Chapter 6

Investigation into Using COPASI to Simulate the Behaviour of the Mathematical Models

6.1 Introduction

Systems biology raises the hope for more effective drug discovery and this area of research involves the integration of experimental and computational data to bring together quantitative data generation, modelling and simulations (Butcher, 2005). An integral part of any systems biology approach to the understanding of a system is the modelling and simulation of that system and is a promising way of understanding complex mechanisms underlying the normal physiological regulations and disease development. Systems biology approaches are particularly useful in identifying effective drug targets and selection of drugs with minimal toxicity (Sahle *et al*, 2008).

Computational biology has two distinct branches: knowledge discovery and simulation-based analysis. Knowledge discovery is extracting the hidden patterns from huge quantities of data often widely used in bioinformatics. Simulation-based analysis involves testing hypotheses with *in-silico* experiments to provide us with predictions to be tested by *in vivo* and *in vitro* studies. Simulations attempt to predict system dynamics and test the validity of underlying assumptions (Kitano, 2002). In this chapter the results obtained from simulations using COPASI[1] will be presented. In using COPASI, we aimed to see if the simulations performed using parameters produced by the mathematical modelling in chapter 5 produced results that matched the original biological data.

[1]COPASI is a stand-alone program that is used to simulated the behaviour of complex biochemical network developed by Hoops *et* al, 2006 and is discussed in more detail in section 6.5

6.2 Using COPASI to Simulate the Behaviour of RHPS4

To re-iterate, application of the data obtained from RHPS4 to the five-compartment model, as in chapter 5, indicates an overall increase in the proportion of cells in both the Σ and A compartments collectively, which is representative of an increase in the proportion of cells in senescence and cell death. Using the five-compartment model, it was identified that this was due to an increase in $k_{x\Sigma}$ and a reduction in $k_{\Sigma A}$. The data regarding the individual rate parameters obtained from the mathematical model was applied to COPASI to determine whether the behaviour of the system could be reproduced. COPASI is a platform-independent biochemical simulator with the ability to integrate the diverse numerical methods used in computational biology. It is a stand-alone program that can be used through two different versions, those being a graphical interface (CopasiUI) and a command line version (CopasiSE) (Hoops *et al*, 2006). CopasiSE only contains the calculation engine and so it was decided for the purpose of simulating the behaviour of RHPS4 in the five-compartment model, CopasiUI would be used. The advantages of CopasiUI are the functions for creating and editing models and plotting the results, although both versions of COPASI are equal (Hoops *et* al, 2006). COPASI requires the flow of cells through the system to be input and the corresponding ODEs are calculated. The corresponding rate parameters for the five-compartment model were entered as shown in table 6.1, for example, k_{xy} was entered as XtoY and COPASI understands this as the flow of cells between compartments X and Y and calculates the corresponding ODE. COPASI simulates the behaviour of the five-compartment model as if it were a series of biochemical reactions, so you begin with a certain number or proportion of cells in each compartment and they behave according to the corresponding rate parameters. The output from COPASI is the change of the proportion or number of cells in each compartment over time. It is important to note that the equation for Z involves two cells entering X because in our mathematical model, compartment Z incorporates mitosis and so there are two cells entering X from Z.

Table 6.1 How the five-compartment model was committed to COPASI

Name	Equation	Rate
XtoY	X →Y	Mass Action
YtoZ	Y→Z	Mass Action
ZtoX	Z→X+X	Mass Action
XtoΣ	X→Σ	Mass Action
ΣtoA	Σ→A	Mass Action

The five-compartment model consists of five compartments named X, Y, Z, Σ and A and what we have termed 'compartments' in our mathematical model, COPASI understands these as 'species' involved in a biochemical reaction as show in table 6.2.

Table 6.2 Species involved in the five-compartment model

Species/Compartment	Reaction the species is involved in
A	Involved in Σ→A
Σ	Involved in Σ→A and X→Σ
X	Involved in X→Σ,X→Y and Z→X+X
Y	Involved in X→Y and Y→Z
Z	Involved in Y→Z and Z→X+X

The initial investigation was to simply understand whether by using the rate parameters obtained from the mathematical model, COPASI was able to replicate the behaviour of the cells in the experiment. To do this, COPASI required the initial proportions of cells in each compartment (i.e. what we observed experimentally) and the rate of transition of cells between these compartments (i.e. the rate parameter values obtained from application of the experimental data to the mathematical model). Application of the data to the model in chapter 5 clearly shows that the rate constants are not 'constant' and are dependent on time and the simplest COPASI model treats them as constants and so for the initial simulation, the values used were the averaged values for each concentration over time and are shown in table 6.3.

Table 6.3 The averaged rate parameters over the 21 days of the experiment according to the five-compartment model

Rate parameter in COPASI	Rate parameter according to mathematical model	Control	50 nM RHPS4	100 nM RHPS4	1 µM RHPS4
		Averaged values of each rate parameter over 21days			
ΣtoA	$k_{\Sigma A}$	0.125	0.032	0.031	0.006
XtoΣ	$k_{x\Sigma}$	0.270	0.881	0.696	1.002
XtoY	k_{xy}	1.6249	2.6094	1.865	2.099
YtoZ	k_{yz}	2.326	2.476	2.889	0.911
ZtoX	k_{zx}	2.281	2.452	2.482	0.866

In the initial simulation, the proportion of cells in each compartment at the start of the simulation was calculated as an average over the 21 days of the experiment. In reality, at the beginning of the experiment, each cell starts as a 'control' cell, however, in an effort to simulate the behaviour of each concentration, the proportions of cells for each concentration were averaged over the 21 days as shown in table 6.4.

Table 6.4 The average compartmental values for RHPS4 over the 21 days of the experiment according to the five-compartment model.

Compartment	Control	50 nM RHPS4	100 nM RHPS4	1 µM RHPS4
	Averaged value of each compartment over the 21 days			
A	0.054	0.034	0.049	0.048
Σ	0.182	0.426	0.419	0.610
X	0.365	0.218	0.203	0.126
Y	0.214	0.171	0.141	0.153
Z	0.187	0.150	0.176	0.126

The data was applied to COPASI as shown in tables 6.4 – 6.5 to generate the graph shown in figures 6.1 – 6.3.

