

Low Density Lipoprotein (LDL) Metabolism

Case Study

- How to enter data from a spreadsheet
- How to simultaneously analyze plasma and urine data
- How to model cumulative and individual urine samples
- How to use change conditions
- How to create a split input

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Prerequisites

The prerequisite for this case study is having worked through the SAAM II introductory tutorial, “Getting Started with SAAM II Compartmental.” It is recommended that you work through the Using SAAM II tutorials “Using Change Conditions” and “Using Experimental Inputs.”

What you will learn in this case study

- How to enter data from a spreadsheet.
- How to simultaneously analyze plasma and urine data.
- How to model cumulative and individual urine samples.
- How to use change conditions.
- How to obtain create split inputs.

Data Required

The data file for this case study is

LDL.xls

which is an Excel® spreadsheet. The contents of this file are included at the end of this case study.

Introduction

Low density lipoproteins (LDL) are particles in the plasma whose primary purpose is to transport cholesterol to cells in the periphery. Its kinetics have been studied by many investigators for many years. Since LDL is operationally defined as particles in the plasma that float in the 1.019 – 1.063g/ml density range, to study them one needs first to isolate them, and then label them normally with radioactive iodine. When radioiodinated LDL was reinjected into an individual, plasma samples were normally taken to 14 days. The decay was always biexponential so a two-compartment model was used to analyze the data.

But LDL was known to be physicochemically heterogeneous, and the model, with its single plasma compartment, could not take this into account. When urine data were collected together with the plasma data, the kinetic heterogeneity was revealed.

The data used in this case study are taken from Foster et al¹. From a modeling point of view, they give an opportunity to discuss modeling strategies in analyzing plasma and urine data simultaneously:

- Instantaneous change conditions to model the individual (24 hours) urine collections;
- Split inputs to deal with the fact that LDL is kinetically heterogeneous; and

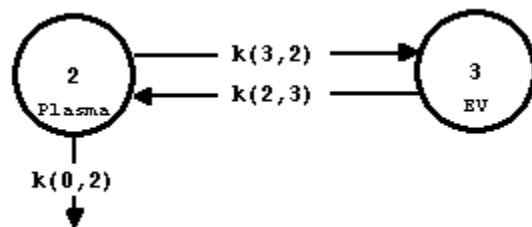
To begin, we will analyze the plasma data using the standard two-compartment model. We will then see this model cannot accommodate the plasma and urine data simultaneously, and that changes in the model structure are required.

1. Foster, DM, A Chait, JJ Albers, C Harris and JD Brunzell. Evidence for kinetic heterogeneity among human low density lipoproteins. *Metabolism* 35:685-696, 1986

Part 1. Create a two-compartment model using the SAAM II Compartmental application.

Low density lipoprotein kinetic described following a bolus injection of radioiodinated LDL is virtually always described by a two-compartment model. This will be the point at which we start the case study.

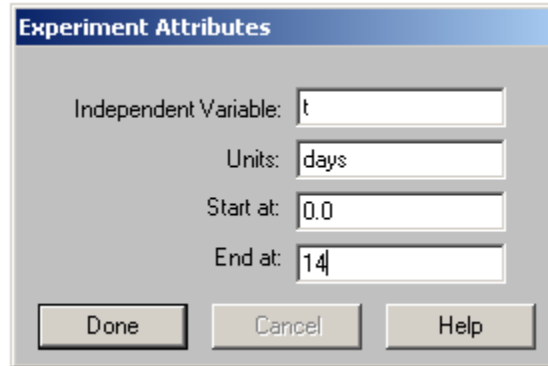
1. **Start the SAAM II Compartmental application.** The **SAAM II Compartmental** main window will open.
2. Create the following two-compartment model.



In creating this model, you compartment numbers will “1” and “2” to begin. You will have to renumber Compartment 2 first, and name it “EV” for extravascular. Then you can renumber and name Compartment 1.

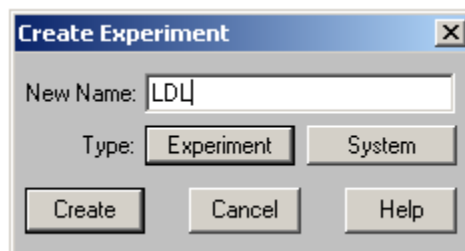
3. Create the experiment.
 - a. In the **SAAM II Toolbox**, click **Experiment**. Notice that the **Model** tools are unavailable and the **Experiment** tools are available. The **Experiment Attributes** dialog box will open.

- b. Change the entry in the **Units** box from “minutes” to “days.”
- c. Enter “14” in the **End at** box. The **Experiment Attributes** dialog box will appear as follows:

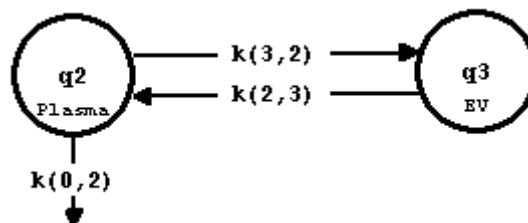


- a. Click **Done**.

The **Create Experiment** dialog box will appear on the **Drawing Canvas**. The choice of experiment **Types** is an **Experiment** or a **System**. **Experiment** is selected with the name “Exper”. Replace “Exper” with “LDL” by typing “LDL” in the **New Name** box. The **Create Experiment** dialog box will appear as follows:




- e. Click **Create**. The model will appear as follows:



4. Add the data from the spreadsheet.

- a. Open the Excel® spreadsheet **LDL.xls**. Select the data as shown in the following:

	A	B	C	D
1	DATA			
2	(FSD 0.1)			
3	t	plasma	cum_urine	ind_urine
4	(/24)	0	0	0
5	0	n	n	n
6	0.16	29745	n	n
7	0.5	31008	n	n
8	1	29114	n	n
9	2	27907	n	n
10	4	27593	n	n
11	6	27483	n	n
12	9	26610	n	n
13	12	25791	n	n
14	0	0	0	0
15	1	20899	6979047	6979047
16	2	13646	2.37E+07	1.67E+07
17	3	9714	3.92E+07	1.56E+07
18	4	6456	5.11E+07	1.19E+07
19	5	4806	5.92E+07	8091944
20	6	3804	6.50E+07	5846717
21	7	3267	6.89E+07	3832041
22	8	2744	7.16E+07	2696780
23	9	2279	7.34E+07	1882786
24	10	2131	7.48E+07	1394947
25	11	1971	7.58E+07	985794
26	12	1720	7.67E+07	876272
27	13	1667	7.74E+07	712898
28	14	1622	7.81E+07	643487
29	END			

- b. Copy the data, and close the spreadsheet.
- c. In the **Show** menu, click **Data**, or alternatively, on the **SAAM II Toolbar**, click **Data** . The **Data** window will open.
- d. In the **Data** window, paste the data copied from the spreadsheet.
- e. In the **Edit** menu, click **Check Data Format**. If there are any errors, correct them. The **Data** window will appear as follows:

```

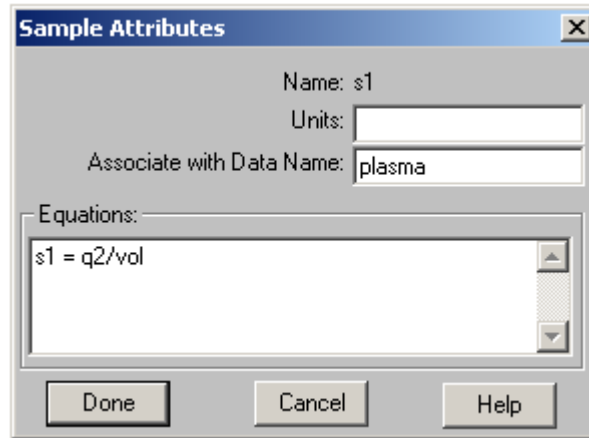
DATA
(FSD 0.1)
t      plasma  cum_urine  ind_urine
(/24)  ()        ()         ()
0      n        n         n
0.16  29745    n         n
0.5   31008    n         n
1     29114    n         n
2     27907    n         n
4     27593    n         n
6     27483    n         n
9     26610    n         n
12    25791    n         n
()     ()        ()         ()
1     20899    6979047  6979047
2     13646    2.37E+07  1.67E+07
3     9714     3.92E+07  1.56E+07
4     6456     5.11E+07  1.19E+07
5     4806     5.92E+07  8091944
6     3804     6.50E+07  5846717
7     3267     6.89E+07  3832041
8     2744     7.16E+07  2696780
9     2279     7.34E+07  1882786
10    2131     7.48E+07  1394947
11    1971     7.58E+07  985794
12    1720     7.67E+07  876272
13    1667     7.74E+07  712898
14    1622     7.81E+07  643487
END
Data Format is okay      Edited

```

Notice the third line of the data file contains operators for the columns. In the column for “t”, the (/24) will divide each entry by 24 converting the units from hours to days. For time “plasma”, “cum_urine” and “ind_urine” there are no operation, but the placeholder “()” must be entered so SAAM II knows which column(s) to operate on. After time “12” the time units switch to days, and division for unit conversion is no longer required.

- f. Close the **Data** window.
5. Create the plasma sample.
 - a. In the **SAAM II Toolbox**, click **Sample**.
 - b. Click Compartment **q2**, and then click on the **Drawing Canvas**. The sample **s1** will appear associated with Compartment **q2**.
 - c. Double-click **s1** to open the **Sample Attributes** dialog box.

- d. In the **Associate with Data Name** box, type “plasma”.
- e. In the **Equations** box, edit the sample equation “s1=q1” to read “s1=q1/vol”. The **Sample Attributes** dialog box will appear as shown below:



- f. Click **Done**.
6. Create the experimental input
 - a. In the **SAAM II Toolbox**, click **Input**.
 - b. Click Compartment **q2**, and then click on the **Drawing Canvas**. An input arrow, **ex1**, will appear on the drawing canvas pointing to Compartment **q2**.
 - c. Double-click **ex1**. The **Exogenous Input** dialog box will open.
 - d. In the **Input Type** pane, be sure **Bolus** is selected.
 - e. Enter “1.0e+08” in the **Initial Amount** box.
 - f. Click **Add**. The **Exogenous Input** dialog box will appear as follows:

Exogenous Input

Name: Reference Name: Units:

Type	Initial	Constant	Start	Stop	Repeat Every	Nr. Repeats
Bolus	1.00e+8	-	0.000	-	-	-

Input Type:

Bolus
 Infusion
 Primed Infusion
 Equation

Initial Amount:

Constant Rate:

Event Start:

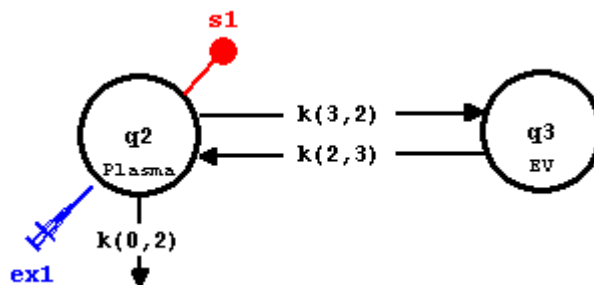
Event Stop:

Repeat Every:

Nr. of Repeats:

Equation:

g. Click **Done**. The model will appear as follows:



Part 2. Analyze the plasma data.

The first step is to analyze the plasma data by themselves using the traditional two-compartment model.

1. Enter the parameter values as shown below:

The Parameters dialog box contains a table with the following data:

Name	Type	Current	Low Limit	High Limit
k(0,2)	Adj	0.2000	0.0200	2.0000
k(2,3)	Adj	0.2000	0.0200	2.0000
k(3,2)	Adj	0.2000	0.0200	2.0000
vol	Adj	3400.0000	340.0000	34000.0000

Below the table, the 'vol' parameter is detailed:

- Name: vol
- Value: 3400.00000000
- Type: Fixed, Adjustable
- Low Limit: 340.00000000
- High Limit: 34000.00000000

Buttons: Done, Cancel, Help, Edit, Save.

How to obtain the initial parameter estimates is the subject of the case studies dealing with the two-compartment model.

