

Modeling Cyclophosphamide's Effect on Leukocytes

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Abstract

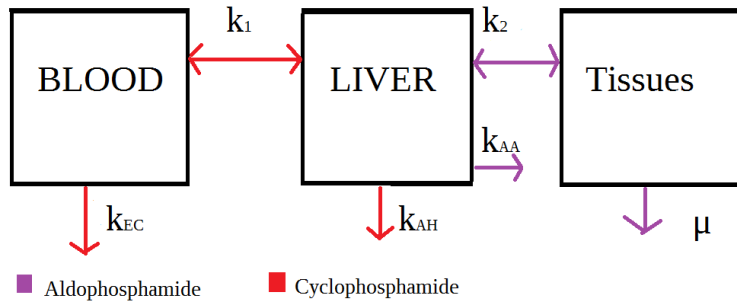
A dangerous side effect of a chemotherapy drug called cyclophosphamide can contribute to the advancement of cancer treatment. Modeling the toxic effect of cyclophosphamide on leukocytes has implications in assisting oncolytic virotherapy, the use of engineered viruses to combat cancerous cells. The pharmacodynamics of cyclophosphamide and its metabolites are modeled as well as its direct and indirect effect on leukocytes numbers. This is accomplished by using compartmental ordinary differential equations. The ultimate goal is optimizing the dosage of cyclophosphamide such that the leukocyte population is suppressed while the cyclophosphamide concentration is maintained in a safe range. The viral response of leukocytes is taken into account as well as the long term effects of the daily dosage. Due to the complexity of these interactions the program Mathematica is used to find the optimal dosing.

1 Introduction

Cyclophosphamide is a pro-drug that is typically used in immune suppression and chemotherapy. However it has been used recently to augment oncolytic virotherapy, the use of engineered viruses to combat tumors. Initially the viral therapy wasn't that successful due to the immune response it elicited. Cyclophosphamide is used to suppress the immune system enough so that the viruses can infect and kill the cancerous cells in the body. (Qi-Xiang et al. [2008]) Similar models have been built, either to model the difference in effectiveness of engineered virus with and without cyclophosphamide dosing, or the effect of chemotherapy drugs on the hematopoiesis. However my model is unique because it specifically targets the reactions of sensitive cells while taking the immune systems response to the viral load into account.

2 Background Biology/Chemistry and Model Development

Cyclophosphamide (CY) is inactive until it reaches the liver and then is metabolized to hydroxycyclophosphamide(HCY) which lives in equilibrium with its tautomer aldophosphamide(AP). About 70% of CY is metabolized to HCY, the rest is primarily excreted unchanged in urine. (McDonald et al. [2003]) HCY is mainly excreted through the reactions of AP. AP is eliminated by its oxidation to an inactive compound carboxycyclophosphamide in the liver. It also freely diffuses into cells where it is converted to phosphoramidate mustard and acrolien which are the main cytotoxic metabolites of CY. Cells that are sensitive to these compounds, such as hematopoietic progenitor cells and lymphocytes, then undergo apoptosis (Emadi et al. [2009]). The model is focused the cyclophosphamide concentrations in the liver and blood (HCY isn't in the model because AP is what interacts chemically in tissues and degrades in the liver). To model that interaction without tracking HCY I assumed that a third of the amount of HCY being activated in the liver is directly converted to AP because in the body there is about a 1:2 ratio of AP to HCY.(Borch et al. [1984])The AP concentration is tracked in the liver and tissue. This is based on the assumption that the tissues is where the cytotoxic activity is happening rather than the blood, it is simply a means of transport between the liver and tissues. In each differential equation the transport is modeled based on a concentration gradient, that the drug dynamically flows to a lower concentration based on the mechanics of passive diffusion. The k_1 and k_2 values approximate these rates per hour.