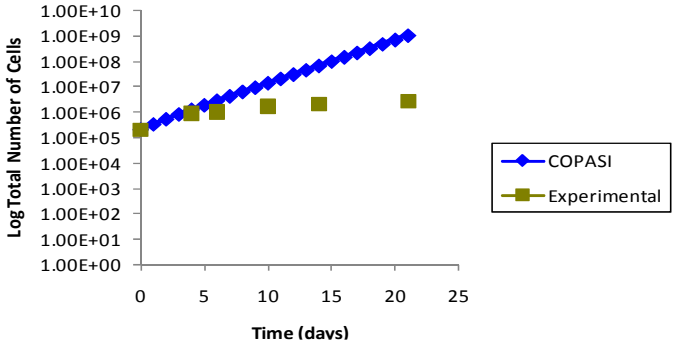


Figure 6.1 Application of the data to COPASI from mathematical model of 50 nM RHPS4. Data from the mathematical model regarding 50 nM RHPS4 was applied to COPASI and the proportion of cells in each phase analysed.

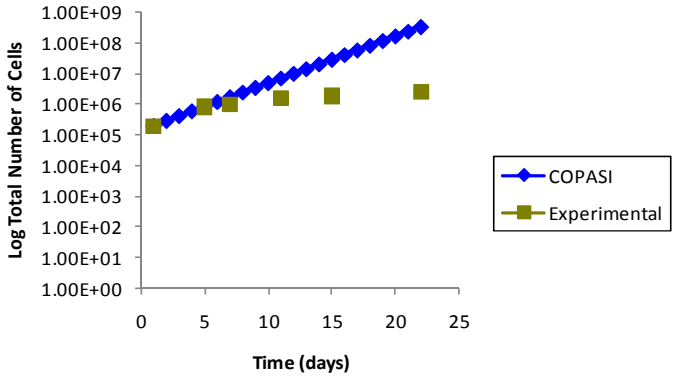


Figure 6.2 Application of the data to COPASI from mathematical model of 100 nM RHPS4. Data from the mathematical model regarding 100 nM RHPS4 was applied to COPASI and the proportion of cells in each phase analysed.

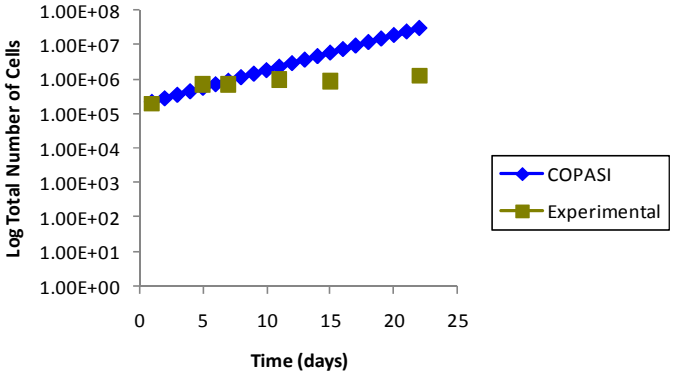


Figure 6.3 Application of the data to COPASI from mathematical model of 1 µM RHPS4. Data from the mathematical model regarding 1 µM RHPS4 was applied to COPASI and the proportion of cells in each phase analysed.

The data shown in figures 6.1 – 6.3 shows the changes in the total number of cells with respect to time according to simulations carried out by COPASI and the data that was obtained experimentally. The simulations with COPASI were carried out using the values for the rate parameters obtained from the mathematical model as shown in table 6.4 for each individual concentration. It is evident from figures 6.1 – 6.3 that the results of the simulation do not reflect those of the experiment. The experimental data clearly shows that the cells grow at a much slower rate than that predicted by COPASI, particularly at long times.

The exact mechanism of action of RHPS4 remains unknown and this may be reflected in the inability at this stage for COPASI to directly simulate the original

biological behaviour. The same concept was applied to data obtained from doxorubicin and again, COPASI required the initial concentration of cells in each compartment and the rate of transition between the compartments and for the initial simulation; these were averaged over the 21 days and are shown in tables 6.5 – 6.6.

Table 6.5 The average starting compartment values for doxorubicin in the five-compartment simulation using COPASI

Compartment	**Control**	**3.15 nM doxorubicin**	**6.3 nM doxorubicin**	**31.5 nM doxorubicin**	**63 nM doxorubicin**
	Averaged compartment concentrations over the 21 day period				
X	0.345	0.302	0.141	0.174	0.132
Y	0.235	0.225	0.229	0.197	0.154
Z	0.199	0.177	0.166	0.171	0.137
Σ	0.164	0.223	0.307	0.378	0.473
A	0.169	0.189	0.241	0.225	0.314

Table 6.6 The average rate parameter values for each concentration of doxorubicin over the 21 day period according to the five-compartment mathematical model as applied to COPASI.

Rate parameter according to COPASI	**Rate parameter according to mathematical model**	**Control**	**3.15 nM**	**6.3 nM**	**31.5 nM**	**63 nM**
		Averaged rate parameter values for doxorubicin over the 21 day period				
XtoY	k_{xy}	0.868	1.197	1.943	1.066	0.972
YtoZ	k_{yz}	1.104	1.317	0.983	0.782	0.730
ZtoX	k_{zx}	1.105	1.456	1.184	0.779	0.747
XtoΣ	$k_{x\Sigma}$	0.190	0.324	0.794	0.493	0.588
ΣtoA	$k_{\Sigma A}$	0.209	0.258	0.159	0.083	0.072

The data was applied to COPASI as shown in tables 6.5 - 6.6 and the results are shown in figure 6.4.

