSTANT   VRC = 0.05  $"#Fraction richly perfused tissue"
CONSTANT   VCC = 0.09  $"#Fraction carcass remaining"
"#ENDCAT"

"#SCAT2- PREFERRED VALUES"
CONSTANT   VLCOP = 0.04 $"#Fraction liver tissue or expected proportion"
CONSTANT   VFCOP = 0.07 $"#Fraction fat tissue or expected proportion"
CONSTANT   VSCOP = 0.75
"#Fraction slowly perfused tissue or expected proportion"
CONSTANT   VRCOP = 0.05
"#Fraction richly perfused tissue or expected proportion"
CONSTANT   VCCOP = 0.09 $"#Fraction carcass or expected proportion"
"#ENDCAT"
```

```
"#CAT- PARTITION COEFFICIENTS"
CONSTANT   PLA = 70.3  $"#Liver/air partition coefficient"
CONSTANT   PFA = 1638.0 $"#Fat/air partition coefficient"
CONSTANT   PSA = 20.0  $"#Slowly perfused tissue/air partition"
CONSTANT   PRA = 70.3  $"#Richly perfused tissue/air partition"
CONSTANT   PB  = 18.9  $"#Blood/air partition coefficient"
"#ENDCAT"

"#CAT- CHEMICAL SPECIFIC DATA"
CONSTANT   MW = 104.16  $"#Molecular weight (g/mol)"
"#ENDCAT"

"#SCAT- CLOSED CHAMBER"
CONSTANT   CC = .FALSE.  $"#Default to open chamber"
CONSTANT   NRATS = 3. $"#Number of rats for closed chamber"
CONSTANT   KLC = 0.  $"#First order loss rate from closed chamber (/hr)"
CONSTANT   VCHC = 9.1 $"#Volume of closed chamber (l)"
"#ENDCAT"


IF (CC) RATS = NRATS  $"Closed chamber simulation"
IF (CC) KL = KLC

IF (.NOT.CC) RATS = 0.  $"Open chamber simulation"
IF (.NOT.CC) KL = 0.
"(Turn off chamber losses so concentration remains constant)"

IF (PDOSE.EQ.0.) KA = 0.  $"#Parenteral dosing"

     "Scaled parameters"

       CINT  = TSTOP/POINTS      $"#Communication interval"
       NSTP = CINT * H + 1       $"#number of integration steps"
       VCH = VCHC-RATS*BW        $"#Net chamber volume (l)"
       AIO = CONC*VCH*MW/24450. $"#Initial amount in chamber (mg)"
       QC = QCC*BW**0.74         $"#Total cardiac output"
       QL = VLC * (QC * QLC) /VLCOP  $"#Blood flow to liver"
       QF = VFC * (QC * QFC) /VFCOP  $"#Blood flow to fat"
       QS = VSC * (QC * QSC) /VSCOP
"#Blood flow to slowly perfused tissue"
       QR = VRC * (QC * QRC) /VRCOP
"#Blood flow to richly perfused tissue"
       QC = QL + QF + QS + QR    $"#Scaled cardiac output"
       QP = (QPC / QCC) * QC     $"#Scaled alvelor ventilation rate"
       VL = VLC*BW               $"#Vol of liver tissue"
       VF = VFC*BW               $"#Vol of fat tissue"
       VS = VSC * BW             $"#Vol of slowly perfused tissue"
       VR = VRC * BW             $"#Vol of richly perfused tissue"
       VMAX = KMAX*BW**0.7       $"#Scaled to body weight vmax"
       KF = KFC/BW**0.3          $"#Scaled to body weight 1st order meta"
       DOSE = PDOSE*BW           $"#Scaled to body weight oral dose"
       IVR = IVDOSE*BW/TINF      $"#Scaled to body weight iv dose"
       PL = PLA / PB            $"#Liver/blood partition coefficient"
       PF = PFA / PB            $"#Fat/blood partition coefficient"
       PS = PSA / PB          $"#Slowly perfused tissue/blood partition"
       PR = PRA / PB          $"#Richly perfused tissue/blood partition"

END     $"End of initial"

DYNAMIC

ALGORITHM IALG = 2  $"#Gear method for stiff systems"
```

```
DERIVATIVE

   CIZONE = RSW((T.LT.TCHNG).OR.CC,1.,0.)
      RAI = RATS*QP*(CA/PB-CI) - (KL*AI)
"#Rate of loss from closed chamber"
       AI = INTEG(RAI,AIO)
       CI = AI/VCH*CIZONE  $"#Concentration in inhaled air (mg/l)"
       CP = CI*24450./MW   $"#Chamber concentration (ppm)"

      RMR = -KA*MR
       MR = DOSE*EXP(-KA*T)  $"#Amount remaining in stomach (mg)"

       CA = (QC*CV+QP*CI)/(QC+(QP/PB))
       "#Concentration in arterial blood (mg/l)"
     AUCB = INTEG(CA,0.)
    "#Area under curve of concentration in blood (mg*hr/l)"