This leads to these equations:

$$\frac{dC_B}{dt} = k_1(C_L - C_B) - C_B k_{EC} + D(t) \quad (1)$$

$$\frac{dC_L}{dt} = -k_1(C_L - C_B) - C_L k_{AH} \quad (2)$$

$$\frac{dA_L}{dt} = k_{AA}C_L - k_2(A_L - A_T) - k_{EA}A_L \quad (3)$$

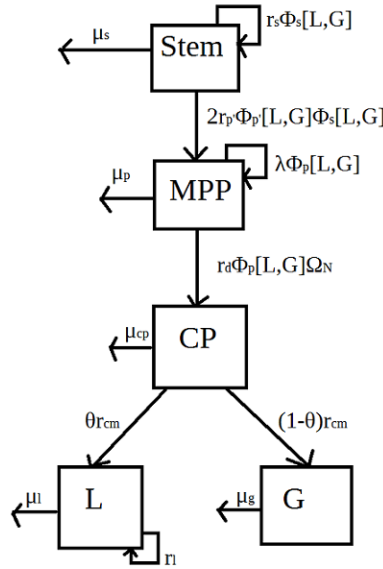
$$\frac{dA_T}{dt} = -k_2(A_T - A_L) - \mu A_T \quad (4)$$

Where $D(t)$ is the controlled dose given every 24 hours. It's a simple piecewise function that is a fixed dose value every 24 hours and is zero all other times.

The model for the leukocyte population is based on Mangel& Bon-sall's article Stem Cell Biology is Population Biology: Differentiation of Hematopoietic Multipotent Progenitors to Common Lymphoid and Myeloid Progenitors. The original model had five compartments, stem cells, multipotent progenitor cells, common lymphoid progenitor cells, common meylod progenitor cells, lymphocytes and meylod cells. I was specifically interested in lymphoid and granulocytes (which are a small subset of the meylod cells). To adapt the model two major assumptions were made. The first was that the ratio of lymphocytes to meylod cells was 1:1000, which was an average ratio found in the original model. The second was a 3:7 ratio of lymphocyte to granulocyte, which is an assumption that was held constant through out the entire model construction. The actual ratio of lymphocytes to granulocytes varies depending on immune response, health, and other factors. (Friberg [2003]) This ratio was around in the middle of the range and the numbers made it easier to convert the old model into a model that was relevant to my focus. The differentiation of multipotent progenitor cells to common lymphoid of meylod progenitor cells was based on a probability function. That probability was fixed based on an assumed a homeostatic state in the body. That constant fixed rate made it easy to combine the two different common progenitor cells into one compartment, especially because their death rate were equivalent. A previous assumption of a 3:7 ratio of lymphocytes to granulocytes in homeostasis meant 30% of the time the common progenitor cells divide they becomes lymphocytes. In order to get a plausible ratio of common progenitor cells to the leukocytes, I assumed a 3:7:2993 ratio of lymphocytes to granulocytes to the other meylod cells. Then the percent of leukocytes (lymphocytes and granulocytes) in total cells is $\frac{10}{3003}$. So in terms of the model that means only $\frac{10}{3003}$ of common progenitor cells created lymphocytes and granulocytes. The ratio also shows that granulocytes are $\frac{7}{3000}$ of the meylod cells, so then $m = \frac{3000}{7}g$ was substi-

tuted in to the feed back functions that were dependent on the meylloid and lymphocyte populations.

The model adapted has 4 groups of cells, stem cells, multipotent progenitor cells, common progenitor cells, lymphocytes, and granulocytes. Stem cells produce themselves, and multipotent progenitor cells, multipotent progenitor cells produce themselves and common progenitor cells. The common progenitor cells in turn produce lymphocytes (which can multiply) and granulocytes (which cannot). However the rates in which the stem cells and multipotent progenitor cells reproduce are dependent on feed back from the lymphocyte and granulocyte concentrations. (Mangel and Bonsall [2013]) In the figure below the ϕ functions of L and G, represent this feedback. This model also incorporates the cytotoxic effects on the multipotent progenitor cells, common progenitor cells, and lymphocytes. Additional parameters are explained more in depth in the table on pg.12



$$\phi_s[L, G] = \text{Max}\left[\frac{1}{1 + 10L}, \frac{1}{1 + \frac{300}{7}G}\right]$$

$$\phi_p[L, G] = \text{Max}\left[\frac{1}{1 + 100L}, \frac{1}{1 + \frac{3}{7}G}\right]$$

$$\phi_{p'}[L, G] = \text{Max}\left[\frac{1}{1 + 20L}, \frac{1}{1 + \frac{600}{7}G}\right]$$

All μ are death rates
 $\lambda, r_s, r_d, r_{p'}, r_{cm}, r_l$
 are just
 rates of cell repro-
 duction or produ-
 ction of another cell
 $\theta = 0.3$