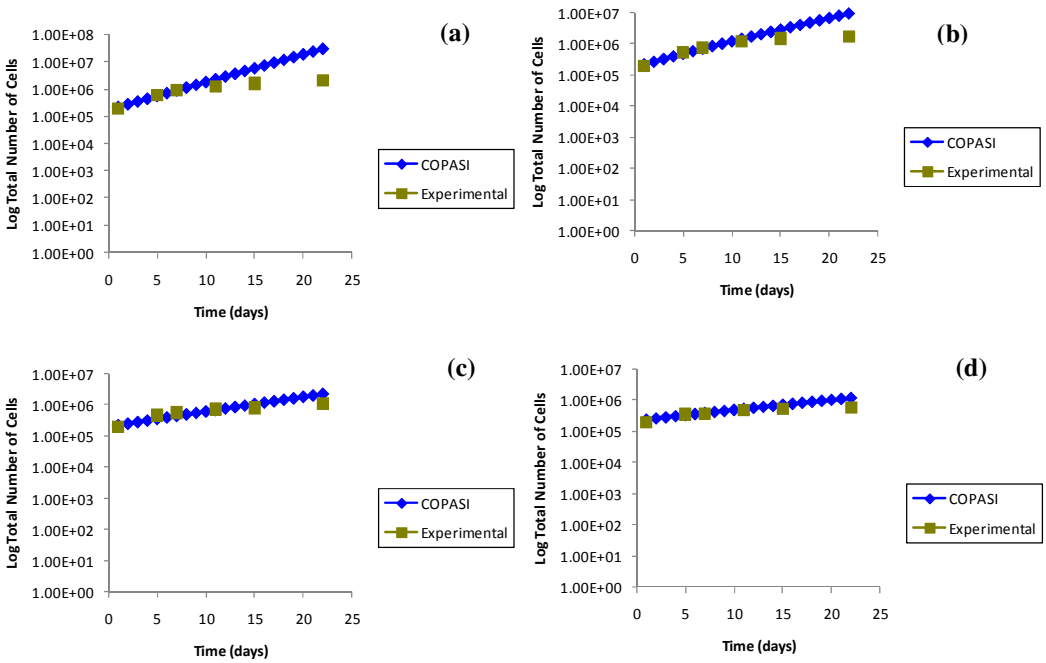


Figure 6.4 Application of the data to COPASI from the mathematical model for doxorubicin at each concentration analysed. Shown here are the results of the simulations of doxorubicin at concentrations of (a) 3.15 nM (b) 6.3 nM (c) 31.5 nM and (d) 63.1 nM. The graphs show the changes in the concentration of each compartment with respect to time up to 21 days.

The results shown in figure 6.4 show that at the higher concentrations, the growth of the system more closely matches that of the experimental data, however, analysis of the actual total numbers of cells, as opposed to the log total number of cells reveals that the growth of the system according to COPASI is at a much faster rate than that observed experimentally. It was also observed that the final total numbers of cells was predicted to be far higher than what was observed experimentally, for example, at a concentration of doxorubicin of 63 nM, COPASI predicts that by day 21 of the experiment, there are 1, 516, 075 cells and experimentally we observed only 568, 275. This shows that COPASI is not simulating the biological behaviour in the manner desired. It appears that this is where COPASI is breaking down as by applying the averaged rate parameter values at the start of the simulation, COPASI is assuming these are the 'constant' rate constants throughout the 21 day period of the simulation. We are using COPASI too simply. Analysis of the rate parameter values obtained from the mathematical models for both RHPS4 and doxorubicin are clearly not constant and

change throughout the duration of the experiment. The data shown in figures 6.1 – 6.5 were generated using the average rate parameter values obtained over the 21 day period and so COPASI assumes this is the rate constant for each day of the simulation. It may also be possible that the results can be explained due to the outgrowth of a resistant population. For example, at the beginning of the simulation, is there a resistant population which outgrows to a sensitive population and therefore could the results in figures 6.1 – 6.5 be due to the growth of two populations of cells? COPASI was used to investigate this concept as in section 6.3.

6.3 Investigation into Resistance of HCT116 Cells to Doxorubicin

Here we investigate the possibility of a resistant population of cells outgrowing a population of sensitive cells in the presence of doxorubicin. To investigate the possibility of a resistant population to doxorubicin, the rate parameters used were the same as those in tables 6.5 – 6.6. Due to changes of the rate parameters through the duration of the experiment, it seemed logical to use the averaged values to ensure accuracy. It is important to note that we are not investigating the emergence of resistance of HCT116 over 21 days, but instead the possibility that at the start of the experiment, there was a sub-population of resistant cells.

In the initial investigation, a simulation was carried out whereby the proportion of resistant cells were 0.01% of the population and a separate simulation was carried out whereby the 0.99% of the cells were sensitive and the total numbers of cells from both simulations added to determine the growth of the system. This was repeated using 0.02% of resistant cells and 0.98% were sensitive and so on and a typical result is shown in figure 6.5. These initial simulations did not reproduce the biological behaviour of the system and is possibly due to the fact that COPASI assumes the growth of each simulation as exponentials, and the sum of two exponentials is an exponential, therefore this method of analysing resistance was never going to reproduce the observed biological behaviour of the system.

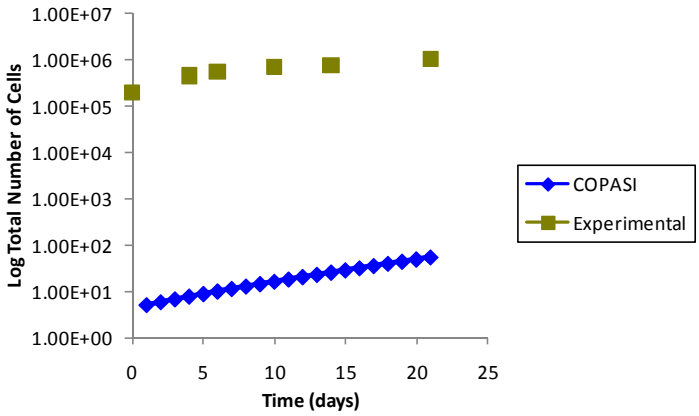


Figure 6.5 Simulation of 0.99 sensitivity and 0.01 resistance of doxorubicin to HCT116 cells. It was assumed that the population of cells was split so that 0.99% of the population was sensitive and 0.01% were resistant. The simulations were carried out separately using the averaged rate parameter values as shown in table 6.6 – 6.7 and the total numbers of cells added to produce the graph shown here against experimental data.

Since the biological behaviour of the system could not be reproduced in this manner, the next logical step was to repeat the simulations using the same idea in that two separate simulations are carried out, however, rather than growing through the same rate parameters, the sensitive population would grow as control cells and would therefore be dominated by the control rate parameters as shown in table 6.6. Again, however, it was proven that the growth of the system did not match that of the experimental data in the desired manner.

To further investigate the possibility of resistance to doxorubicin, the proportions of cyclings cells at each time point through the experiment were calculated to determine the proportion of cells that were therefore still in the system. The proportions of cycling cells were considered to be those in the X, Y and Z compartments of the mathematical model and the results with respect to each concentration are shown in figure 6.6.

