

Analysis of Toxicity Effects from Molecular to Population Level: Circadian Oscillator Case Study

José-Manuel Zaldívar Comenges*

European Commission, Joint Research Centre, Institute for Health and Consumer Protection, Ispra, Italy

Abstract: To analyze how toxic effects propagate from molecular to population level, a simple model of a circadian oscillator has been coupled with a cyanobacteria growth model. It has been assumed that the circadian oscillator synchronizes with light-dark environmental conditions and toxicants affect this synchronization reducing the population growth. Then dose-response curves have been developed. The results evidence that to understand toxic effects we have to focus our study on the interactions that a certain compound may have with proteins, and/or DNA, since the result of these interactions will determine the final mode of action at macroscopic level. Therefore an intermediate step for assessing toxic effects has been proposed.

INTRODUCTION

Computational toxicology has been defined in [1] as “the integration of modern computing and information technology with the technology of molecular biology and chemistry to improve risk assessment for toxic chemicals”. Therefore, computational toxicology should consider several computational disciplines including [1]:

- to improve our understanding of the linkages between a chemical and its adverse effects from a continuum prospective, i.e. from molecular to ecosystem level, see Fig. (1);
- to provide predictive models for chemical prioritization, screening and testing, able to reduce animal testing; and

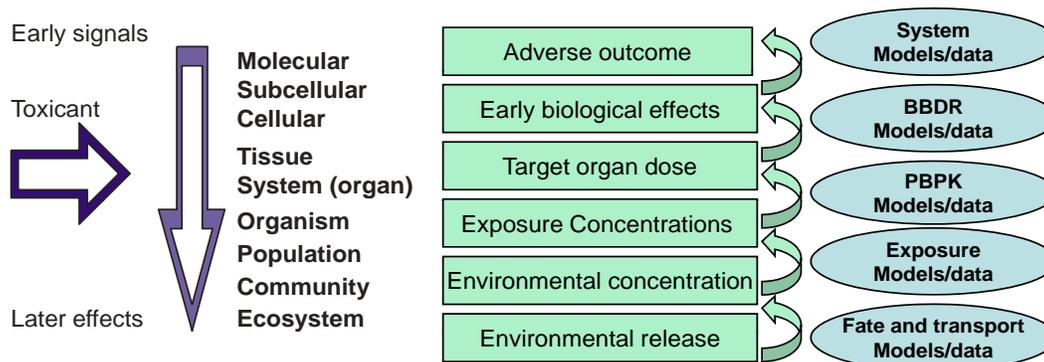


Fig. (1). Sequential order of contaminant effects and cause-effect continuum and Computational Toxicology approaches, modified from [1] and [3]. (BBDR: Biology-based dose response; PBPK: Physiologically based pharmacokinetic).

- Computational chemistry: physical-chemical modeling at the molecular level (quantum and molecular mechanics simulations) and chemo-informatics, e.g. QSAR.
- Bioinformatics: development of molecular biology databases as well as data processing, storage, distribution, analysis and interpretation.
- Molecular systems biology: application of mathematical modeling and reasoning to the understanding of complex biological phenomena [2].

According to [1], the objectives of Computational Toxicology are:

- to provide quantitative risk assessment methodologies able to integrate human and ecological risk assessment.

Therefore, the final objective of computational toxicology is to be able to describe the response of a functioning organism to toxicants at all levels of biological organization and complexity by combining the information from several sources to gain a deeper understanding on the mechanisms of toxic action and by developing approaches allowing a more sensitive and earlier detection of adverse effects in toxicity studies. This constitutes a big challenge since it requires to study interactions between genes, proteins and metabolites, and to integrate the results from different theoretical and experimental set-ups. However, a better understanding of the molecular mechanisms of toxicity may contribute to the extrapolation between experimental conditions, from low to high doses, from short term to chronic exposures, etc; thus,

*Address correspondence to this author at the European Commission, Joint Research Centre, Institute for Health and Consumer Protection, Ispra, Italy; E-mail: jose.zaldivar-comenges@jrc.ec.europa.eu

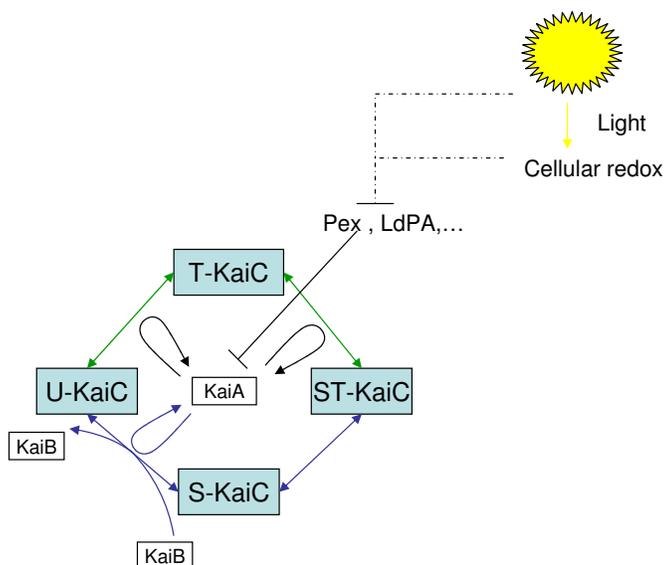


Fig. (2). A model of KaiC phosphorylation oscillations with the role of KaiA and KaiB on the process and the effects of solar radiation, modified from [6].

reducing the need for animal testing as well as the dose levels needed for toxicity assessment.