This picture gives rise to the following set of differential equations:

$$\frac{dS}{dt} = S \ln\left(\frac{K}{S}\right)(r_s - r_{p'}\phi_{p'}[L, G])\phi_s[L, G] - \mu_s S \quad (5)$$

$$\frac{dMPP}{dt} = S \ln\left(\frac{K}{S}\right)(r_s + 2r_{p'}\phi_{p'}[L, G])\phi_s[L, G] + MPP((\lambda - r_d)\phi_p[L, G] - \mu_p - \alpha_1 A_t(t)) \quad (6)$$

$$\frac{dCM}{dt} = MPP r_d \phi_p[L, G] \Omega_N - CM(\mu_{cm} + r_{cm} + \alpha_2 A_t(t)) \quad (7)$$

$$\frac{dL}{dt} = CM r_{cm} \theta \frac{10}{3003} + L(r_l - \mu_l - \mu_{l*} I_{vt > vth} - \alpha_3 A_t(t)) \quad (8)$$

$$\frac{dG}{dt} = CM r_{cm} (1 - \theta) \frac{10}{3003} + G \mu_g \quad (9)$$

The term $\ln\left(\frac{K}{S}\right)$ expresses the assumption that there is a maximum density K of stem cells in a niche. The α terms takes the toxic effect of AP into account where α is the kill rate of the cells dependent on the concentration of AP in the tissues. The cells that AP actually kills are progenitor cells and leukocytes, stem cells are comparatively resistant to AP toxicity. (Emadi et al. [2009]) So additional parameters $\alpha_1, \alpha_2, \alpha_3$ were added as rates that AP kills MMP, CM and L (respectively). Locating definite values for α_1 and α_2 was possible, however a value for α_3 remained unattainable. So several values were inputted to the model to run a rudimentary sensitivity analysis. The immune response is also modeled by a slightly higher death rate of lymphocytes which in turn stimulates the stem cells and multipotent progenitor cells to replicate more causing an overall increase in the cells. The term $\mu_{l*} I_{vt > vth}$ models this interaction. I is a piecewise function that is zero when the viral concentration is less than the threshold concentration of viruses that it takes to elicit an immune response and one when the viral concentration is greater than the threshold concentration. The term μ_{l*} is the additional death rate that ultimately leads to a greater number of lymphocytes. (Mangel and Bonsall [2013])

3 Tools and Techniques

Given the complexity of solving a nonlinear seven differential equation system, the primary tool in using and understanding these equations was the program Mathematica. The first result was using the NDSolve function on the leukocyte model, without the effect of aldophosphamide or viral load. From looking at the long term behavior through various initial conditions

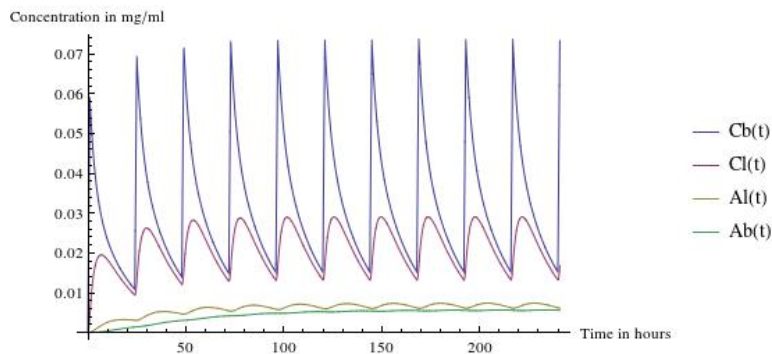
I found hesitant equilibrium values which were used as the initial conditions in the combined model. Starting with equilibrium would get a more accurate view of aldophosphamide's effect on the cells. Then the cyclophosphamide model was looked at specifically. All my parameters values were found except for the k_1 and k_2 values. These values were found by fitting data in to the model. Ultimately the entire system was initially inputted as a function where the variables were the time (in hours) and doses (mg/kg). Within the function it was converted to mg/ml of blood, assuming a 100% body absorption, and the doses were introduced at $t=0$ and every 24 hours after that. After that the system was set as a function of a loading dose, maintenance dose, and time, with the same units as the original function. A loading dose is a high dose given to achieve a drastic effect quickly, while a maintenance dose is a lower dose given to maintain that effect. Within the function the loading dose was given for the first three days and the maintenance dose the following seven days. This function was my main tool to ultimately getting results.