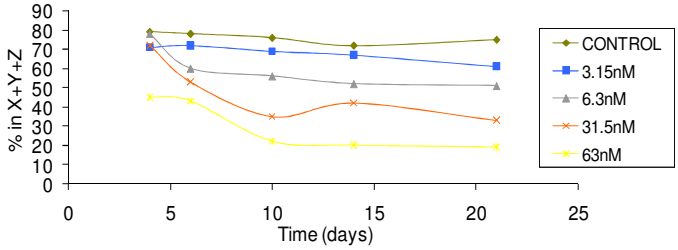


Figure 6.6 The proportion of cycling cells throughout the experiment. To further investigate the possibility of resistance from doxorubicin, the proportions of cycling cells throughout the 21 days of the experiment was calculated and is shown here.

Figure 6.6 clearly shows that under control conditions, the proportion of cycling cells does not change significantly over the duration of the experiment and is sustained between 72 – 79%. The same is true when HCT116 cells were incubated with doxorubicin at a concentration of 3.15 nM. At the higher concentrations of doxorubicin, i.e. 31.5 nM and 63 nM, there is a marked reduction in the proportion of cycling cells between day 4 and 10 from 72% to 35% and 45% to 22%, respectively. After day 10, the proportions of cycling cells remain stable. Following this, the next logical step was to assume that over the 21 day period, resistance was gained and so that the sensitive proportions of cells exceeds the resistant population at the beginning of the experiment and by day 21 it is the resistant population which exceeds the sensitive population. Figure 6.7 was produced assuming that for each condition, at the start of the experiment there are 100% sensitive cells which by intermediate time points reduces to 30% and then to 10% meaning that 70% and 90% of the cells are considered resistant, respectively at these time points. We assume that by day 21, there are 0% sensitive cells and so 100% of the cells are resistant.

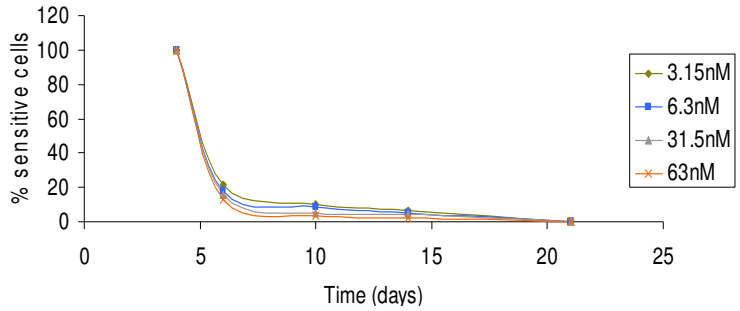


Figure 6.7 Proportion of sensitive cells with respect to concentration. The graph shown here depicts the proportion of sensitive cells with respect to concentration based on the assumption that at the beginning of the experiment the cells are 100% sensitive to doxorubicin and by the end of this experiment, there is 0% sensitive cells within the population.

The assumption that resistance is gained over the 21 days of the experiment and the cells are 100% sensitive at day 0 and 0% sensitive by day 21 was applied to the mathematical model to calculate new rate parameter values for the sensitive population of cells and the simulations were carried out assuming that the sensitive population grew in accordance with the rate parameters in table 6.7 and the resistant population by the actual rate parameters calculated for day 21 by the mathematical model.

Table 6.7 Rate parameters calculated when applying 0.1x, 0.15x and 0.3x compartment data to the five-compartment mathematical model.

Rate parameter	3.15 nM doxorubicin			6.3 nM doxorubicin		
	0.1x	**0.15x**	**0.3x**	**0.1x**	**0.15x**	**0.3x**
k_{xv}	1.451	1.480	1.569	1.424	1.351	1.424
k_{vz}	1.611	1.626	1.672	1.691	1.651	1.691
k_{zx}	1.881	1.881	1.881	1.811	1.811	1.811
$k_{\Sigma A}$	0.031	0.047	0.094	0.079	0.039	0.079
$k_{x\Sigma}$	0.044	0.065	0.131	0.155	0.077	0.155
Rate parameter	**31.5 nM doxorubicin**			**63 nM doxorubicin**		
	0.1x	**0.15x**	**0.3x**	**0.1x**	**0.15x**	**0.3x**
k_{xv}	1.322	1.342	1.024	0.946	0.959	0.996
k_{vz}	1.111	1.119	1.146	0.525	0.528	0.537
k_{zx}	1.095	1.095	1.095	0.963	0.963	0.963
$k_{\Sigma A}$	0.022	0.034	0.066	0.009	0.014	0.029
$k_{x\Sigma}$	0.074	0.111	0.223	0.063	0.095	0.189

As a final investigation into the concept of resistance of HCT116 cells to doxorubicin, the total number of cells throughout the experiment was calculated

based on the number of doublings and the number of cells at the beginning of the experiment and it was again assumed that 100% of the cells at the end of the experiment were resistant. Utilising the knowledge of the total numbers of cell throughout the experiment, simulations were carried out using the exact numbers (as opposed to the average values shown in tables 6.4 and 6.5) of cells in each compartment at the beginning and then simulated for 21 days. From the number of cells that is predicted from COPASI, it was then worked backwards to calculate the number of cells that would be required at the beginning. The behaviour of the simulations did not mirror those of the experiment and so it was concluded that the results obtained from the application of the data from doxorubicin to the five-compartment model in chapter 5 were possibly not the result of the growth of two sub-populations of resistant and sensitive cells.

6.4 Parameter Fitting

The results obtained from doxorubicin could not be explained in terms of resistance and so could the behaviour of doxorubicin or RHPS4 be mirrored exactly in COPASI using parameter fitting, and which parameters would have to be changed and to what degree? Our initial COPASI model assumes the rate parameter values to be constant, and it is obvious from the application of the data to the five-compartment mathematical model in chapter 5, that in fact the rate parameters are not constant, and their values do change throughout the duration of the experiment. To investigate using COPASI to simulate the biological behaviour of doxorubicin and RHPS4, each rate parameter was considered separately and the exact total numbers of cells that were observed experimentally were applied as opposed to the average values as used in section 6.3. The experiments were set up using a cell density of $2x10^5$ and so using the population doublings data, an accurate estimate of the numbers of cells at each time point can be calculated. In the experiments carried out as outlined in chapters 4 and 5, observations of the cells in the absence and presence of RHPS4 and doxorubicin at different concentrations were carried out at days 4, 6, 10, 14 and 21. To reflect this, and increase the accuracy of the simulations, the simulations were carried out

assuming that between day 0 and day 4, the cells were cycling by the same rate parameter values as the control cells as at the beginning of the experiment. The cells have not been exposed to agent and therefore essentially begin the experiment as if they were a 'control cell'. Between days 4 and 6, the cells were assumed to be cycling in accordance with the rate parameter values calculated at day 4, and between days 6 and 10, the cells were cycling via the rate parameters calculated for day 6 and so on until day 21.