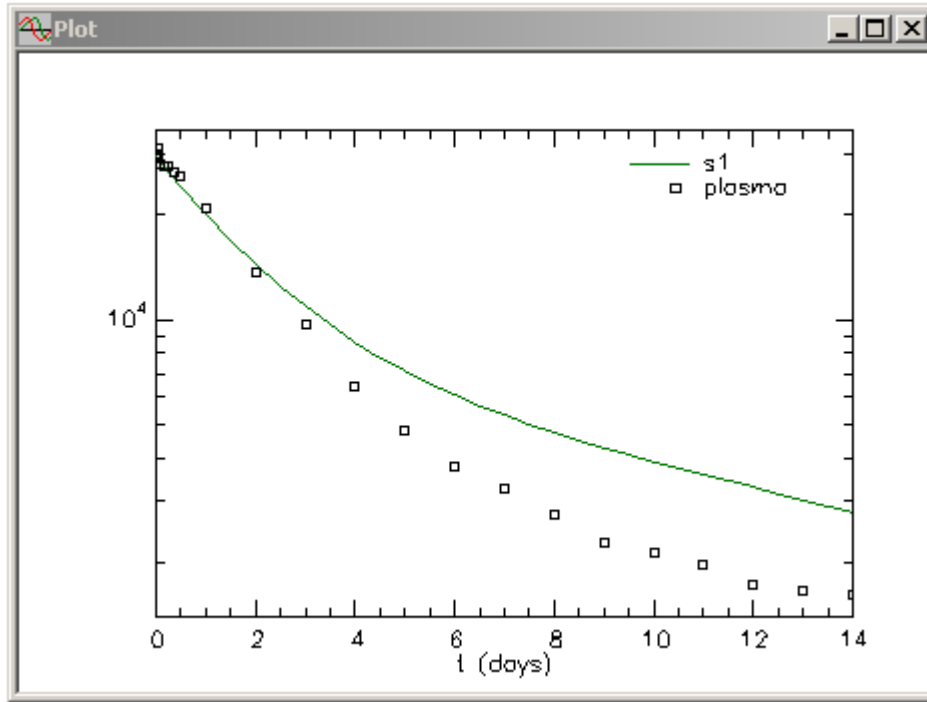
2. Solve and view the solution. When you plot your solution, it will be useful to set the **Plot and Table Scale** as follows:

The Plot and Table Scale dialog box shows the following settings:

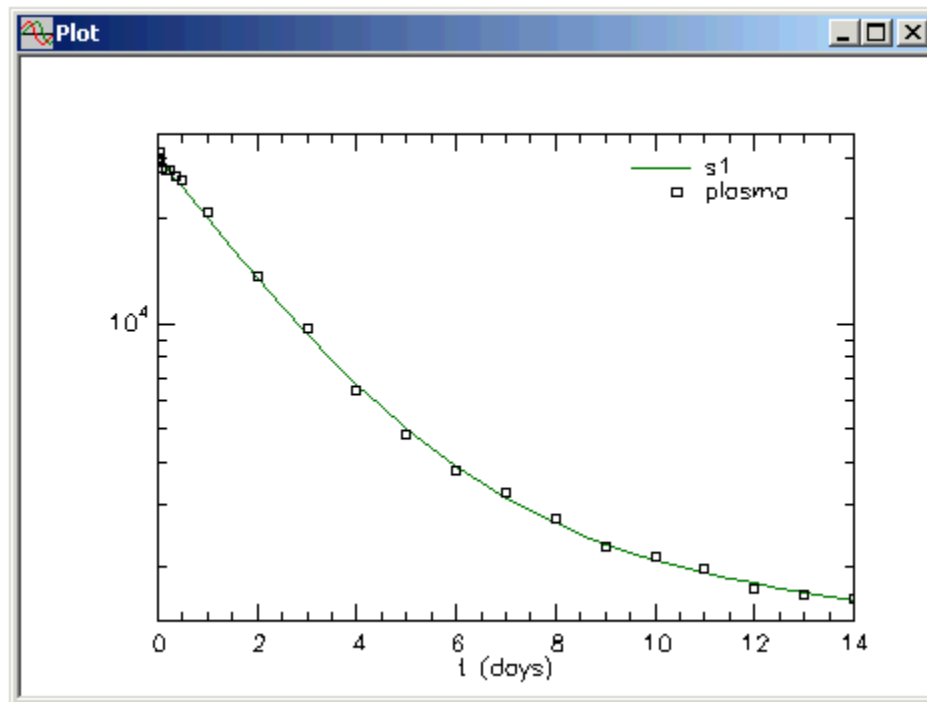
Axis	Mode	Minimum	Maximum
X Axis	<input checked="" type="radio"/> AutoScale	0.0	14.00000000
	<input type="radio"/> Set	0.0	14.00000000
Y Axis	<input type="radio"/> AutoScale	1622.00000000	31008.00000000
	<input checked="" type="radio"/> Set	1400.00000000	35000.00000000

Buttons: Done, Cancel, Help.

Your plot in semi-log mode will appear as follows:



3. Fit the model and view the solution. Your plot will be updated as follows:



The statistics associated with this Fit are shown as follows:

Parameter/Variable	Value	Std.Dev.	Coef. of Var.	95% Confidence Interval	
k(0,2)	0.23687	2.35653e-002	9.94866e+000	0.18736	0.28638
k(2,3)	0.07702	1.73766e-002	2.25610e+001	0.04051	0.11353
k(3,2)	0.18603	1.64011e-002	8.81637e+000	0.15157	0.22049
vol	3290.27012	3.96907e+001	1.20631e+000	3206.88311	3373.65713

	Objective	Scaled Data Variance
sl : plasma	1.191241e+001	1.083518e-001

Total objective	1.191241e+001	
AIC	7.102417e+000	
BIC	7.226399e+000	

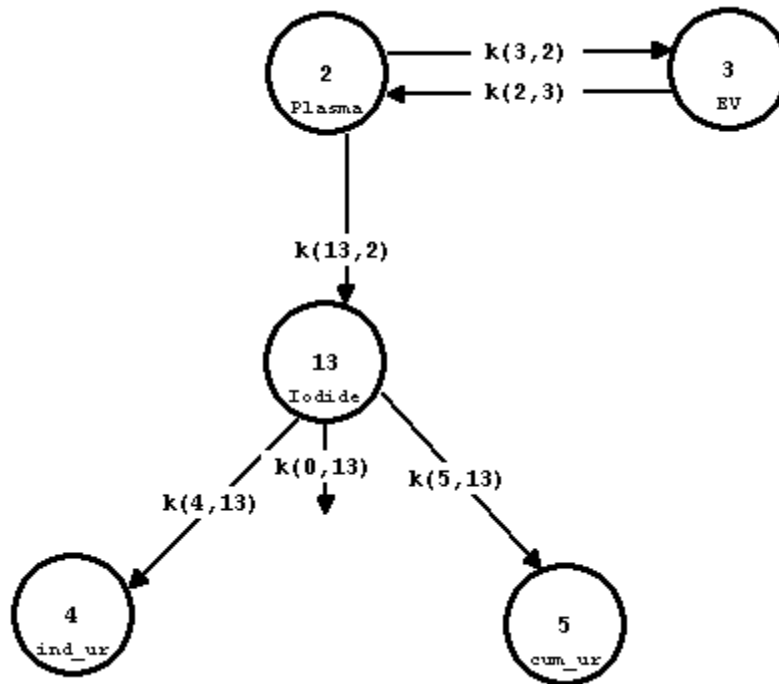
Close all open windows.

If you wish, before proceeding to the next part of this case study, you can examine the residuals (or weighted residuals) associated with this Fit; you can also perform a test for goodness-of-fit. If you add a third compartment to your model, you will find this is not supported by the data.

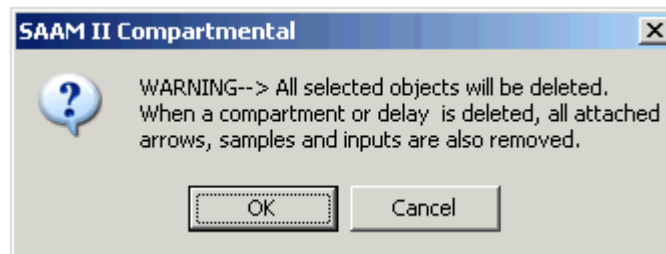
Record the parameter values as they will be used in the next part of the case study.

Part 3. Analyze the plasma and urine data simultaneously using the two-compartment model to describe LDL kinetics

In analyzing the urine data, you must remember that the tracer is radioiodinated LDL. The label coming out in the urine is radioactive iodide. Thus when LDL is metabolized, the radioactive iodide enters the total body iodide pool, and then moves into the urine. Iodide kinetics are very rapid compared to LDL; it is known that the body iodide pool turns over at about 2.5/day. Thus a body iodide pool will be added to the model, and from this pool the radioactive iodide will enter the urine. The model to be created is shown below:



1. Create the model shown above.
 - a. In the **SAAM II Toolbox**, click **Model** so these tools are available.
 - b. Click $k(0,2)$.
 - c. Press **Delete**. The following message will appear:



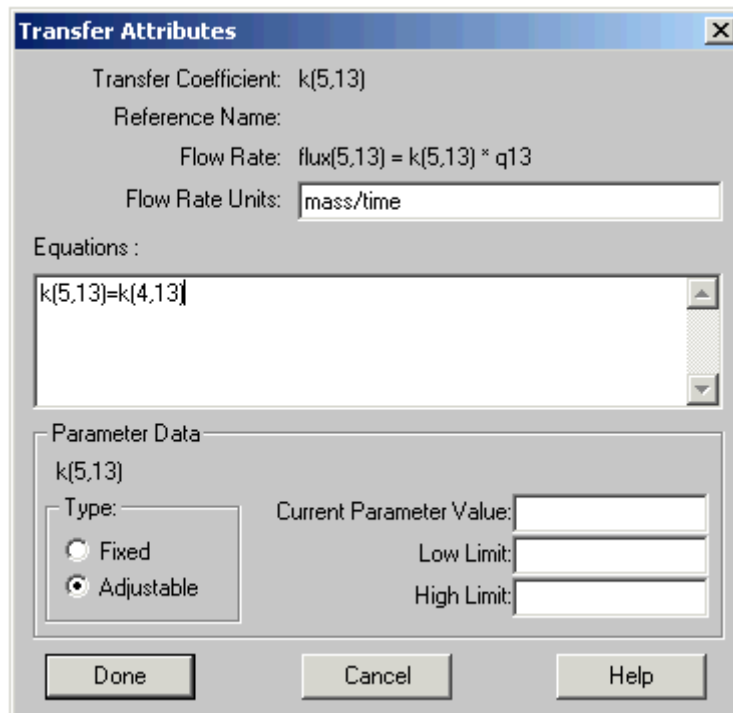
- d. Click **OK**.
- e. Add the compartments and transfers as shown above. Compartment **13** will appear first as Compartment **1**. If you add Compartments **4** and **5** next, then you can just renumber Compartment **1** to Compartment **13**.
- f. In the **SAAM II Toolbox**, click **Experiment** to make these tools available. Remember the experimental attributes, input and sample information have already been defined, and do not change because you added the new compartments to your model.

2. Define the parameters describing urinary loss of iodide.

In the model, the loss from Compartment **q13** will be set equal to 2.5 since this is the turnover of the total body iodide pool. The rate constant $k(13,2)$ replaces $k(0,2)$ from the first model.

However, the individual and cumulative urine samples are two different ways of describing the same data. Obviously $k(4,13)$ and $k(5,13)$ must be the same (equal to 2.5). But this will double the loss from Compartment **q13**. Thus we must introduce a loss $k(0,13)$ from Compartment **q13**, and set it equal to “ $-k(4,13)$ ”.

- a. Double-click $k(5,13)$ to open the **Transfer Attributes** dialog box.
- b. Type “ $k(5,13) = k(4,13)$ ” in the **Equation** pane. The **Transfer Attributes** dialog box will appear as follows:



- c. Click **Done**.
- d. Double-click $k(0,13)$ to open the **Loss Attributes** dialog box.
- e. Type “ $k(0,13)=-k(5,13)$ ” in the **Equation** pane.
- f. Click **Done**.



Balancing losses. When modeling both individual and cumulative urine samples, you will have a transfer rate into the two compartments representing the urine collections. The question arises: why look at both modes of representing urine? The answer is that they provide different kinds of information. The cumulative urine provides a measure of how much material is actually excreted. In radioactive studies, this is important since one wants to account for the injected material. As more urine is collected, the counts are higher and the data generally “better.”

The individual urine counts will indicate how well the subject adhered to the protocol. If they failed to collect a sample at a specified time, then all future cumulative urine data will not be accurate. If they collected at a different than specified time, this will appear as an “error” in the individual datum, but the cumulative data will still be okay.

One has to balance the loss from the compartment feeding the two urine compartments. In this case, the two compartments are **4** and **5** with input from Compartment **13**. The differential equation for the losses from Compartment **13** is:

$$\frac{dq_{13}}{dt} = -(k(4,13) + k(5,13) + k(0,13))q_{13} + \dots$$

If both $k(4,13)$ and $k(5,13)$ were present by themselves, this would double the loss from Compartment **q13** making it inaccurate. Setting “ $k(0,13) = -k(4,13)$ ” accomplishes the following:

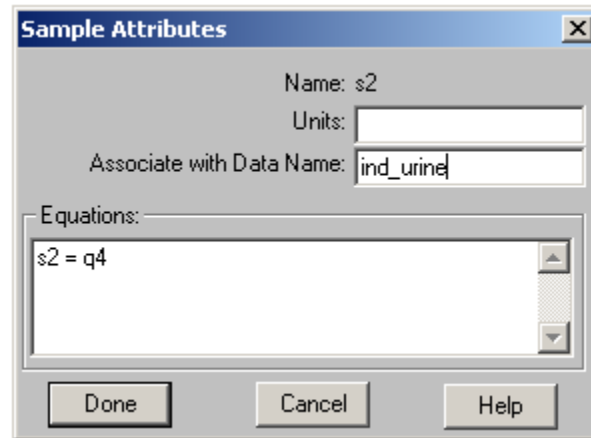
$$\begin{aligned} \frac{dq_{13}}{dt} &= -(k(4,13) + k(5,13) + k(0,13))q_{13} + \dots \\ &= -(k(4,13) + k(5,13) - k(5,13))q_{13} + \dots = -k(4,13)q_{13} + \dots \end{aligned}$$

and the differential equation for Compartment **q13** is correct.

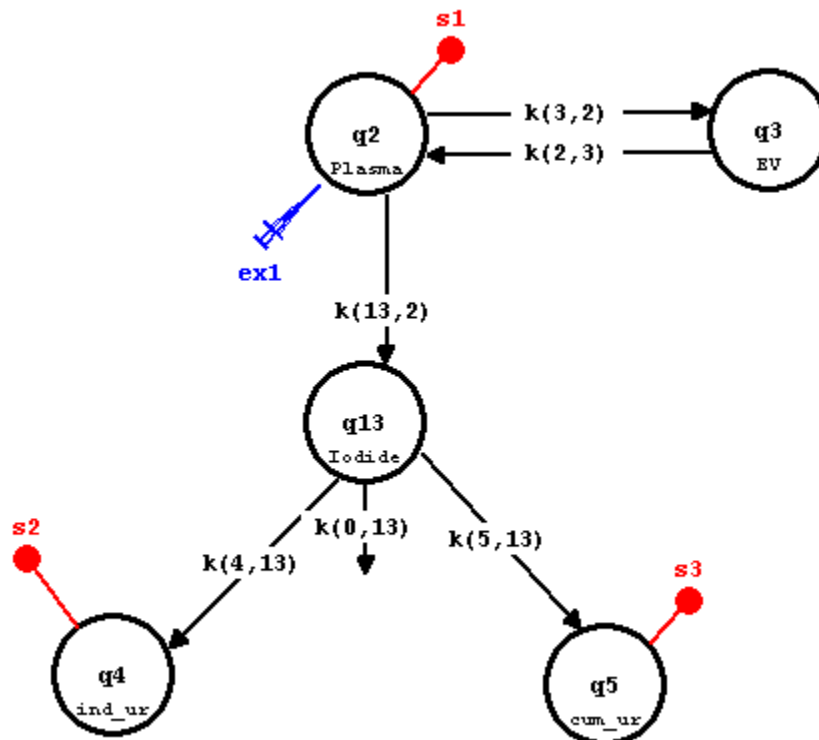


3. Add the samples for the urine collections.
 - a. In the **SAAM II Toolbox**, double-click the **Sample** tool.
 - b. Click Compartment **q4** and the **Drawing Canvas** to create **s2**; click Compartment **q5** and the **Drawing Canvas** to create **s3**.
 - c. In the **SAAM II Toolbox**, click **Select**.