4 Results

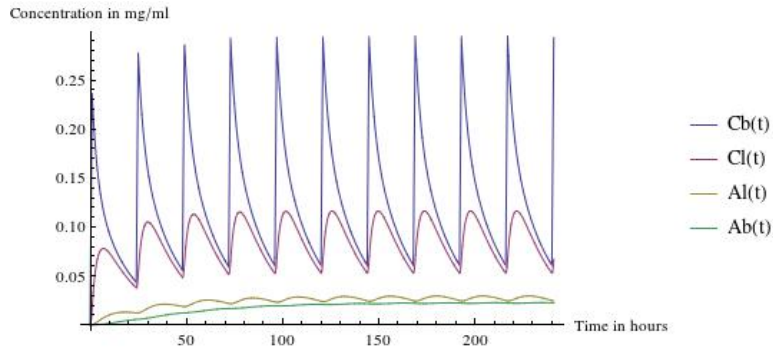
Here is an example of what the curves for equations (1-4) looked like with a fixed dose given every day for 10 days:

Different Dosages over 10 days

Dose of 5 mg/kg



Dose of 20 mg/kg

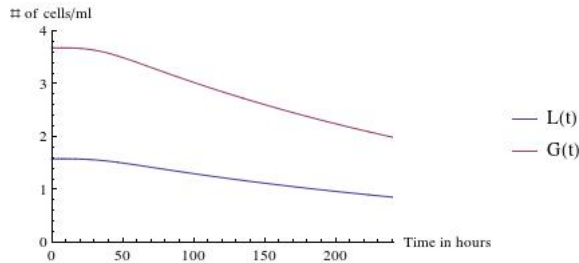


Due to the lack of information in the literature I was unable to find a definitive α_3 value. So the Monte Carlo Method was utilized in order to analyze how sensitive the lymphocyte decrease is due to different values of α_3 on the percent decrease of L in Mathematica. This was achieved by writing a loop that takes α_3 as a random real number between 0 and 2 and applies it to the output of the percent decrease. I ran this loop 10,000 times and took the mean and standard deviation of the percent decrease of L. From this I was able to ascertain that this alpha value affects the change in L a great deal. The first function was used (with variables of a fixed dose for ten days) and inputted various α_3 values, shown below.

This shows the various effects on lymphocytes with different α_3 values with a fixed 5 mg/ml dose for ten consecutive days.