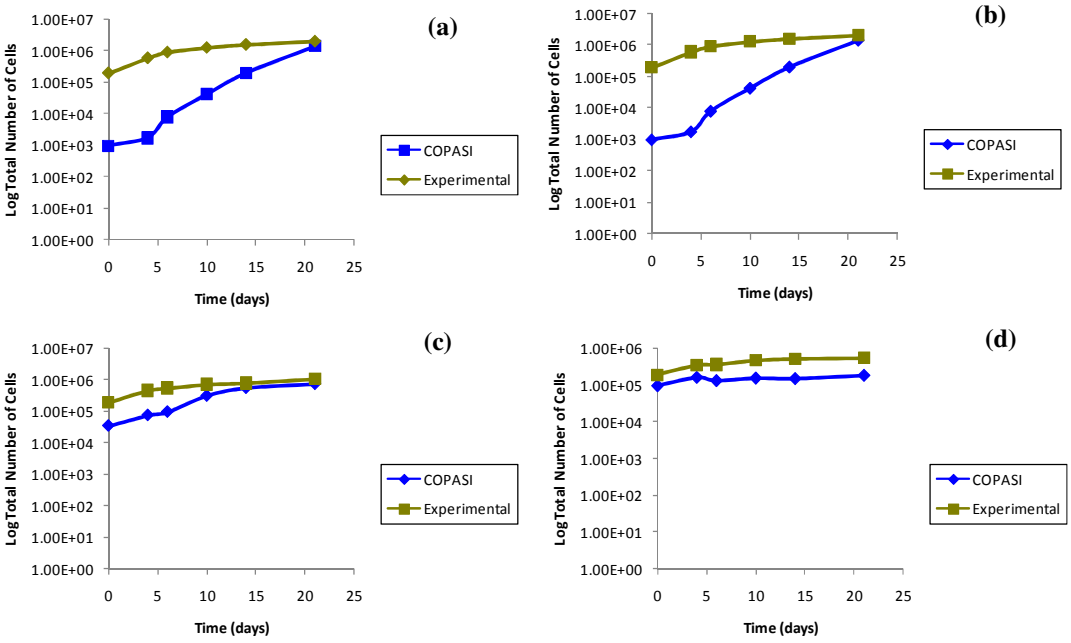


Figure 6.8 Simulations with COPASI whereby each rate parameter is considered separately. Parameter fitting was carried out to determine whether we could simulate the behaviour of doxorubicin exactly for each concentration (a) 3.15 nM, (b) 6.3 nM, (c) 31.5 nM and (d) 63 nM. The rate constants that were calculated using the five-compartment mathematical model were initially considered to be constant, however, closer analysis reveals that in fact they are not constant and change over the duration of the 21 days of the experiment. The simulations shown here were carried out by changing the rate parameters in accordance with how the experiment was carried out and show the total numbers of cells at each time point according to COPASI and what was observed experimentally. Each parameter was changed at day 4, 6, 10, 14 and 21 of the simulation.

Figure 6.8 illustrates the total numbers of cells at each time point according to COPASI and what was observed in the laboratory and simulations were carried out so that each rate parameter was changed in accordance with the time points that

were used experimentally. The graphs show that COPASI has failed to simulate the experimental results exactly and underestimates the numbers of cells at the early time points and overestimates the final total numbers of cells at the end of the experiment. At the highest concentration of doxorubicin analysed (i.e. 63 nM), COPASI underestimated the total numbers of cells throughout the duration of the simulation from that observed experimentally.

The next approach was to identify one rate parameter that changes to the greatest degree over the duration of the experiment according to the five-compartment mathematical model and repeat the simulations changing this rate parameter only on a daily basis in accordance with the line of best fit. The other rate parameters that do not change to the same degree were kept at the same value during the simulation and would be the average value for that rate parameter obtained from the mathematical model.

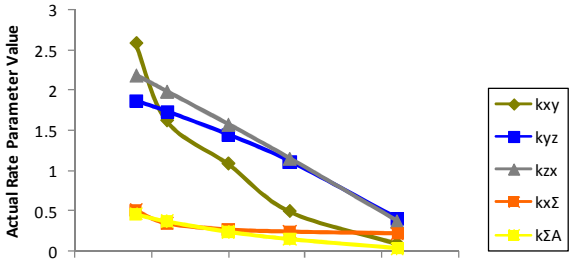


Figure 6.9 Changes in the rate parameters over the duration of the experiment at a concentration of doxorubicin of 6.3 nM. The change in each of the rate parameters obtained by the mathematical model is depicted here over the duration of the experiment. The values shown are the actual values and the control values are not considered.

Figure 6.9 shows the changes in the actual rate parameter values, irrespective of control, and shows that there are two groups of rate parameters; those which are time-dependent, and those that are not. Using figure 6.9 we wanted to identify one rate parameter that changed with respect to time with the greatest degree. The graph in figure 6.9 shows the actual values generated from the application of data from doxorubicin at a concentration of 6.3 nM to the five-compartment

mathematical model. It is important to note that control values are not taken into account. It is evident from figure 6.9 that the actual calculated values of $k_{\Sigma A}$ and $k_{x\Sigma}$ do not significantly change for the duration of the experiment. Although k_{yz} and k_{zx} do change, as evident in figure 6.9, they do not change to the same degree as k_{zx} which at day 4 of the experiment is 2.1 and by day 21 has decreased to 0.32 meaning there is a 6-fold difference in k_{xy} over the duration of the experiment and therefore is considered to change the most. In the initial simulation, whereby only the value of k_{xy} was changed, the line of best fit function was applied and the corresponding values at each time point were used. In the simulation, k_{xy} was change on a daily basis to reflect a linear change of k_{xy} with respect to time, whilst the values of k_{yz}, k_{zx}, $k_{\Sigma A}$ and $k_{x\Sigma}$ were kept constant as explained previously. These simulations revealed that again the biological behaviour of the system was not reproduced and so if the values of k_{xy} do change over the duration of the experiment and we highlight where the mathematical model is breaking down. The results of figure 6.9 clearly show a linear change in the rate parameters, however, COPASI reveals that it is not as simple as this as the biological behaviour is not reproduced. In this concept, we are using COPASI in a more complex manner and so highlight problems with the mathematical model. It is possible that a linear change in time in k_{xy} in accordance with the line of best fit might not actually be true because the values come from the five-compartment mathematical model which assumes exponential growth, and experimentally we did not observe a true exponential growth in the population of cells in the presence or absence of doxorubicin or RHPS4. The next logical approach was to apply a 'decay function' to the values of k_{xy} and again keep the values of the other rate parameters constant, however, change k_{xy} in accordance with the following equation:

$$\frac{dk}{dt} = -\alpha(k - k_0) \qquad \textbf{(6.4.1)}$$

Where $\frac{dk}{dt}$ represents the rate of change of k_{xy} with respect to time; α is the gradient of the decay of k_{xy}; k_0 is the end value of k_{xy} and k is the value of k_{xy} determined by the equation.

The aim of using this function is to change the value of k_{xy} in accordance with a decay function, and equation (6.4.1) is solved as in equations (6.4.2) – (6.4.3).

$$\int \frac{1}{(k-k_0)}\, dk = \int -\alpha\, dt \qquad \textbf{(6.4.2)}$$

$$\ln(k - k_0) = -\alpha t + c \qquad \textbf{(6.4.3)}$$

Where c = t0 – end value (i.e. the initial value of k_{xy} – the final value of k_{xy}).

And so, $k - k_0 = e^{-\alpha t + c}$, therefore $k = ce^{-\alpha t} + k_0$.

At t=0, $k = c + k_0$ and $c = k_0 - k$.

By changing the value of α (the gradient of the change in k_{xy}) and the k_0 (end value at which k_{xy} reaches), we were able to simulate the behaviour of doxorubicin as shown in figure 6.10 in terms of the growth of the system. It was desired that the biological behaviour of the system be reproduced in COPASI as accurately as possible, so in addition to reproducing the growth of the system, we wanted to ensure that at the end of the experiment, the predicted proportions of cells in each phase according to COPASI matched those of the experimental data and are shown in figure 6.10. When carrying out this 'trial and error' method of analysing the change in k_{xy}, it was evident that irrespective of the changes in α or the end value at which k_{xy} reached, the conclusions with respect to the proportions of cells in each phase were the same, even if the values obtained from COPASI were not the same as those of the experiment.

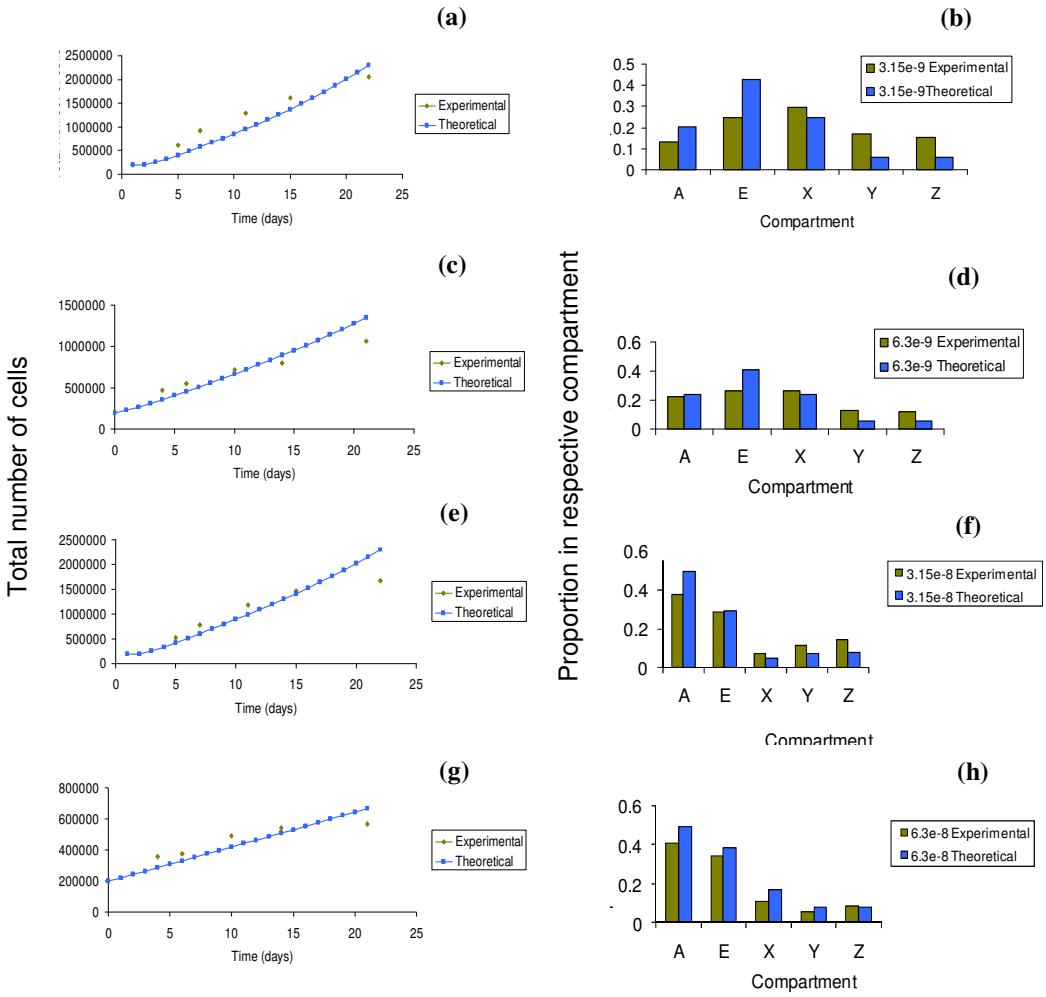


Figure 6.10 Total number and proportion of cells according to COPASI after parameter fitting for doxorubicin. Using parameter fitting it was found that not only could the growth of the systems be closely matched, but also the proportion of cells predicted in each phase. Graphs (a), (c), (e) and (g) show the growth of the system according to COPASI and experimentally for concentrations of doxorubicin at 3.15 nM, 6.3 nM, 31.5 nM and 63 nM respectively. Graphs (b), (d), (f) and (h) show the proportions of cells in each compartment at day 21 according to COPASI and experimentally, again for concentrations of doxorubicin of 3.15 nM, 6.3 nM, 31.5 nM and 63 nM.