       CX = CA/PB
   CXPPM = (0.7*CX+0.3*CI)*24450./MW $"#Exhaled air concentration (ppm)"
     RAX = QP*CX             $"#Rate of elimination in lungs"
      AX = INTEG(RAX, 0.)    $"#Amount exhaled (mg)"

     RAS = QS*(CA-CVS)     $"#Rate of accumulation in slowly perfused"
      AS = INTEG(RAS,0.)   $"#Amount in slowly perfused tissues (mg)"
     CVS = AS/(VS*PS)      $"#Slowly perfused venous blood conc (mg/l)"
      CS = AS/VS           $"#Slowly perfused tissue conc (mg/l)"

     RAR = QR*(CA-CVR)     $"#Rate of accumulation in richly perfused"
      AR = INTEG(RAR,0.)   $"#Amount in rapidly perfused tissues (mg)"
     CVR = AR/(VR*PR)      $"#Richly perfused venous blood conc (mg/l)"
      CR = AR/VR           $"#Richly perfused tissue conc (mg/l)"

     RAF = QF*(CA-CVF)      $"#Rate of accumulation in fat"
      AF = INTEG(RAF,0.)    $"#Amount in fat tissue (mg)"
     CVF = AF/(VF*PF)       $"#Leaving fat venous blood conc (mg/l)"
      CF = AF/VF            $"#Conc in fat (mg/l)"

     RAL = QL*(CA-CVL) - RAM + RAO   $"#Rate of accumulation in liver"
      AL = INTEG(RAL,0.)     $"#Amount in liver tissue (mg)"
     CVL = AL/(VL*PL)        $"#Leaving liver venous blood conc (mg/l)"
      CL = AL/VL             $"#Liver conc (mg/l)"
    AUCL = INTEG(CL,0.)
"#Area under curve of concentration in liver"

     RAM = (VMAX*CVL)/(KM+CVL) + KF*CVL*VL
     "#Rate of metabolism in liver"
      AM = INTEG(RAM, 0.)     $"#Amount metabolized (mg)"

     RAO = KA*MR          $"#Rate of input from stomach"
      AO = DOSE-MR        $"#Total mass input from stomach (mg)"

  IVZONE = RSW(T.GE.TINF,0.,1.)
      IV = IVR*IVZONE      $"#Intravenous infusion rate (mg/hr)"

      CV = (QF*CVF + QL*CVL + QS*CVS + QR*CVR + IV)/QC
    "# Mixed venous blood concentration (mg/l)"
   TMASS = AF+AL+AS+AR+AM+AX+MR   $"#mass balance (mg)"

  TERMT(T.GE.TSTOP)