For $\alpha = 0$

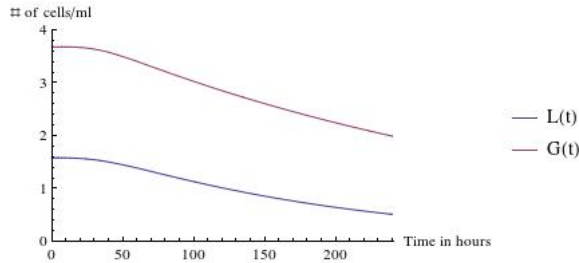
L and G decreases 46%



When $\alpha = 0.5$

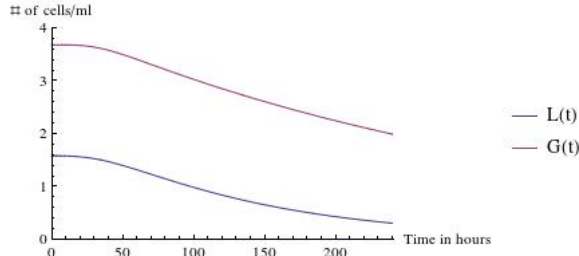
L decreases 68%

, but G decreased the same amount



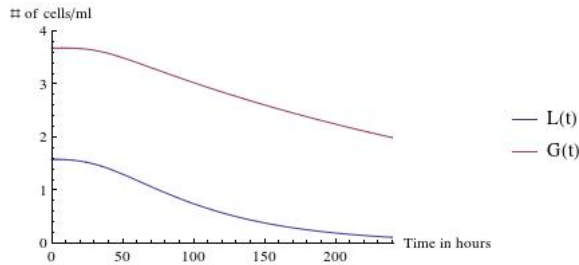
For $\alpha = 1$

L decreases 81% and has no effect on G



$\alpha = 2$

L decreases 93% which is extremely dangerous

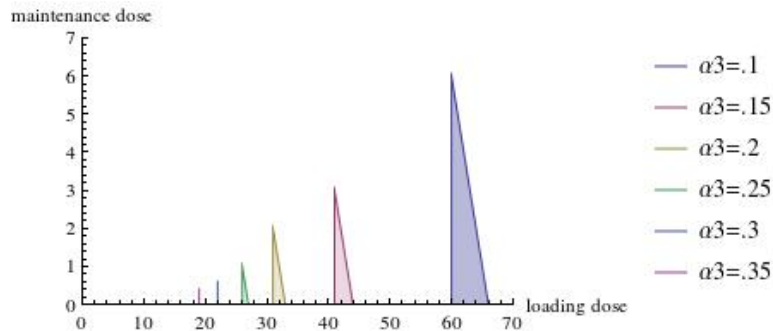


The above graphs shows how to get a reasonable α_3 value range. 5 mg/kg of CY given once a day for 10 days shouldnt decrease the lymphocyte population over 60%, because that is a relatively small dose(Alan Boddy [2000]). So for the model to have a reasonable output the α_3 value should be between 0 and 0.5. First I split the difference and choose $\alpha_3=.25$. Next the loading dose was found that would decrease about 30% in the first three days. Then the corresponding maintenance dose was found that would keep the percent decrease at the end of 10 days and tried to keep it above 75%, and kept adjusting both. The results ended up as a loading dose of 25 mg/kg as a loading dose and 1.5 mg/kg as a maintenance dose.The percent decrease in lymphocytes after 3 days was approximately 29.9% and at

the end of the 10th day was 74.6%. The decrease of leukocytes (lymphocytes+granulocytes) at the end of the 3rd day was approximately 19.5% and at the end of the 10th day was 56.6%. Slightly increasing α_3 to .3 lead to a need to adjust the maintenance dose to 0 which still lead to a 76.9% decrease in lymphocyte population. My conjecture is that this phenomenon is caused by the build up of aldophosphamide in the tissues with its relatively slow rate of degradation in tissues. On the other hand decreasing alpha to .2 lead to having to adjust the loading dose but not maintenance dose. With the new loading dose as 30 mg/kg, this yielded a 29.6% decrease after 3 days and a 73.8% decrease after 10 days. Here is a table of some α_3 values and the corresponding maintenance and loading doses.

α_3 value	LD	MD	ΔL 3 days	ΔL 10 days
0	200	10	17.3%	50.2%
.1	60	1	30.3%	73.6%
.2	30	1.5	29.6%	73.8%
.25	25	1.5	29.9%	74.6%
.3	22	0.5	30.4%	74.9%
.35	19	0	30.3%	74.5%

I wanted to generalize more on what the optimal loading and maintenance doses could be given different α_3 values. This doesn't include the case where $\alpha_3 = 0$ because it didn't output reasonable maintenance and loading doses and also I already knew via trial and error that $\alpha_3 = 0$, as a solution, will not work. At day three the approximate minimum loading dose it took to suppress the lymphocyte population at least 30% (in the graphs below this is the point on the triangle on the x-axis line closer to the origin) was found. Then the maintenance dose was increased to find the maximum possible maintenance dose such that the lymphocyte population decreases less than 75% at the end of the 10th day (in the graph below this is the highest point on the triangle). Finally the maintenance dose was kept equal to zero and increased the loading dose until the maximum loading dose possible was found, such that at the end of ten days the decrease in lymphocytes doesn't exceed 75%



This gave me an approximate (I cannot verify the linearity between the three points I found to draw the triangles) range of combinations of loading doses and maintenance doses given different α_3 values. This graph is supposed to illustrate that as α_3 gets higher the range of combinations of loading and maintenance doses gets smaller.

5 Discussion

In my research I was only able to find vague references to the sensitivity of lymphocytes to aldophosphamide. It doesn't indicate a direct effect on the lymphocyte population. However I attempted to ignore the α_3 value and just use the α_1 and α_2 values my model didn't work and produce reasonable output. This may be due to the α_1 and α_2 values because they were found in a study that showed the effect on the cells in vitro with HCY, rather than in vivo. I began to adjust the different values but the change in values made very little difference to this model. So I assumed that there is some α_3 value which is how I obtained results. However, this outcome could be caused by an potentially erroneous assumption that α_1 and α_2 are fixed rates, when in fact it is entirely possible that they are not, and rather than simple values, α_1 and α_2 could be functions of time instead.