Figure 6.10 shows the original experimental data alongside the data obtained following parameter fitting in COPASI. At the higher concentrations of doxorubicin, the data obtained from COPASI appears to more closely match that of the original experimental data and is true both in terms of the total numbers of cells and the proportions of cells. The data used to obtain the results is shown in table 6.8 and illustrates the original rate parameter values obtained from the application of the data to the five-compartment model alongside the data used

following parameter fitting in COPASI which was obtained in a 'trial and error' manner. Table 6.8 also shows the values of α and k_0 used to generate the results in figure 6.10 and shows that α appeared to change in a concentration-dependent manner, however, the value of k_0 appeared not to be concentration-dependent.

Table 6.8 The original data from doxorubicin and the data used in COPASI following parameter fitting.

Rate parameter	**Concentration of doxorubicin original rate parameter value (rate parameter value according to COPASI)**			
	3.15 nM	**6.3 nM**	**31.5 nM**	**63 nM**
k_{xy}	1.2	1.3	1.2	0.6
k_{yz}	1.1 (1.1)	1.13 (1.2)	0.7 (0.7)	0.4 (0.4)
k_{zx}	1.1 (1.1)	1.1 (1.1)	0.6 (0.7)	0.6 (0.6)
$k_{x\Sigma}$	0.25 (0.2)	0.34 (0.2)	0.5 (0.5)	0.3 (0.3)
$k_{\Sigma A}$	0.2 (0.05)	0.16 (0.05)	0.1 (0.1)	0.07 (0.08)
α	0.6	0.6	0.8	10.0
k_0 (end value of k_{xy})	0.29	0.27	1.34	0.32

The same concept was applied to the data obtained from the data obtained from application of the data obtained from RHPS4 to the five-compartment mathematical model. Initial observation of the change in the actual values of each rate parameter revealed that for RHPS4, it is the value of $k_{\Sigma A}$ that changes the most over the duration of the experiment. Again these are the actual values of the individual rate parameters, not changes relative to control. As stated previously, the parameter fitting was performed in a 'trial and error' manner, and when applying the same concept to RHPS4, it was proven that more deviation from the original data was required to obtain the results shown in figure 6.11.

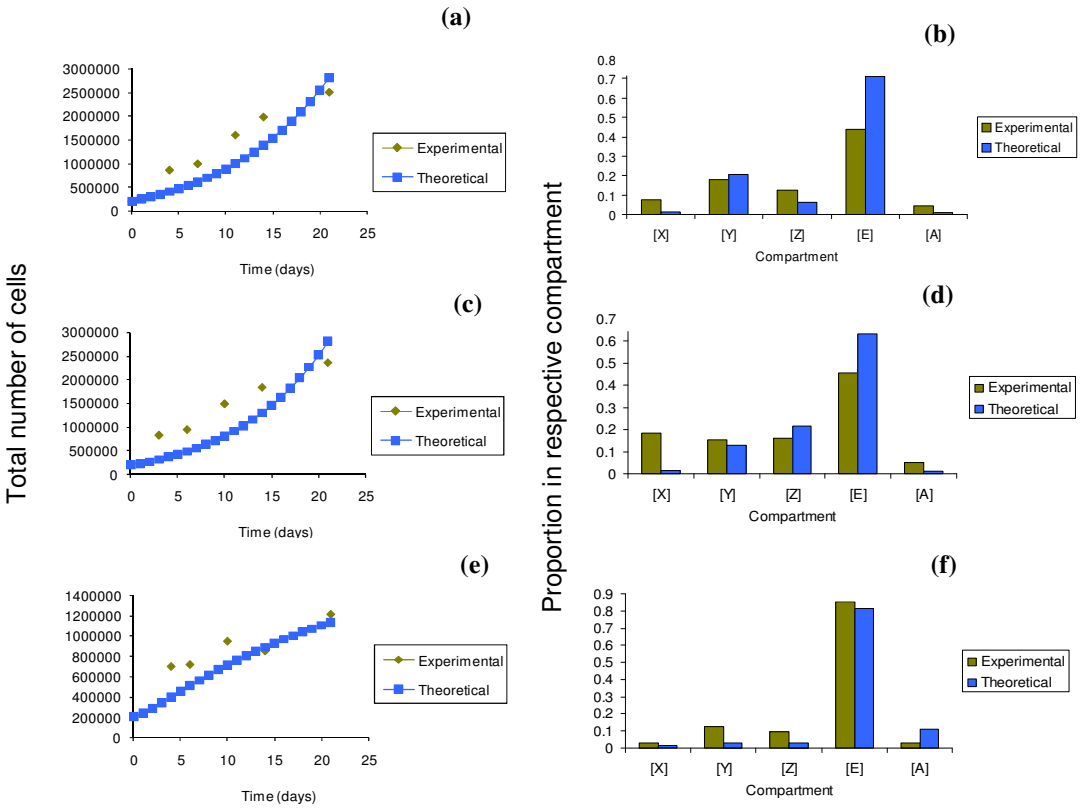


Figure 6.11 Total numbers of cells and the proportion of cells in each compartment when RHPS4 data is applied to COPASI. Using parameter fitting it was found that not only could the growth of the systems be closely matched, but also the proportion of cells predicted in each phase. Graphs (a), (c), and (e) show the growth of the system according to COPASI and experimentally for concentrations of RHPS4 at 50 nM, 100 nM and 1 µM, respectively. Graphs (b), (d), and (f) show the proportions of cells in each compartment at day 21 according to COPASI and experimentally, again for concentrations of RHPS4 of 50 nM, 100 nM and 1 µM.

Figure 6.11 show that although we were able to closely match the growth of the system using COPASI in terms of the number of cells, the proportions of cells that are predicted in each compartment are not as closely matched. It is also evident that at a concentration of RHPS4 of 1 µM, the growth of the system is more closely matched than at the lower concentrations of 50 nM and 100 nM. The GI_{50} of RHPS4 in HCT116 cells was calculated as 1 µM over a 72 hour period and which is short term compared to the 21 days upon which the data for the mathematical model and COPASI was based. In theory this concentration of RHPS4 causes 50% growth inhibition in HCT116 cells and so it is likely we are 'blasting' the cells with a high concentration of RHPS4 over a long period of time and so we are observing the simpler effects of RHPS4. At this concentration of

RHPS4 we may be observing a simpler mechanism of action and we are possibly 'masking' the more complicated effects. The data in table 6.9 shows the values of each rate parameter that was applied to COPASI to obtain figure 6.14 and shows the original rate parameter values and the values to which they were changed to. At the higher concentration of RHPS4, it is evident that there was no requirement to change the values of k_{xy}, k_{yz} or k_{zx}, however, the value of $k_{\Sigma A}$ had to be changed from 0.009 to a lower value of 0.001.