- d. You will probably want to move **s2**. Double-click **s2** to open the **Sample Attributes** dialog box.
- e. Type “ind_urine” in the **Associate with Data Name** box. Leave the sample equation along. The **Sample Attributes** dialog box will appear as follows:



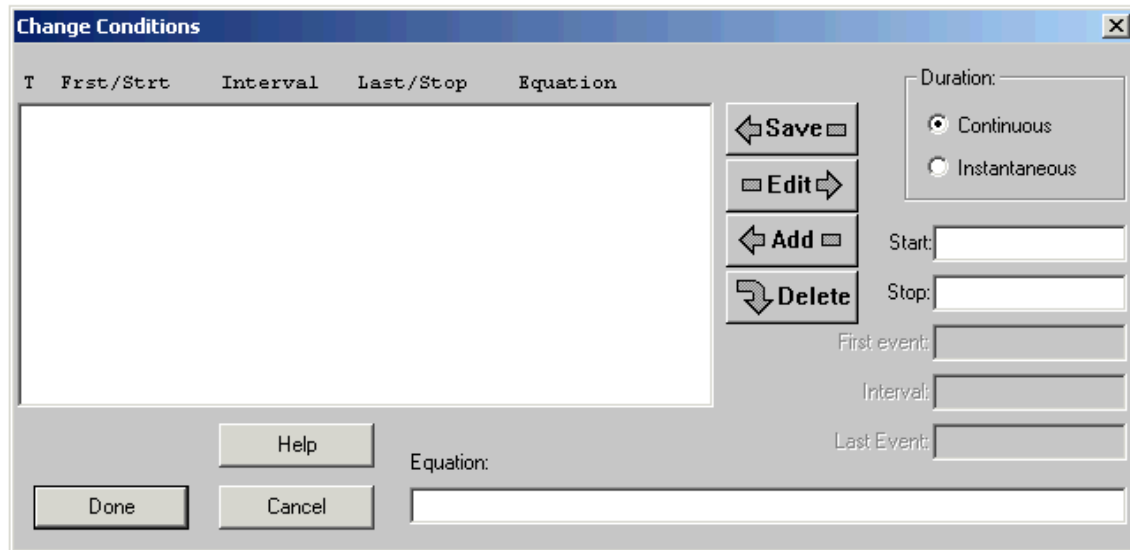
- f. Associate “cum_urine” with sample **s3**. Your model will appear as follows:



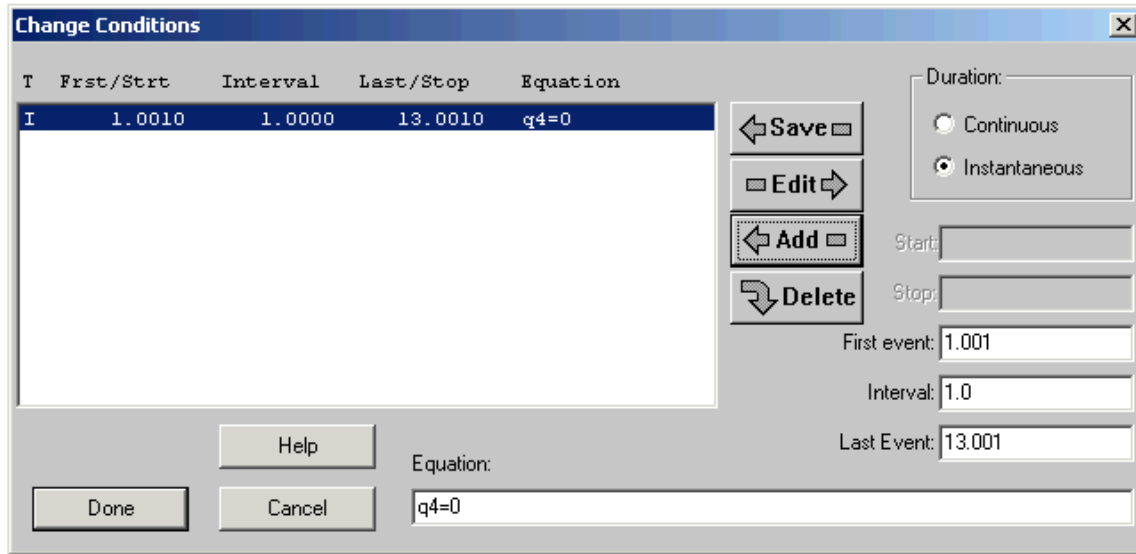
4. Create the individual urine samples.

To create the individual urine samples, it is necessary to reset the value of Compartment **q4** equal to zero following the daily collection period. This will be done using the **Change Condition** tool.

- a. In the **SAAM II Toolbox**, click **Change Condition**. The **Change Condition** dialog box will open as follows:



- b. In the **Duration** pane, select **Instantaneous**.
- c. In the **First event** box, enter “1.001”.
- d. In the **Interval** box, enter “1.0”.
- e. In the **Last Event** box, enter “13.001”.
- f. In the **Equation** pane, type the equation “ $q4 = 0$ ”.
- g. Click **Add**. The **Change Condition** dialog box will appear as follows:



- h. Click **Done**. What this will do is reset the value of Compartment **q4** equal to zero daily just after the urine collection period. Thus on the first day, the collection is made at time “1”; the resetting is just after that at “1.001.”
5. Enter the parameter values.

The first step in analyzing the urine data is to fix the plasma parameters, and see if the urine data can be described. Thus enter the parameters as shown in the following **Parameter** dialog box. All parameters are fixed to those you obtained from the best fit of the plasma data by the two-compartment model with $k(13,2)$ taking the place of $k(0,2)$; this assumes all iodide is lost into the body iodide pool.

Name	Type	Current	Low Limit	High Limit
k(2,3)	Adj	0.0770	0.0200	2.0000
k(3,2)	Adj	0.1860	0.0200	2.0000
k(4,13)	Fix	2.5000		
k(13,2)	Adj	0.2370	0.0237	2.3700
vol	Adj	3290.2701	340.0000	34000.0000

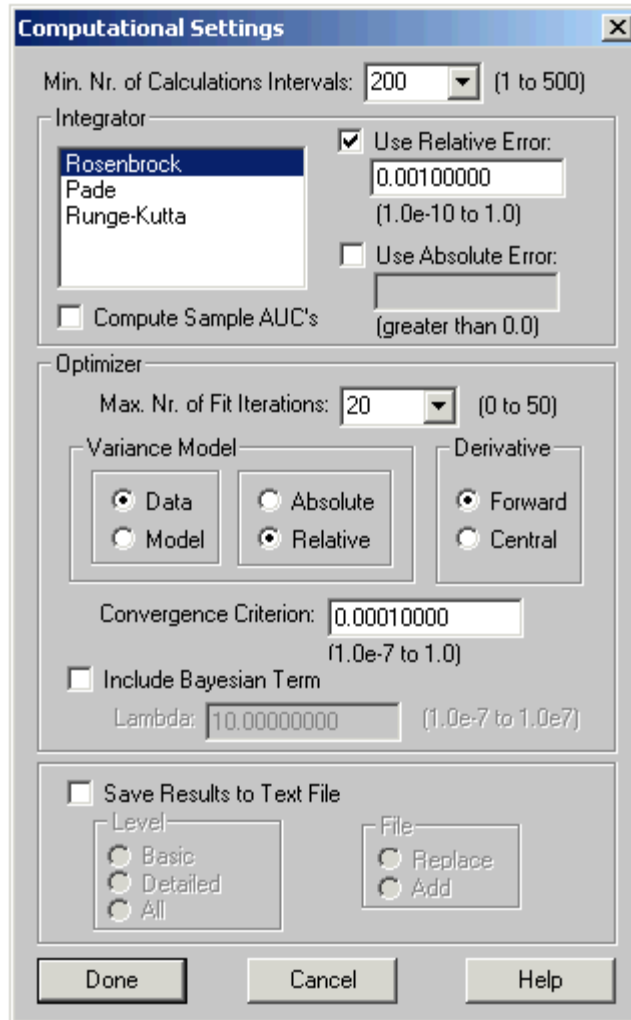
Name: k(4,13) Value: 2.5

Type: Fixed Adjustable

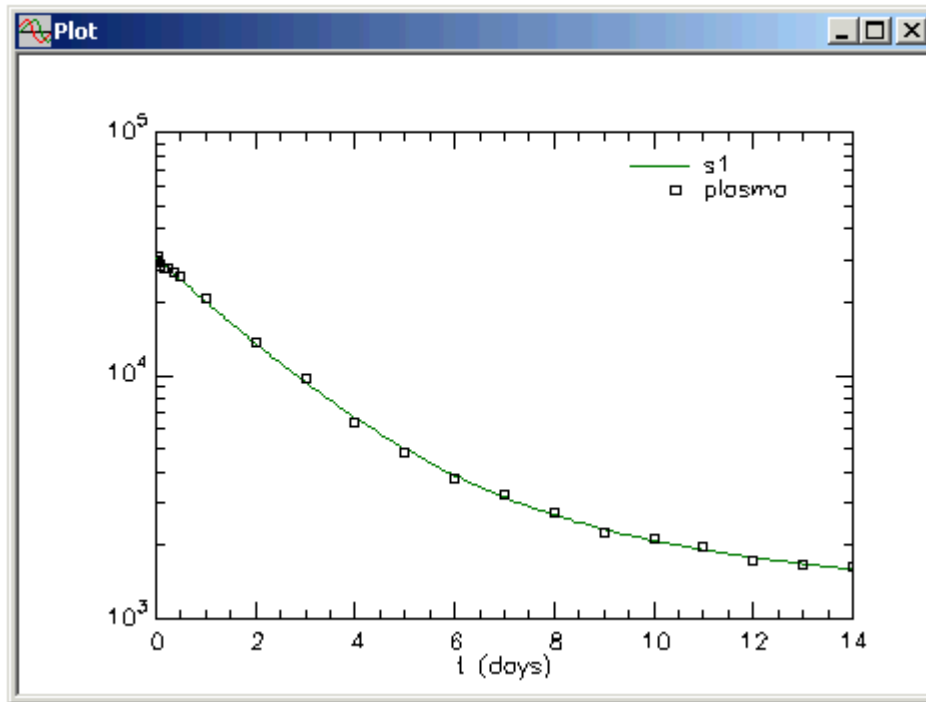
Low Limit: 0.25000000 High Limit: 25.00000000

Buttons: Done, Cancel, Help, Edit, Save

6. Solve the model and view the solution.
 - a. It will be useful to have a better resolution of the plots. In the **Compute** menu, click **Settings**. The **Computational Settings** dialog box will open.
 - b. Enter “200” in the **Min. Nr. Calculations Intervals** box. The **Computational Settings** dialog box will appear as follows:



- c. Click **Done**.
- d. Solve your model, and view the plasma data. The plot should appear as follows:



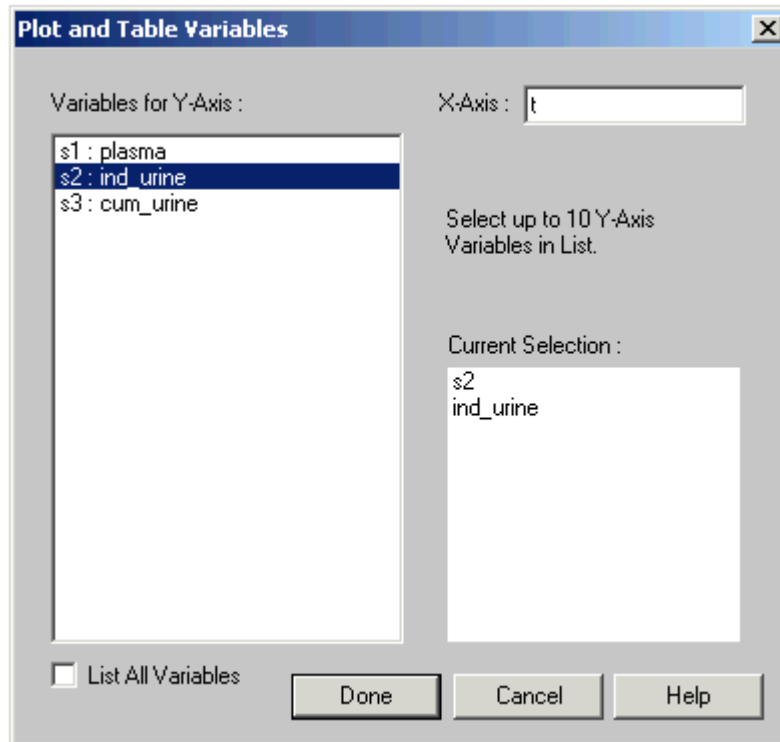
This is the same plot as that obtained from the best fit of the two-compartment model to the plasma data. Notice, however, when you changed the model structure, SAAM II has reverted to the default values for the plot scale.

Leave the **Plot** window open.