END       $"End of derivative"
END       $"End of dynamic"
END       $"End of program"
```

Parsing the CSL File

The PBPK_SIM program uses the same CSL file that is used by ACSL. By the time the CSL file is parsed in PBPK_SIM it must have already met all of the syntactical requirements imposed by ACSL. It must also meet the requirements stated in the CSL File Rules section of this manual to be parsed correctly by PBPK_SIM.

When the cReate option is selected from the Options function of the Data window menu bar and a valid data file name has been provided, the CSL file selected from the MAIN MENU window will be read in for parsing. The PBPK_SIM program searches through the CSL file looking for key words such as, CONSTANT, INTEGER, REAL, CAT, SCAT, etc. It also searches for the '=' sign so that variables to the left of it can be retrieved. The PBPK_SIM program will not extract variables for ARRAYS or TABLE, so these should not be used in the CSL file.

The following describes what occurs when the parsing finds certain key words, the purpose of each key word and the set up of the CMD file are discussed in the CSL file section of this manual.

CONSTANT — moves right until the first non-blank character is encountered. A maximum of six characters (until a blank is encountered) are then picked up to form the name of the variable. An '=' sign is then searched for and, if found, the first non-blank character past the '=' is located. Characters are then retrieved to form the value of the variable. These characters are retrieved until a maximum of 20 have been obtained or a blank, '$', or end of line is found. If a '$' is located, then PBPK_SIM checks to see if the next character is the '#' sign. If the '#' sign does follow, this indicates the comment is for the PBPK_SIM program. The first 40 characters, or until a '(', or a "" is found, are picked up as the comment for the variable. If a '(' was discovered this indicates that a unit is being specified. The next 10 characters (or until the ')' is found) are picked up as the unit description for the variable. If the variable just parsed is discovered again during the parsing process, it is ignored.

INTEGER, REAL, LOGICAL — These are handled just as the CONSTANT key word except that no value is retrieved.

#CAT, #SCAT, #SCAT2 — When one of these is found, the program searches for the first non-blank character that occurs after it. The next 40 characters (or until the "" is found) are picked up as the category name. The CONSTANT declarations within the CATS are picked up as described above and are associated with the category title.

#ENDCAT — Terminates a category, as described above.

'='

When an equal sign is discovered that is not located in the CONSTANT declaration, the program back tracks until the first non-blank character is located. It then back tracks again until the beginning of the variable name is found. The characters of the variable (up to 6) are then picked up as the variable name. A search is then made to find the beginning of the comment, the '$', or the end of line. If the end of line is found, a check is made to see if the next line is a comment line. If it is, it is read as the comment for the variable. If '$' is found, the comment and units are stripped out as discussed in the CONSTANT declaration. If a duplicate variable is found, it is not saved. If this duplicate variable contains a different comment or unit value, the original comment or unit value is retained.

Dose-Response Models

The possible quantal models in PBPK_SIM use data on the numbers of animals in the experimental groups, numbers of animals with the responds of interest and doses to estimate the probability, P(d), that a response will occur when a subject is exposed to an amount (d) of the chemical in question. The doses used in the model fit procedure are the dose surrogates calculated by ACSL using the PBPK model chosen.

MULTISTAGE

$P(d) = 1 - \exp(-q_0-q_1d-...-q_kd^k)$ for $q_i \geq 0$, i = 0,...,k, and k = # of dose groups - 1.

ONE-STAGE

$P(d) = 1 - \exp(-q_0-q_1d)$ for $q_i \geq 0$, i = 0,1.

TWO-STAGE

$P(d) = 1 - \exp(-q_0-q_1d-q_2d^2)$ for $q_i \geq 0$, i = 0,...,2.

THREE-STAGE

$P(d) = 1 - \exp(-q_0-q_1d-q_2d^2-q_3d^3)$ for $q_i \geq 0$, i = 0,...,3.

FOUR-STAGE

$P(d) = 1 - \exp(-q_0-q_1d-q_2d^2-q_3d^3-q_4d^4)$ for $q_i \geq 0$, i = 0,...,4.

FIVE-STAGE

$P(d) = 1 - \exp(-q_0-q_1d-q_2d^2-q_3d^3-q_4d^4-q_5d^5)$ for $q_i \geq 0$, i = 0,...,5.

SIX-STAGE

$P(d) = 1 - \exp(-q_0-q_1d-q_2d^2-q_3d^3-q_4d^4-q_5d^5-q_6d^6)$ for $q_i \geq 0$, i = 0,...,6.

Parameter Estimation

Each model contains unknown parameters (denoted by q_i) that are estimated by values which make the models correspond most closely to the experimental data. The specific estimation method used is the maximum likelihood method. Suppose there are g treatment groups in an animal study and d_i is the dose delivered to the ith group. Let s_i be the total number of animals in the ith group and r_i the number of animals in the ith group having the response of interest. Then for a particular dose response function P(d) the logarithm of the likelihood of the experimental data is

$$\ln L = C + \sum_{i=1}^{g} \{r_i \ln P(d_i) + (s_i - r_i) \ln [1 - P(d_i)]\}$$

where C does not involve the unknown parameters. The maximum likelihood estimates (MLEs) of the parameters are those which maximize the likelihood, or equivalently, ln L. These estimates are calculated using a deflected gradient search employing a variation of the quasi-Newton method (Broyden, 1970; Fletcher, 1970; Goldfarb, 1970; Shanno, 1970; see also Bertsekas, 1982). The search is confined to the parameter space determined by the restrictions on the parameters in the definition of the dose responses, P(d).

After each step in the search, the appropriate Kuhn-Tucker conditions are checked (see Guess and Crump, 1976). The correct set of parameters is considered to be the maximum likelihood estimates if each of the Kuhn-Tucker conditions is satisfied to within a tolerance of 10^{-8}, based on a normalization of experimental doses so that the highest dose is taken to be 1.

The p-value that is used to express the goodness-of-fit of a model is derived as the p-value associated with the chi-square statistic

$$\sum_{i=1}^{g} \frac{[r_i - s_i\ P(d_i)]^2}{s_i\ P(d_i)\ [1-P(d_i)]}$$

and based on the chi-square distribution. Degrees of freedom are assumed to be g - (number of parameters whose MLEs are not on the boundary of the parameter space) (see Crump and Crockett, 1985, for a discussion of this heuristic approach).

Maximum likelihood estimates of additional or extra risk for a given dose, d, are calculated using these formulas and the maximum likelihood parameter estimates in the dose response function P(d). Additional risk is defined as

$$P(d) - P(0)$$

and extra risk as

$$\frac{P(d) - P(0)}{1 - P(0)}$$

Confidence Limits

The likelihood method (see Cox and Lindley, 1974, and Crump and Crockett, 1985) is used to compute upper bounds on risk and lower bounds on dose producing a given level of risk. Let ln L_m be the maximum value of the log-likelihood, that is, the log-likelihood ln L evaluated at the maximum likelihood parameter estimates. A upper 95% confidence limit for the extra risk, π,

corresponding to a given dose, d, is calculated as the largest π for which model parameters exist that satisfy the model constraints on the parameters and for which

$$\pi = \frac{P(d) - P(0)}{1 - P(0)}$$

and

$$2(\ln L_m - \ln L) = 2.70554$$

are both satisfied. The obvious changes are made if additional risk is involved rather than extra risk.

For 90%, 97.5%, and 99% bounds, 2.70554 is replaced, respectively, by the cumulative 80th percentage point (1.64237), 95th percentage point (3.84146), and 98th percentage point (5.4119) of the chi-square distribution with one degree of freedom.

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