Table 6.9 The original data from RHPS4 when applied to the five-compartment mathematical model and when used in COPASI following parameter fitting.

Rate parameter	**Concentration of RHPS4 original rate parameter value (rate parameter value according to COPASI)**		
	50 nM	**100 nM**	**1 µM**
k_{xy}	2.9 (8.0)	4.4 (10)	1.8 (1.8)
k_{yz}	2.5 (0.5)	2.9 (1.0)	0.8 (0.8)
k_{zx}	2.7 (1.0)	2.3 (0.5)	0.8 (0.8)
$k_{x\Sigma}$	0.9	1.3	0.7
$k_{\Sigma A}$	0.05 (0.001)	0.01 (0.001)	0.009 (0.001)
α	0.9	3.0	0.5
k_0 (end value of $k_{x\Sigma}$)	3.9	4	2.3

At the lower concentrations of RHPS4 (i.e. 50 nM and 100 nM) each of the rate parameters had to be changed, with k_{xy} having the greatest degree of change. RHPS4 is known to have a complex mechanism of action and so at these low concentrations of RHPS4 it is likely we are observing different kinds of changes within the cells that, at the moment, the mathematical model cannot distinguish between. We are observing a time-dependent change in the rate parameters with respect to RHPS4 using COPASI; however, there appears to be no consistent concentration-dependent trends. Using the mathematical modelling and COPASI has allowed us to establish the there are changes occurring within the cells almost instantly, and changes occurring at long times and it is these that are more dominant and are therefore the ones being modeled in chapter 5. Their exact mechanisms at present remain unknown. The aim was never to establish the mechanism of action of RHPS4 using mathematical modelling and COPASI, but

to link the emergent properties of the experimental results to a possible molecular target. It is clear that at the moment we are able to distinguish that there are both short-term and long-term changes within the cells and adds to the complexity of RHPS4 as an anti-tumour agent and it is possible to use the information derived from the mathematical modelling and COPASI in this chapter to focus further investigations regarding the underlying mechanisms involved.

6.5 Conclusions

An integral part of any systems biology study is the modelling and simulation of the system under investigation (Sahle *et al*, 2008). Simulation and modelling are greatly becoming the standard approaches to the understanding of complex biochemical processes. COPASI is one such program which allows the simulation and analysis of biochemical networks and here allowed us to analyse the accuracy of the five-compartment mathematical model derived in chapter 4 following the application of experimental data in chapter 5 (Hoops *et al*, 2006). When applied to the five-compartment model, it was initially thought that there was resistance of HCT116 cells to doxorubicin, particularly at the higher concentrations. COPASI enabled us to model the data and reveal whether the population of HCT116 cells was composed of a resistant population and sensitive population. If there was in fact, a resistant population, COPASI would allow us to distinguish this and the proportion of resistant cells to be calculated. If we model a system of resistant and sensitive cells using the methods described in section 6.3, each sub-population still grows in an exponential manner and so the total growth rate is a sum of exponentials- which can never be the behaviour we observed. In the experiments, the cells did not grow in an exclusively exponential fashion, nor did they grow exclusively linear, however, the methods used to investigate resistance assumed true exponential growth, and therefore would never be able to reproduce the biological behaviour we observed.

Finally, COPASI was used to clarify the five-compartment model and prove that the results can be reproduced using parameter fitting. In order to do this, for each concentration, the rate parameters were analysed and it was recognized that the

rate 'constants' were in fact not constant and so the line of best fit for each parameter was calculated to obtain the values at each time point. From the line of best fit, the initial proportion of each compartment was applied and the rate parameter values were changed daily. Again, the simulations were not reflective of the observed experimental results and so the rate parameters that were considered not to change significantly over the 21 days were kept constant and the value of the rate parameter that was considered to change the most was changed daily according to the line of best fit. From the application of the data to the five-compartment model, it appeared that the value of k_{xy} was changing the most as the day 4 value (according the five-compartment model) was 1.88 at a concentration of doxorubicin of 3.15 nM and reduced to 0.32 by day 21 and appeared to be the case for each of the concentrations of doxorubicin observed. This approach appeared to reduce the final numbers of cells to be more in line with the experimental values, however, the system was still growing in an exponential manner and so maybe the value of k_{xy} was not changing in a linear manner. The same approach was applied to the data obtained from RHPS4, and it was noticed that $k_{x\Sigma}$ was changing at a faster rate, and to the greatest degree than the other rate parameters, and again was not occurring in a linear fashion. The concept of a non-linear change, and instead a 'decay function' applied to changes in the rate parameters k_{xy} and $k_{x\Sigma}$ for doxorubicin and RHPS4, respectively was applied to COPASI using a parameter fitting methodology and this allowed the data to be simulated more accurately. It was found, however, that the changes in the values of the rate parameters for RHPS4 deviated from the original values more than those obtained from doxorubicin and is possibly due to RHPS4 having the more complex mechanism of action. With RHPS4, we were also unable to determine a concentration-dependence in the changes of the rate parameters; however, we could establish a time-dependence. At the lower concentrations of RHPS4, it is possible that there are different kinds of changes occurring within the cells upon exposure to RHPS4, with some occurring at earlier time points than others and the model is unable to distinguish between these. At the highest concentration, whilst different kinds of changes are occurring, it is possible that this concentration is

'blasting' the cells and so that we may only be observing the simpler effects of RHPS4 and the model is able to distinguish these and so when trying to reproduce the biological behaviour of this concentration in COPASI, the rate parameters did not have to be changed to the same degree as those for the lower concentrations of 50 nM and 100 nM. In addition to reproducing the growth of the system in COPASI, we also wanted to reproduce the proportions of cells in each compartment that was observed experimentally, and whilst we were unable to match them directly, what was obvious, was that irrespective of the rate parameter being changed and to what degree of change, the overall conclusions were the same.