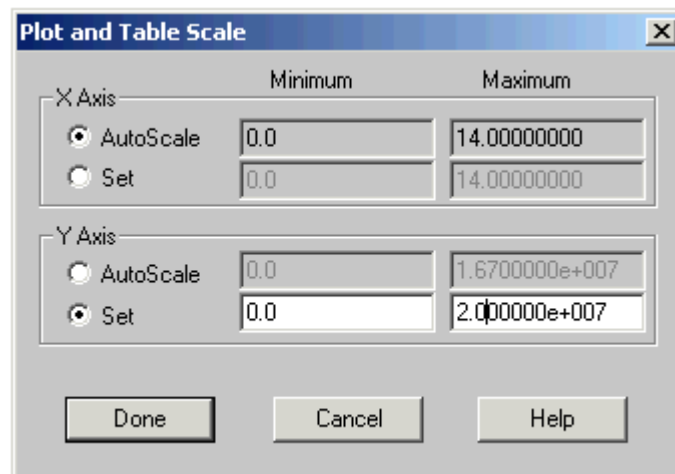
e. Evaluate the individual urine simulation.

(1) In the **Set** menu, click **Plot/Table Variables**. The **Plot and Table Variables** dialog box will open.

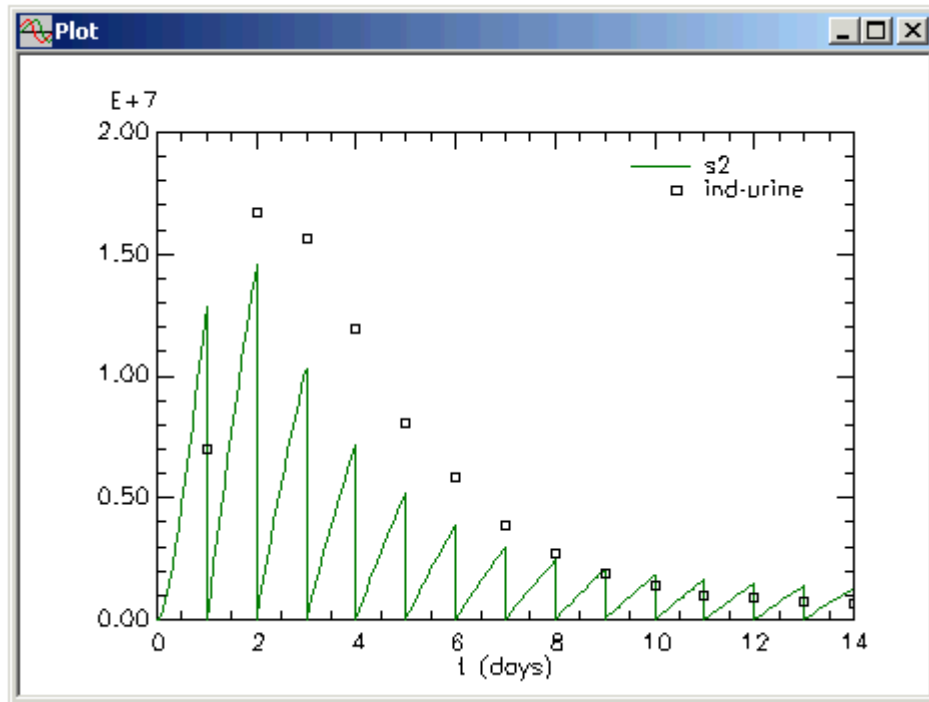
(2) Click **s2:ind_urine** to move this to the **Current Selection** pane. The **Plot and Table Variables** dialog box will appear as follows:



- (3) Click **Done**. Set the plot in linear mode, and change the **Plot Scale** as follows:



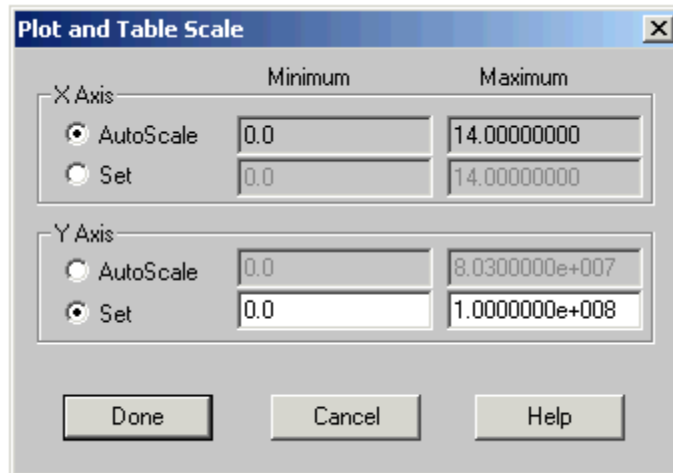
- (4) Click **Done**. The plot of the individual urine samples and the model simulation will appear as follows:



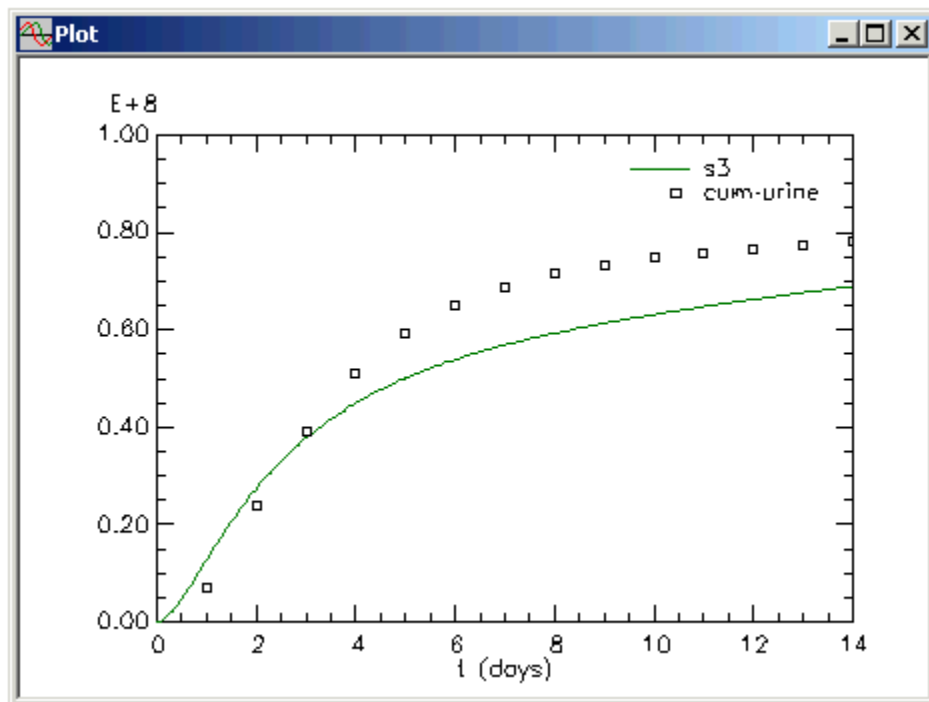
Remember this is the model predictions knowing that the model accurately describes the plasma data. It is clear that the model over-predicts the first datum, then under-predicts to day 8 after which the model over-predicts. It is possible that the first datum is spurious; this will be resolved as part of the modeling process.

One might think there is a loss pathway from Compartment **q2**, i.e. a $k(0,2)$. While this might help with the first datum, it will only make the under-predictions worse. After we examine the cumulative urine, we will fit the model to the data and see what happens.

- f. Evaluate the cumulative urine simulation.
 - (1) In the **Set** menu, click **Plot/Table Variables**. The **Plot and Table Variables** dialog box will open.
 - (2) Click **s3:cum_urine** to move this to the **Current Selection** pane.
 - (3) Click **Done**. Set the plot in linear mode, and change the **Plot Scale** as follows:



- (4) Click **Done**. The plot of the cumulative urine samples and the model simulation will appear as follows:



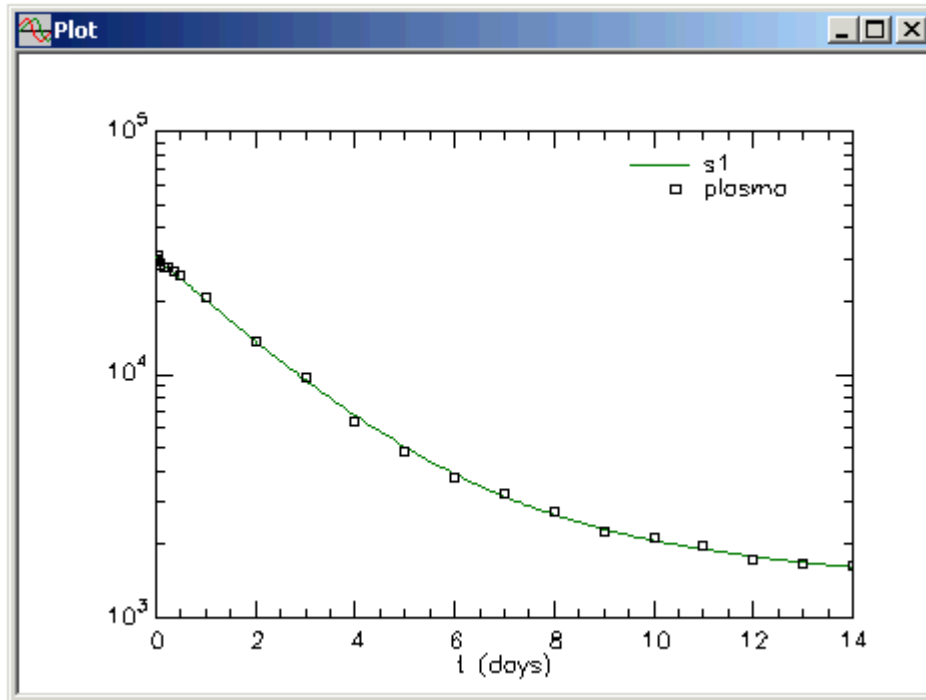
- (5) Close the **Plot** window.

7. Fit the model to the data, and view and evaluate the solution.

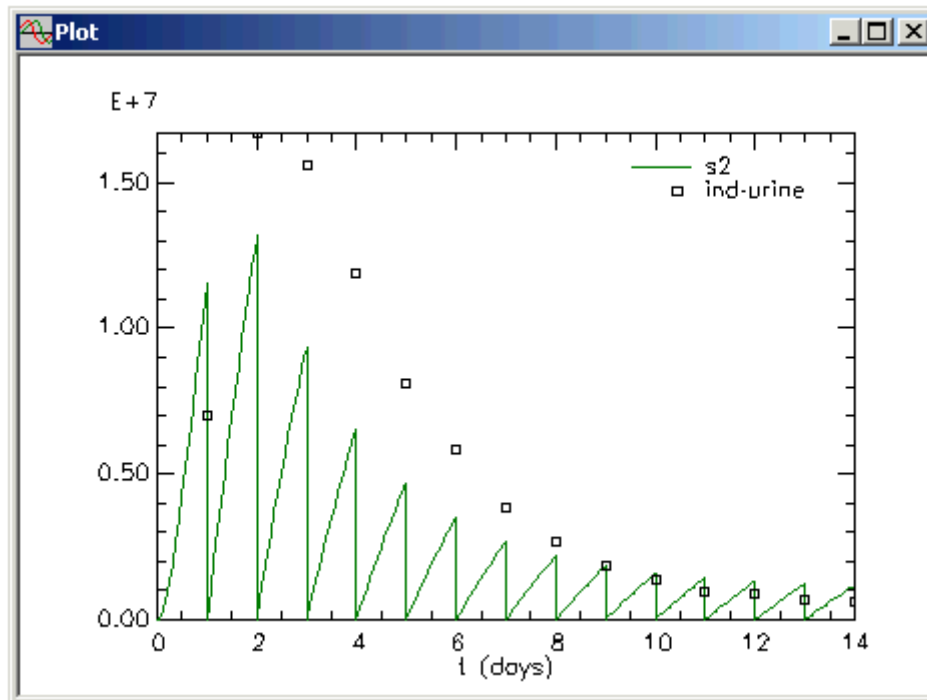
It is clear from the simulation that the model cannot possibly describe all of the data simultaneously. It is instructive, however, to Fit the model to the data and see what happens.

- a. Fit the model to the data.