6 Further Research

I would like to either find a more definitive α_3 value or find α_1 and α_2 values or functions that produce reasonable output. There could also be a longer loading dose time period that is necessary to accomplish the effect of the lowered immune response perhaps three days is not sufficient to lower the

lymphocyte levels due to the need for a build of of aldo phosphamide in the tissues. It would be interesting to look at different loading dose time periods and find how many days is optimal to suppress the immune system to a reasonable level. Ideally I would really like some hard data to fit my model to in order to accomplish this. Particularly looking at the immune response to different dose schedules of CY. However finding data is extremely difficult especially since the chemical reactions happen in the tissues rather than the blood where most white blood cell counts are quantified, and even those are few and far between when it comes to CY studies. Eventually the inclusion of the viral response to the virotherapy treatment will need to be taken into account because the effect of the AP on leukocytes have been studied with an assumption of homeostasis in the body, which will not be the case in terms of an immune response to viruses. I did try to approximately take this into account by decreasing the lymphocytes 75% which is actually a higher rate than is necessary. This may take the influx of white blood cells due to a viral load down to a more reasonable amount. Ultimately once I am satisfied that my model has sufficient parameters I would like to use control theory to mathematically find the optimal loading and maintenance doses. I would first solve equations 5-9 for an ideal A_t that would suppress the immune system. Then I would try to minimize the difference between the actual A_t curve and the ideal A_t , which will let me end up with an ideal dose of CY given.

Parameter	Interpretation	Value	Reference
K	Max S in a Niche	10	Mangel and Bonsall [2013]
r_s	Max rate of S Self-Renewal	2.5	Mangel and Bonsall [2013]
$r_{p'}$	Max rate of S asymmetrical division	0.001	Mangel and Bonsall [2013]
μ_s	Rate of S death	0.004	Mangel and Bonsall [2013]
λ	Rate of MPP multiplication	0.25	Mangel and Bonsall [2013]
θ	Fixed rate of L production	0.3	Friberg [2003] ₁
μ_p	Rate of MPP death	0.2	Mangel and Bonsall [2013]
Ω_N	Combination of intermediate constants	1	Mangel and Bonsall [2013]
r_{cm}	Rate of division of CM into L&G cells	0.01	Mangel and Bonsall [2013]
μ_{cm}	Rate of CM death	0.001	Mangel and Bonsall [2013]
r_l	Rate of multiplication of L	0.025	Mangel and Bonsall [2013]
μ_l	Rate of L death when IS is inactive	0.028	Mangel and Bonsall [2013]
μ_{l*}	rate of L death when IS is active	0.01	Mangel and Bonsall [2013]
v_{th}	Min concentration of virus to activate IS	0.025	Mangel and Bonsall [2013]
r_d	Rate of division of MPP into CM cells	0.2	Mangel and Bonsall [2013]
α_1	Rate of MPP death due to A_T	36.101	Siena et al. [1985] ₃
α_2	Rate of CM death due to A_T	25.63175	Siena et al. [1985] ₃
α_3	Rate of cell death due to A_T	unknown	unknown
μ_g	Rate of G cell death	0.003	Friberg [2003] ₁
k_1	Diffusion rate of CY between blood and liver	.15	Joy et al. [2012] ₂

k_2	Diffusion rate of AP between tissues and liver	.036	Joy et al. [2012] ₂
k_{ec}	Elimination rate of CY in blood	0.0319846	CCO [2014] ₃
k_{ah}	Activation rate of HCY	0.0746308	McDonald et al. [2003] ₃
k_{aa}	Activation rate of AP	.0248	Borch et al. [1984] ₁
k_{ea}	Elimination rate of AP in Liver	0.07254	Joy et al. [2012] ₃
μ	Elimination rate of AP in tissues	0.00806	Joy et al. [2012] ₃

1 Based on assumptions mentioned in paper

$-\mu_g$ was solved for in order to maintain the assumption of a fixed θ

2 Found by fitting data to model

3 Found using calculations based on data or fact

$-\alpha_1$ and α_1 were found by converting percent change in cell death per $\mu\text{mo/Liter}$ to percent change in cell death per mg/ml

$-k_{ec} = .693/t_{1/2}$ where $t_{1/2}$ is based on the half life of CY and this was a similar calculation for k_{ah}

$-\mu$ and k_{ea} are based on $t_{1/2}$, together they add up to $.693/t_{1/2}$ but the different values combinations of these don't impact the model

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