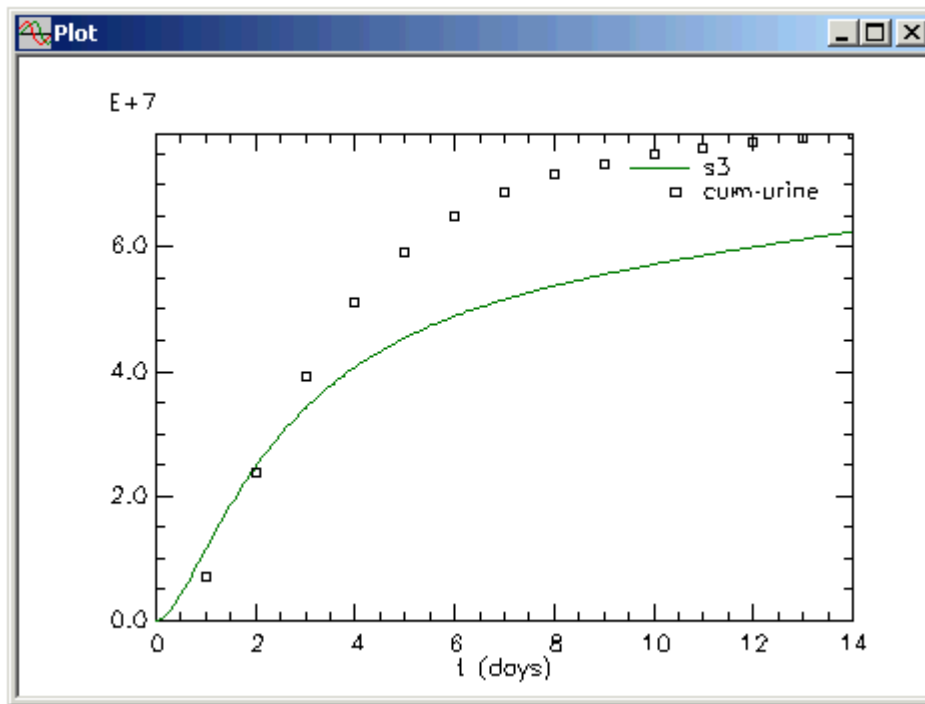
- b. View **s1:plasma**. Your plot will appear as follows (in semi-log mode; set the **Plot Scale** to **AutoScale**):



- c. View **s2:ind_urine**. Your plot will appear as follows (in linear mode):



d. View **s3:cum_urine**. Your plot will appear as follows (in linear mode):



e. View the statistics. The **Statistics** window will appear as follows:

Parameter/Variable	Value	Std.Dev.	Coef. of Var.	95% Confidence Interval	
k(2,3)	0.06213	6.90337e-003	1.11110e+001	0.04822	0.07604
k(3,2)	0.20159	1.16945e-002	5.80099e+000	0.17803	0.22516
k(4,13)	2.50000	** Fixed **	** Fixed **	** Fixed **	** Fixed **
k(13,2)	0.21303	1.36489e-002	6.40688e+000	0.18553	0.24053
vol	3301.59598	3.69163e+001	1.11814e+000	3227.21531	3375.97664
----- Derived Variables -----					
k(0,13)	-2.50000	0.00000e+000	0.00000e+000	-2.50000	-2.50000
k(5,13)	2.50000	0.00000e+000	0.00000e+000	2.50000	2.50000

		<input type="radio"/> Correlation Matrix		<input type="radio"/> Covariance Matrix	
		<input checked="" type="radio"/> Objective			
		Objective		Scaled Data Variance	
s3 : cum_urine	9.503779e+000			7.983406e+000	
s2 : ind_urine	8.241777e+000			2.395371e+001	
s1 : plasma	5.261308e+000			1.008064e-001	

Total objective	2.300686e+001				

AIC	1.256237e+001				
BIC	1.269621e+001				

In terms of evaluating the fit, the plasma does not change much. Clearly the individual and cumulative urine data can never be described by this model, despite the fact that the statistics are reasonable.

Thus the two-compartment model for LDL kinetics must be rejected, and a new model postulated.

Part 4. Expand the model.

To expand the model, we will take into account the fact that LDL is a heterogeneous collection of particles. Thus rather than a single plasma compartment for the labeled LDL, we will postulate two kinetically distinct compartments whose sum is the data. What happens is the following. LDL is radioiodinated, and we know how much radioactivity is injected initially. What we do not know is how much radioactivity is associated with the two plasma compartments. To accommodate this, we will take advantage of SAAM II split input capability in the **Exogenous Input** dialog box.



Split inputs. Split inputs can occur in metabolic and pharmacokinetic situation in a variety of ways. When they occur, it is important that you take them into account or your model will be incorrect.

One common situation is when the substance you are injecting is contaminated by an amount large enough to be detected in your analysis. You may be injecting a substance with an unknown amount of contaminate where the kinetics of the contaminate are different from the substance in which you are interested. This can be modeled following the philosophy described below.

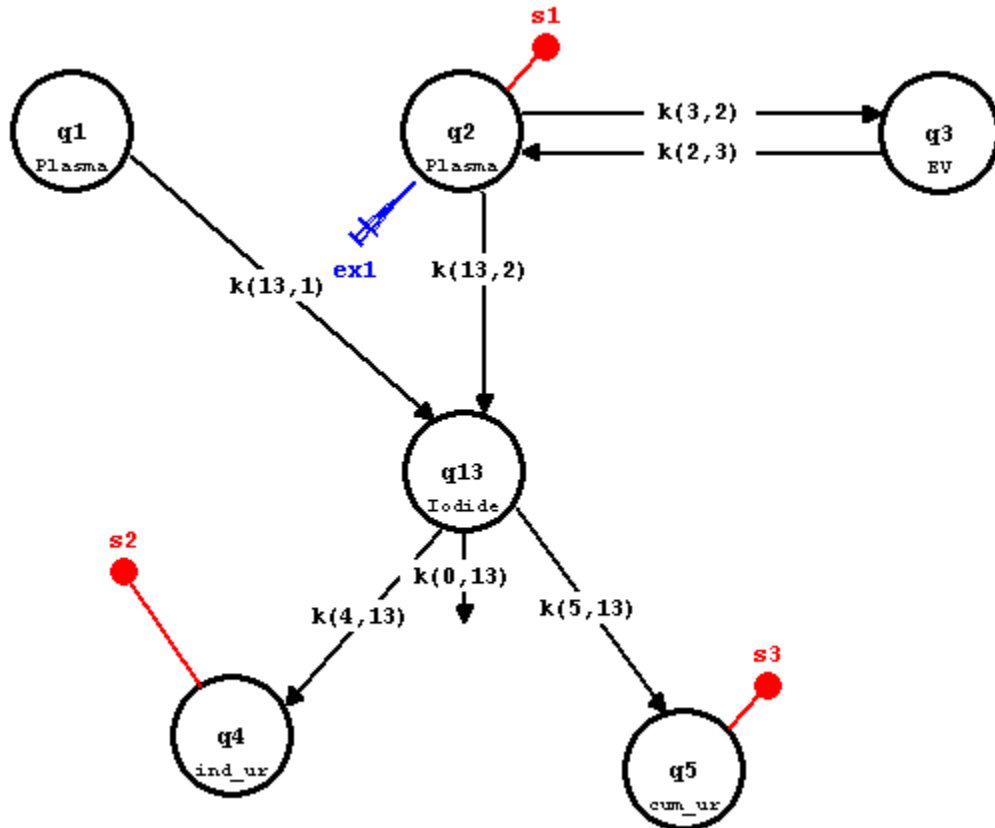
Another situation is when you are injecting a substance with the characteristics of a “parent” and “metabolite”, where there may actually be some conversion of parent into metabolite before you actually inject the substance. Using the split input feature of SAAM II, you can actually estimate the fraction of the injected substance that has been converted to the metabolite.



The system model we will create is shown below:

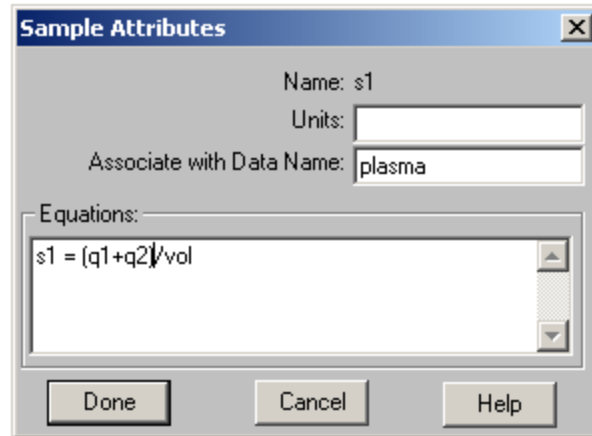


1. In the **SAAM II Toolbox**, click **Model** to make these tools available. Add Compartment 1 and the flux $k(13,1)$ as shown in the model above.
2. In the **SAAM II Toolbox**, click **Experiment** to make these tools available. Your model will appear as follows:

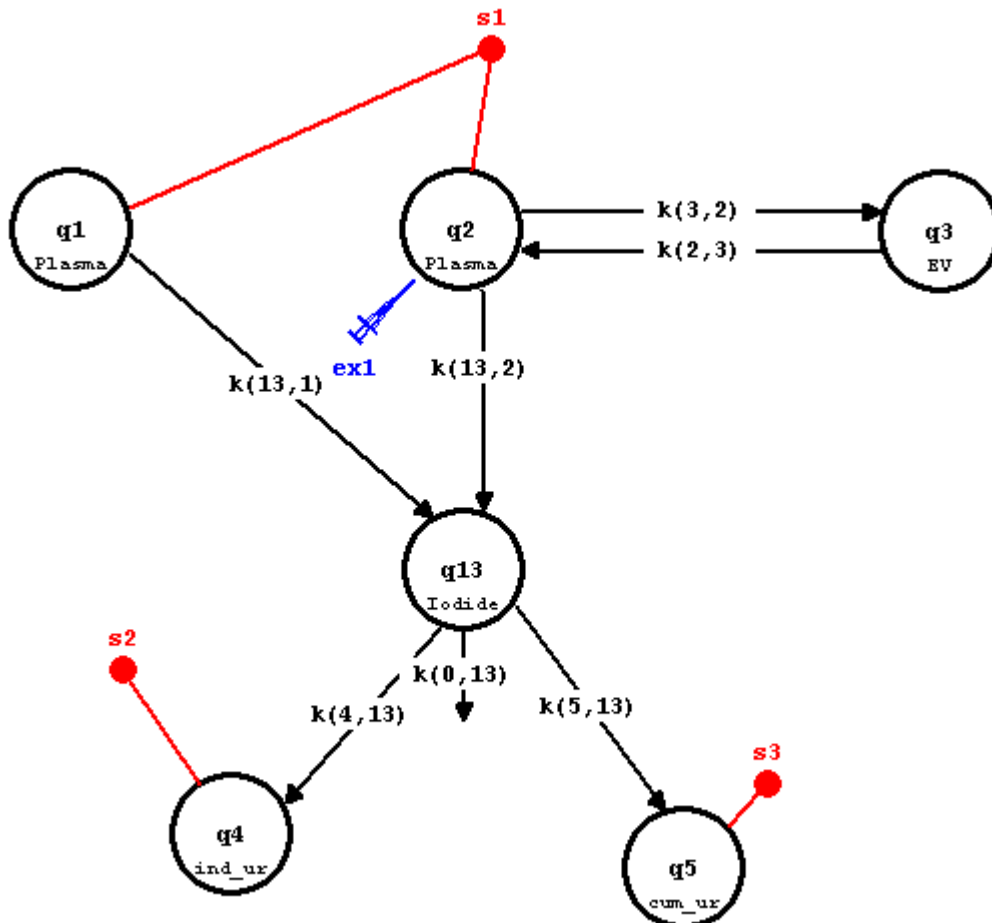


The **Experimental Attributes** have not changed, and the model for the urine has not changed. But the experiment on the system model has to be modified to take into account the two plasma compartments, **q1** and **q2**, and the split input. That is, **ex1** needs to be re-specified as does the sample equation.

3. Double-click **s1** to open the **Sample Attributes** dialog box.
 - a. Modify the sample equation to read “ $s1 = (q1 + q2)/vol$ ”. The **Sample Attributes** dialog box will appear as follows:



- b. Click **Done**. Your model will appear as follows (you may need to move the **s1** bullet):



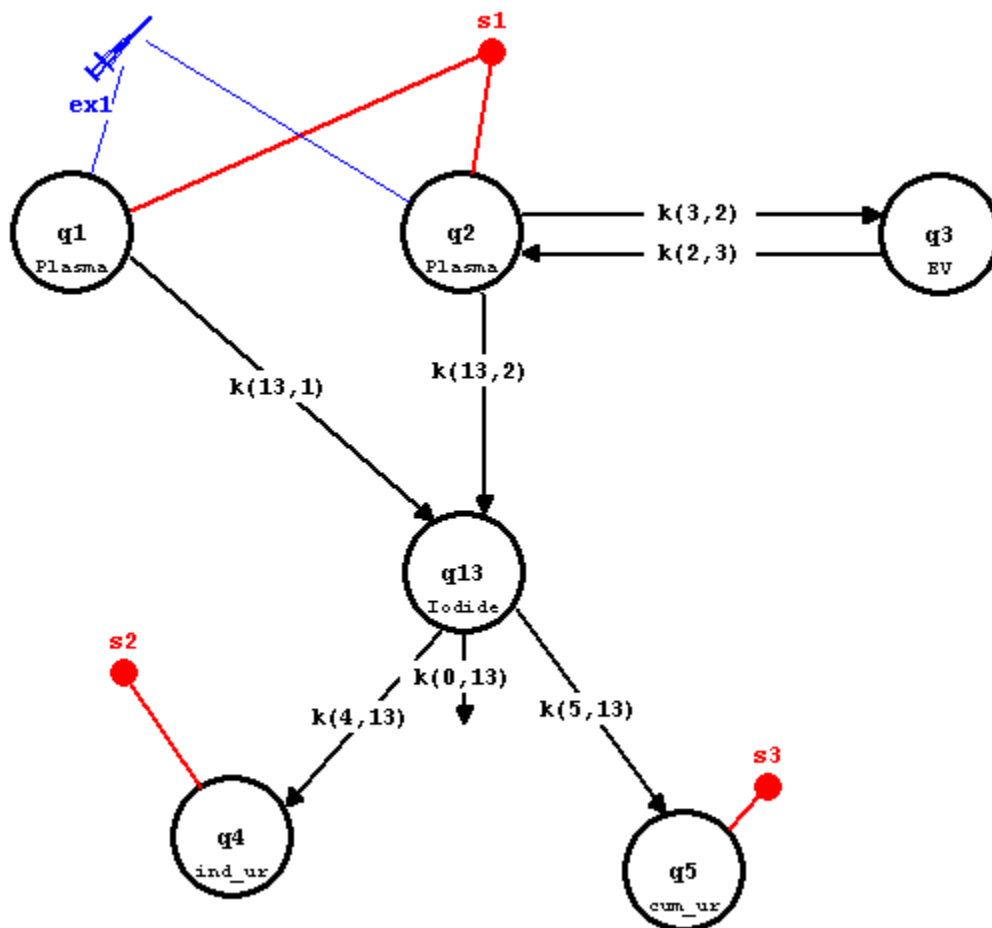
Notice that **s1** is now connected to Compartments **q1** and **q2**. This is because both **q1** and **q2** appear in the measurement equation for **s1**. Thus the sample calculation to be compared with the data is actually the sum of the

radioactivity in the two compartments divided by the volume. The two plasma compartments have different kinetics, and have different initial conditions. How the total radioactivity is split between the two compartments is dealt with in the next section.

4. Specify the split input.

To specify the split input, you must first delete the current **ex1**.

- Click **ex1**.
- Press **Delete**. Click **OK** on the warning message. **ex1** will be removed from the model.
- In the **SAAM II Toolbox**, click **Input**.
- Click Compartment **q1**, then Compartment **q2** and then the **Drawing Canvas**. After moving the **ex1** tool around, your model will appear as follows:



ex1 is now connected to the two plasma compartments **q1** and **q2**. SAAM II will recognize this as a split input situation as described below.

- e. Double-click **ex1** to open the **Exogenous Input** dialog box. The dialog box will appear as follows:

Exogenous Input

Name: Reference Name: Units:

Type	Initial	Constant	Start	Stop	Repeat Every	Nr. Repeats
------	---------	----------	-------	------	--------------	-------------

Input Type:

Bolus
 Infusion
 Primed Infusion
 Equation

Initial Amount:
Constant Rate:
Event Start:
Event Stop:
Repeat Every:
Nr. of Repeats:

Equation:

Save Edit Add Delete

Split Input... Done Cancel Help

Notice the **Split Input....** Option is available in the lower left hand corner of the box.

- f. Enter 1.0e+08 in the **Initial Amount** box, and click **Add**. The **Exogenous Input** dialog box will appear as follows:

Type	Initial	Constant	Start	Stop	Repeat Every	Nr. Repeats
Bolus	1.00e+8	-	0.000	-	-	-

This looks exactly like the situation with the two-compartment model except **Split Input...** is available.

- g. Click **Split Input...** to open the **Split Input** dialog box. The **Split Input** dialog box will appear as follows:

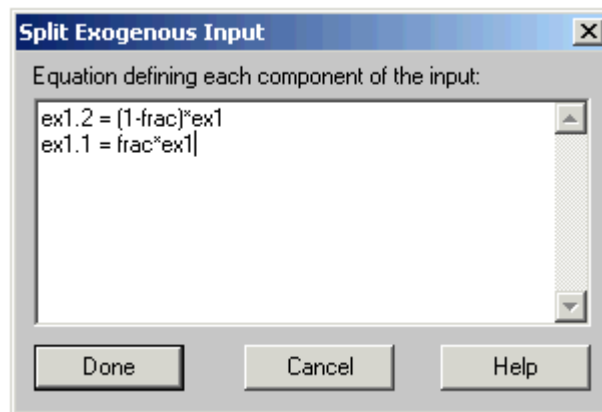


Split inputs. Split inputs are specified in the **Split Input** dialog box. If **ex_i** is a split input, then depending upon the number of compartments to which the input is attached, there will be **ex_{i,j}** for the initial amount in Compartment **j**.

For the case here, there is an **ex₁** that is split between Compartments **q₁** and **q₂**. **ex_{1,1}** is the amount in Compartment **q₁** and **ex_{1,2}** is the amount in Compartment **q₂**. The user must specify what the relationship between the two is.



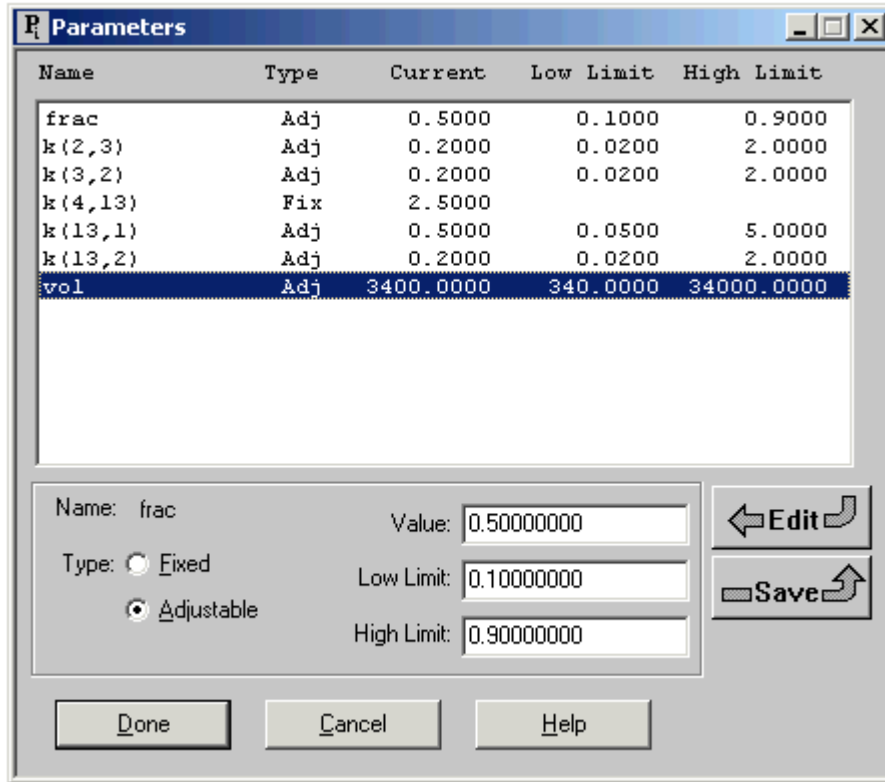
- h. Modify the **Equation defining each component of the input** as follows:



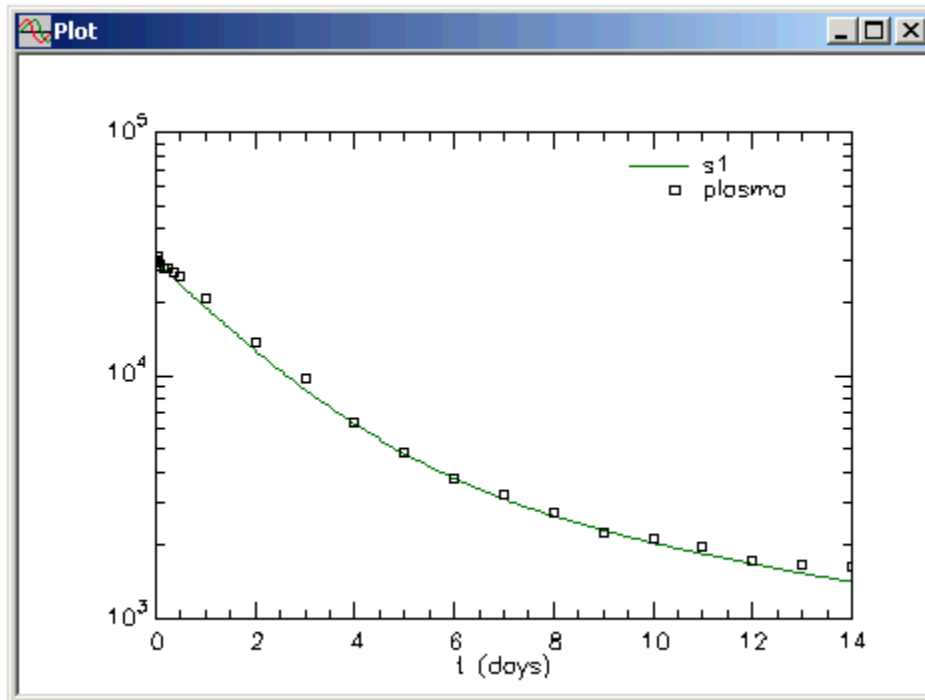
frac will become a parameter to be estimated when you fit your model to your data. *Frac* is the fraction of the total injected dose, **ex₁**, in Compartment **q₁**. “1-*frac*” is the remainder of the total dose; it is in Compartment **q₂**. *frac* will thus lie between zero and 1.

- i. Click **Done** to close the **Split Exogenous Input** dialog box.
 - j. Click **Done** to close the **Exogenous Input** dialog box.
5. Enter the parameter values

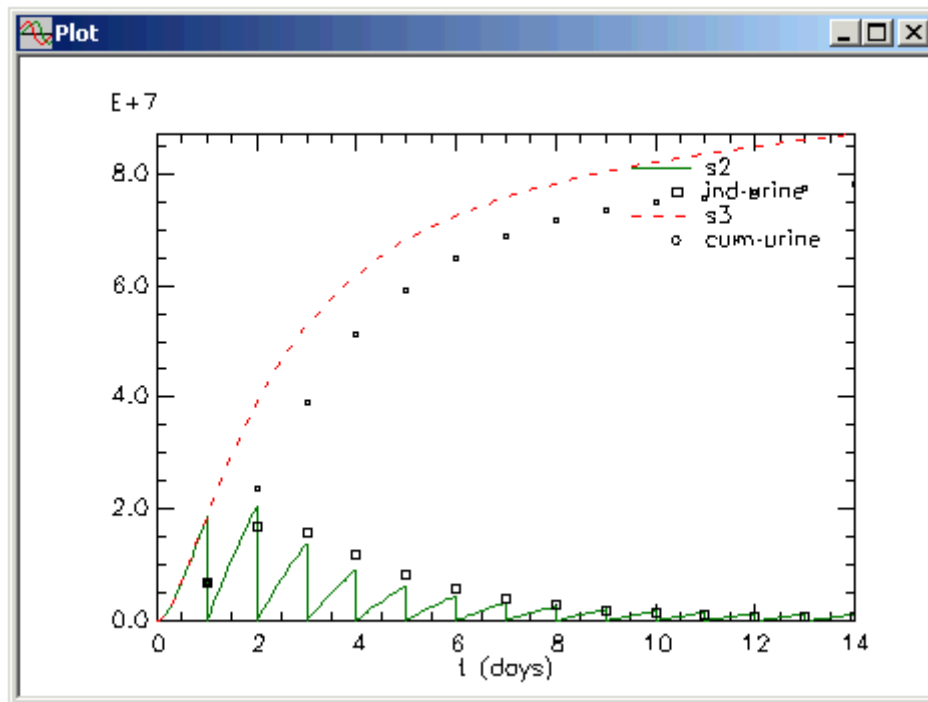
Enter the parameters as shown in the following **Parameters** dialog box.



- Solve the model, and view the solution. The plot of the plasma data will appear as follows:



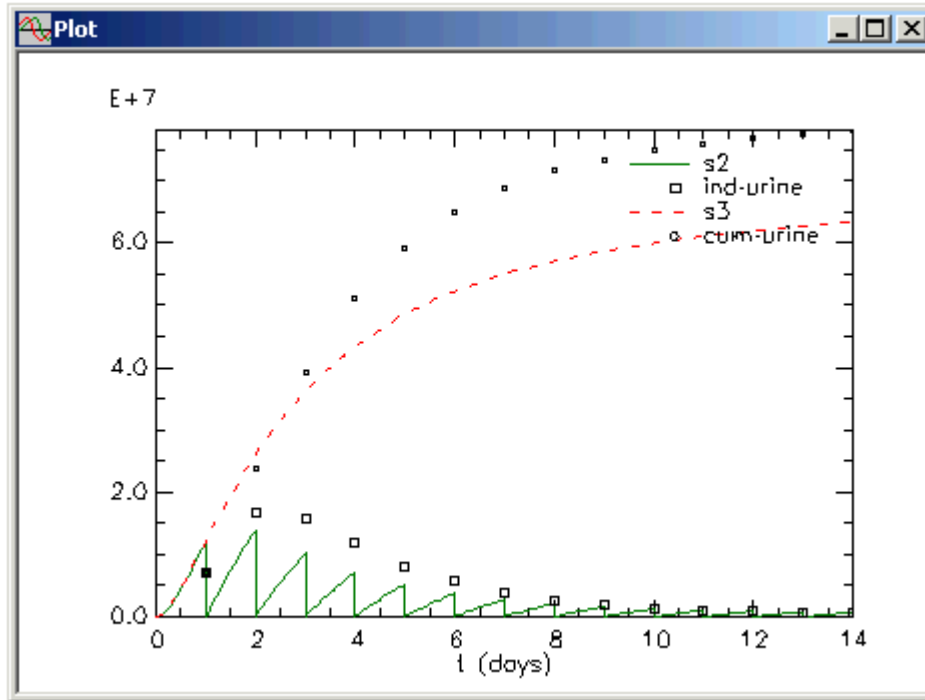
The plot of the individual and cumulative urine (together, in linear mode) will appear as follows:



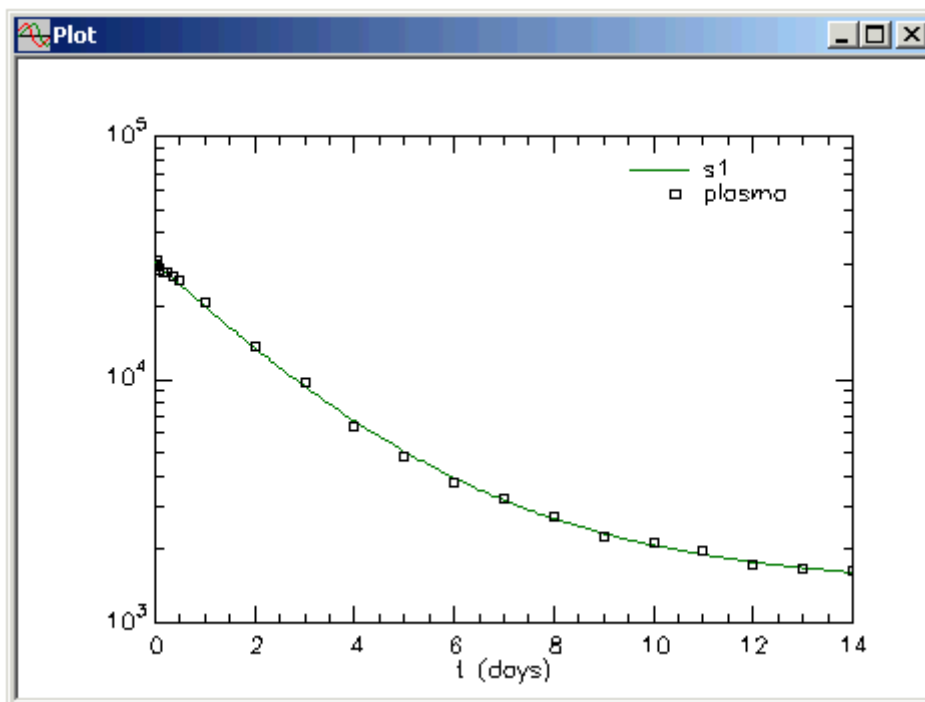
The solution is close enough to Fit the model to the data. However, you should notice that the first individual urine datum is way over-estimated. This is what causes all of the cumulative data to be poorly described. Thus if the first individual urine datum is spurious but the remaining data are okay, then none of the cumulative data can be used because of the problems caused by the first urine datum.

Leave the **Plot** window open.

7. Fit the model to the data, and evaluate the solution. The plot of the urine data will be updated as follows:



The plasma data will appear (in semi-log mode) as follows:



What is happening? The plasma data are well-described by the model. If you look carefully at the individual urine collections, towards the end of the collection period, from day 8 to day 14, the model fairly well describes the data. Since there is essentially no radioactivity left at this point in Compartment **q1** (as it decays

monoexponentially – you can verify this by plotting **q1**) this means that the Compartment **q2** and **q3** system is fairly well describing the latter stages of LDL metabolism.

There are two strategies that can be applied to see if the model can be resolved. One is to treat the first individual urine datum as spurious. The other is to recognize that low value for the first individual urine datum could be caused by a slight delay in the appearance of radioactivity from Compartment **q1** into the body iodide pool. We will investigate both.

Part 5. Resolve the model.

1. Treat the first individual urine datum as spurious.

To do this, we will unweight the first datum. As noted previously, if this datum is spurious, then all cumulative data will also be spurious, and also need to be unweighted.

- a. Unweight the first individual urine data and all of the cumulative urine data. The first part of the **Data** window will appear as follows:

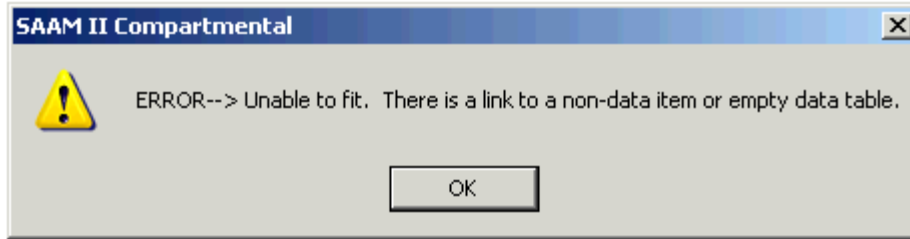
```

DATA
(FSD 0.1)
t      plasma  cum_urine  ind_urine
(/24)  ()        ()          ()
0      n       n          n
0.16   29745    n          n
0.5    31008    n          n
1      29114    n          n
2      27907    n          n
4      27593    n          n
6      27483    n          n
9      26610    n          n
12     25791    n          n
()     ()        ()          ()
1      20899    6979047(-+)  6979047(-)
2      13646    2.37E+07    1.67E+07

```

In the “cum_urine” column, the entry (-+) following the datum on day 1 will unweight all subsequent data. In the “ind_urine” column, the (-) following the datum on day 1 will unweight only this datum.

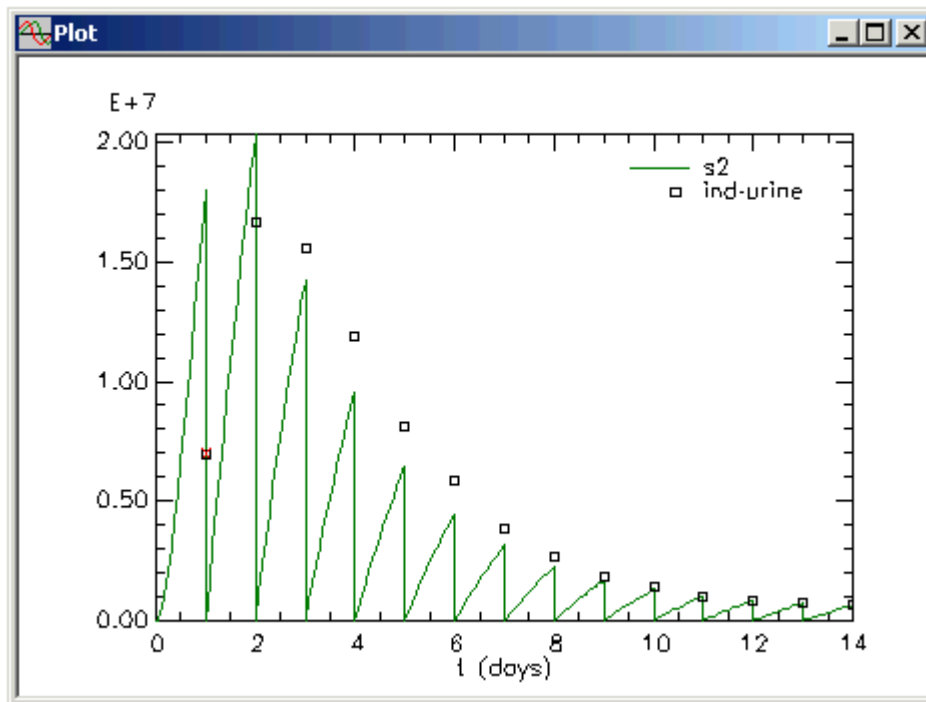
- b. Solve the model; the solution will be the same as that obtained at the end of Part 4.
- c. Fit the model to the data. The following warning will appear:



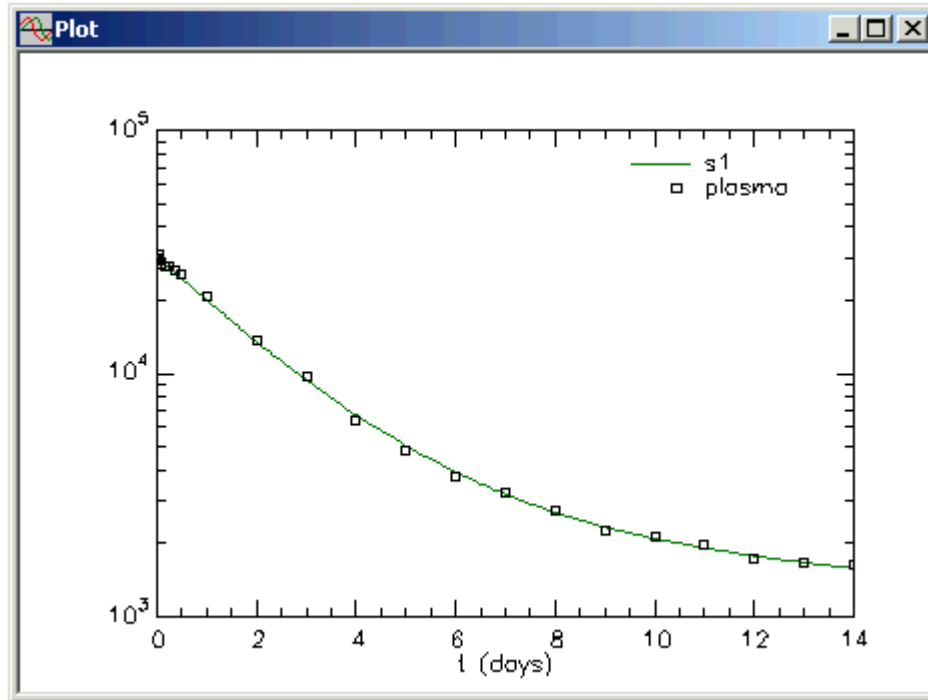
The reason why this warning appears is that sample **s3** is associated with the cumulative urine data. Since these are all unweighted, SAAM II thinks there are no data available for fitting purposes.

Click **OK**.

- d. Double-click **s3** to open the **Sample Attributes** dialog box.
- e. Remove “cum_urine” from the **Associate with Data Name** box.
- f. Click **Done**. Note the bullet **s3** is now an open circle.
- g. Re-Fit the model to the data. The individual urine data will appear as follows:

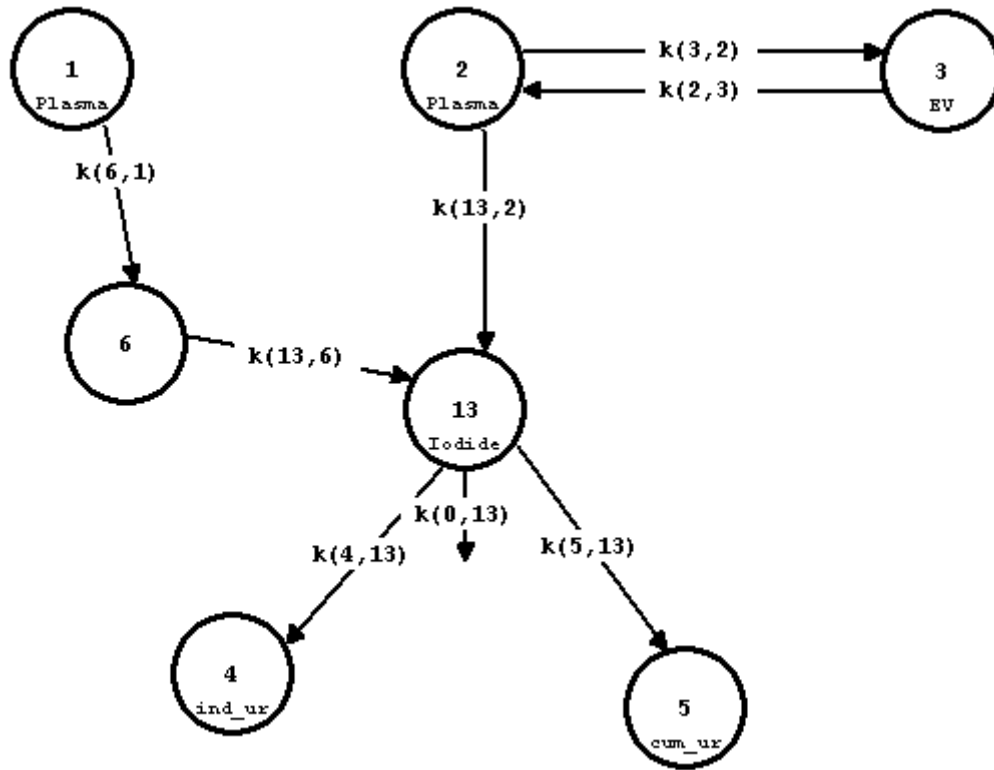


and the plasma data as follows:

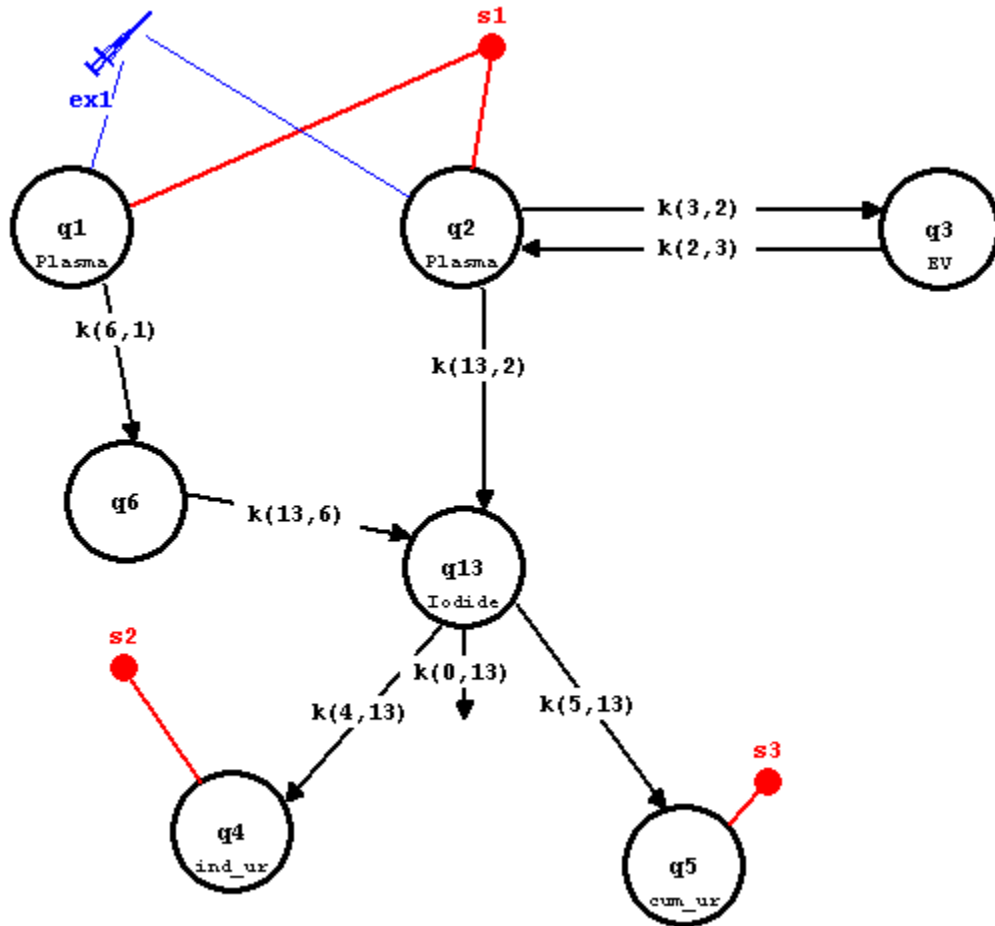


While the plasma data are quite reasonable, it is clear there are still difficulties with the urine data. In this situation, clearly more than the first datum is causing a problem. The over-prediction of the first two individual urine samples indicates a slight delay in the appearance of radioactivity from Compartment **q1** to Compartment **q13**.

- h. Close any open windows and dialog boxes. Reweight all data. Re-associated **s3** with **cum_urine**.
2. Modify the model structure by adding a compartment between Compartments **q1** and **q13**.
 - a. In the **SAAM II** toolbox, click **Model** so these tools are available.
 - b. Delete the flux $k(13,1)$.
 - c. Add a compartment between Compartments **1** and **13**; it will appear as Compartment **6**.
 - d. Add the fluxes $k(6,1)$ and $k(13,6)$. Your system model will appear as follows:



- e. In the **SAAM II Toolbox**, click **Experiment**. Your model will appear as follows:



All experiment attributes and experiment design remain the same. The only difference between this and the previous model is the addition of Compartment **q6**. Thus only the parameters will need to be added before you can test this model structure.

- f. Enter the parameter values as shown in the following **Parameters** dialog box:

Name	Type	Current	Low Limit	High Limit
frac	Adj	0.5000	0.1000	0.9000
k(2,3)	Adj	0.2000	0.0200	2.0000
k(3,2)	Adj	0.2000	0.0200	2.0000
k(4,13)	Fix	2.5000		
k(6,1)	Adj	0.5000	0.0500	5.0000
k(13,2)	Adj	0.2000	0.0200	2.0000
k(13,6)	Adj	0.1000	0.0100	1.0000
vol	Adj	3400.0000	340.0000	34000.0000

Name: vol Value: 3400

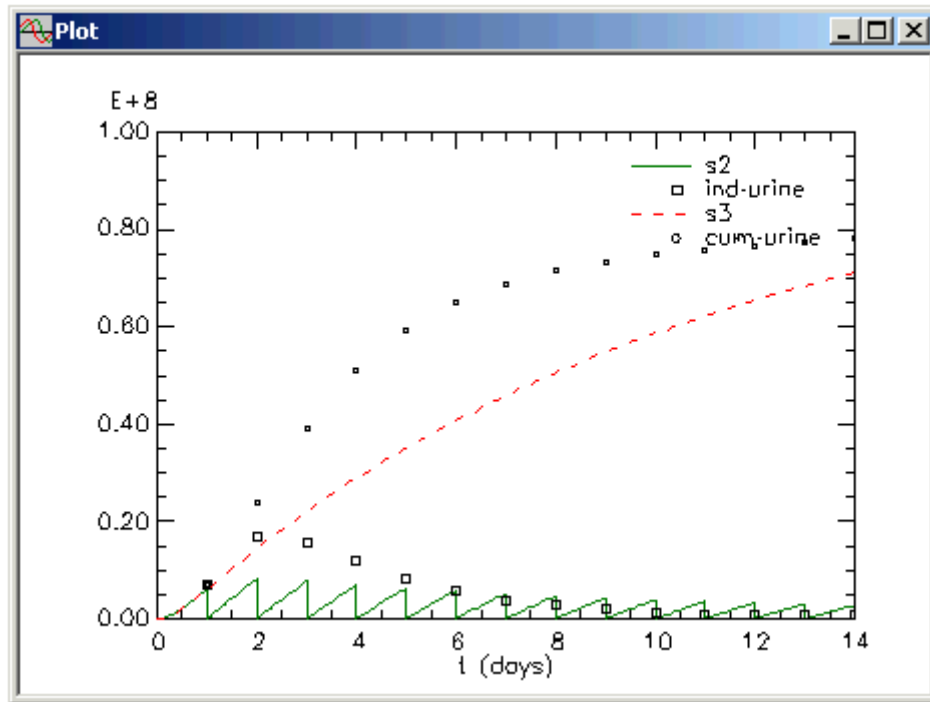
Type: Fixed Adjustable

Low Limit: 340.00000000

High Limit: 34000.00000000

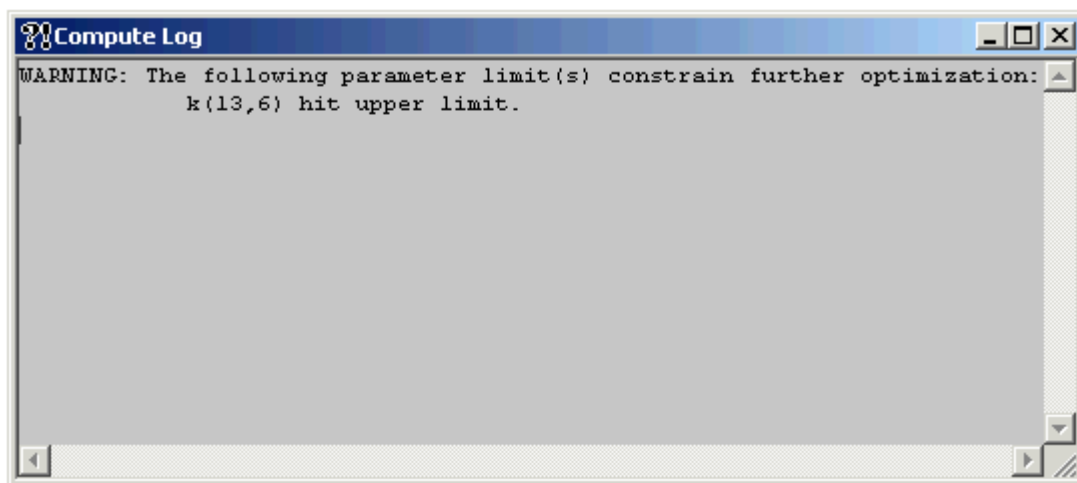
Buttons: Edit, Save, Done, Cancel, Help

- h. Solve the model, and view the solution. The plot of the plasma data is quite reasonable. The simultaneous plot of the individual and cumulative urine data will appear as follows (with the Y Axis scale changed to a maximum of $1.0e+08$):



The plot of the first individual urine datum is now quite good. The fact that the other data are not that well described is a combination of the amount of material initially in Compartment **q1**, and the rate constants. You can Fit your model to your data.

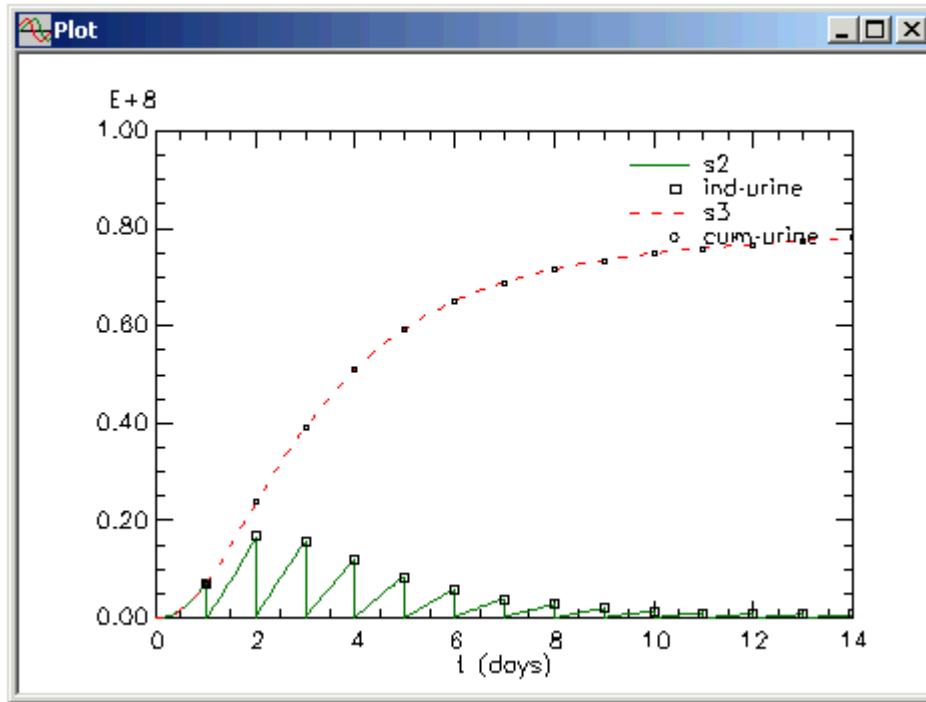
- i. Fit the model to the data. You will get the following warning message:



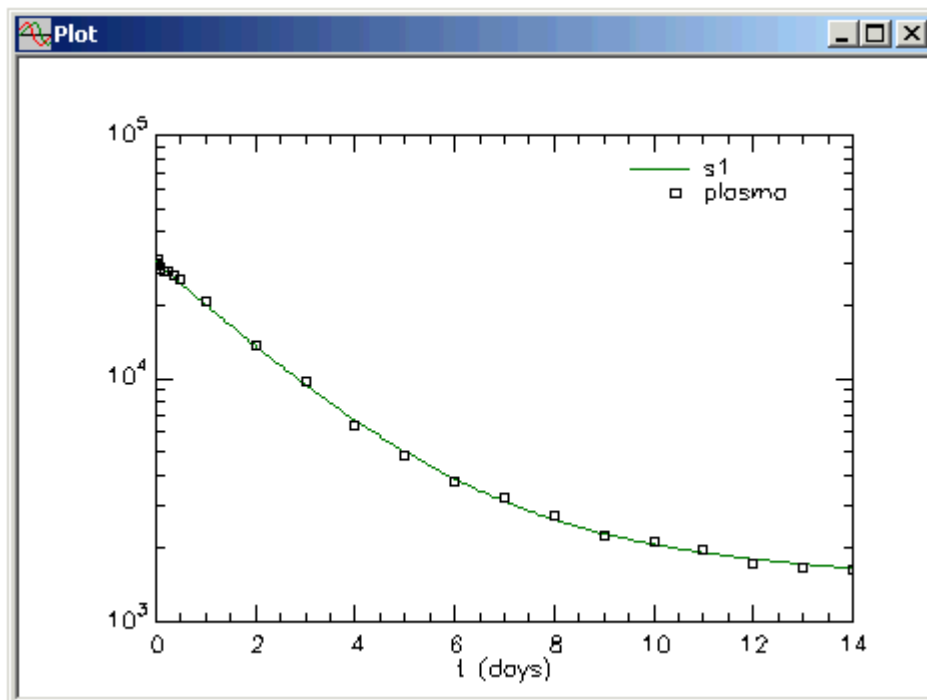
Close the **Compute Log** dialog box.

You will need to readjust the limits on $k(13,6)$. Open the **Parameters** dialog box, and set the **Low Limit** and **High Limit** for $k(13,6)$ equal to 0.1 and 10 respectively.

Re-Fit the model to the data. This time the Fit will be successful, and the plot of the urine data will be updated as follows:



The plasma data, plotted in semi-log mode and with **AutoScale** on, will appear as follows:



The addition of Compartment **q6** which provides a slight delay in the appearance of radioactivity from Compartment **q1** into Compartment **q13** is sufficient to resolve the problem of the first individual urine datum appearing a little low.

- j. View the statistics. The **Statistics** window will appear as follows:

Parameter/Variable	Value	Std.Dev.	Coef. of Var.	95% Confidence Interval	
frac	0.65532	1.59384e-002	2.43217e+000	0.62316	0.68748
k(2,3)	0.10407	1.14007e-002	1.09546e+001	0.08107	0.12708
k(3,2)	0.25186	3.30276e-002	1.31133e+001	0.18522	0.31851
k(4,13)	2.50000	** Fixed **	** Fixed **	** Fixed **	** Fixed **
k(6,1)	0.45518	1.83017e-002	4.02075e+000	0.41825	0.49211
k(13,2)	0.08581	5.34820e-003	6.23258e+000	0.07502	0.09660
k(13,6)	1.10936	6.74795e-002	6.08275e+000	0.97319	1.24553
vol	3311.80881	4.00609e+001	1.20964e+000	3230.96737	3392.65025

	Objective	Scaled Data Variance
s3 : cum_urine	7.337257e+000	3.724698e-003
s2 : ind_urine	6.705869e+000	1.062645e-001
s1 : plasma	5.313299e+000	1.213650e-001

Total objective	1.935642e+001	
AIC	1.079715e+001	
BIC	1.098835e+001	

The statistics associated with the Fit are also very reasonable with the largest coefficient of variation equal to 13%. Thus you can conclude that this model can simultaneously describe the plasma and urine data where an estimated 66% of the initial dose is in Compartment **q1** and the remainder is in Compartment **q2**.

The kinetics of this model are consistent with LDL being physicochemically heterogeneous. Without the urine data, you would only have been able to use the plasma data, and work with a two-compartment model.

Quit the SAAM II Compartmental application.

Modeling Notes:

This is an exercise in the step-wise procedure to develop a model which will simultaneously describe several different data sets. It illustrates how to start from the “simple”, and proceed to the more “complex” as the need arises. It also illustrates how known physiology and/or biochemistry can be accommodated in a model structure.

Essential Points to Remember

- Start simple and get more complex as the need arises.
- Plasma samples representing different homogeneous moieties can be accommodated via the sample tool.
- Split inputs can be accommodated where the fraction of the input distributed among different compartments can be known, or estimated from the data.

Data for this case study

DATA

(FSD 0.1)

t	plasma	cum_urine	ind_urine
(/24)	()	()	()
0	n	n	n
0.16	29745	n	n
0.5	31008	n	n
1	29114	n	n
2	27907	n	n
4	27593	n	n
6	27483	n	n
9	26610	n	n
12	25791	n	n
()	()	()	()
1	20899	6979047	6979047
2	13646	2.37E+07	1.67E+07
3	9714	3.92E+07	1.56E+07
4	6456	5.11E+07	1.19E+07
5	4806	5.92E+07	8091944
6	3804	6.50E+07	5846717
7	3267	6.89E+07	3832041
8	2744	7.16E+07	2696780
9	2279	7.34E+07	1882786
10	2131	7.48E+07	1394947
11	1971	7.58E+07	985794
12	1720	7.67E+07	876272
13	1667	7.74E+07	712898
14	1622	7.81E+07	643487

END