The introduction of new developments of Computational Toxicology into Systems Toxicology may be able to support *in vitro* experiments by discovering the underlying molecular mechanisms of toxicity and to provide a better link better between *in vitro* models to *in vivo* studies. Finally, these developments should serve to improve human and ecosystem risk assessment, after a new set of standardization, regulations and guidelines have been developed. Of course, this process will require extensive experimental validation before its application in a regulatory context and probably sequential approach. However, with the development of Integrated Testing Strategies (ITS) that allow the integration of diverse types of toxicological information for toxicological hazard identification and characterization; computation toxicology may play an increasingly important role in this process helping in the 3Rs (reduce, refine or replace) process on the use of laboratory animals.

In this work, as a first attempt to analyze toxic effects from molecular level to population level, we have coupled a model of the circadian clock of cyanobacterium [4], see Fig. (2), with a bioenergetics growth model of phytoplankton [5]. Contaminants' effects are modelled by modifying the amount of proteins available in the circadian oscillator. Assuming that the circadian oscillator is synchronized with the external light-dark cycles, the change in protein concentrations propagates in the ability of cyanobacteria to deal with solar radiation and their variation over a yearly cycle and, subsequently, in the growth of the population. Based on these assumptions, dose-response relationships have been also modelled.

METHODS AND APPROACH

Circadian Oscillator Model

Studies on the cyanobacterium *Synechococcus elongatus* have shown that it has an internal biological clock for

measuring circadian time. Tomita *et al.* [7] and Nakajima *et al.* [8] showed that circadian oscillations in the cyanobacterium *Synechococcus elongatus* could be reproduced *in vitro* using three proteins: KaiA, KaiB and KaiC plus ATP. KaiA is a dimeric enzyme that enhances the autophosphorylation of KaiC, whereas KaiB antagonizes the activity of KaiA. KaiC (U-KaiC) is a hexameric enzyme that can phosphorylate and dephosphorylate at two positions: serine 431 (S-KaiC) and threonine 432 (T-KaiC, and on both ST-KaiC), see Fig. (2).

The model to simulate circadian oscillations was developed in [4] as an interlinked positive and negative feedback loop, see Fig. (2). Even though a simple negative loop is able to generate sustained oscillations, Tsai *et al.* [9] demonstrated that to have a more robust system able to generate constant amplitude oscillations over a wide range of frequencies positive and negative loops were necessary. Following [4], the model equations can be written as:

$$\frac{dT}{dt} = k_{UT} \cdot U + k_{DT} \cdot D - k_{TU} \cdot T - k_{TD} \cdot T \quad (1)$$

$$\frac{dD}{dt} = k_{TD} \cdot T + k_{SD} \cdot S - k_{DT} \cdot D - k_{DS} \cdot D \quad (2)$$

$$\frac{dS}{dt} = k_{US} \cdot U + k_{DS} \cdot D - k_{SU} \cdot S - k_{SD} \cdot S \quad (3)$$

where T, D and S denote T-KaiC, ST-KaiC and S-KaiC, respectively, and the rate constants k_{XY} are defined as:

$$k_{XY} = k_{XY}^0 + \frac{k_{XY}^A \cdot A}{K_{1/2} + A} \quad (4)$$

with k_{XY}^0 the rate constant when KaiA is not present and k_{XY}^A the influence of KaiA on this rate constant, i.e. positive promotion, negative inhibition; and A the active monomers of KaiA which are inhibited *via* S through KaiB, $A = \max\{0, [KaiA] - 2S\}$. The total concentration of KaiC can be expressed as:

$$\begin{aligned}
 [KaiC]_{total} &= [U - KaiC] + [KaiC]_p = \\
 &[U - KaiC] + [S - KaiC] + [T - KaiC] \\
 &+ [ST - KaiC]
 \end{aligned}
 \tag{5}$$

The original values of the model obtained in [4] are summarized in Table 1.

Table 1. Parameters Used in the Model, from [4]

Parameter	Value	Units	Parameter	Value	Units
k_{UT}^0	0	h ⁻¹	k_{UT}^A	0.479	h ⁻¹
k_{TD}^0	0	h ⁻¹	k_{TD}^A	0.213	h ⁻¹
k_{SD}^0	0	h ⁻¹	k_{SD}^A	0.506	h ⁻¹
k_{US}^0	0	h ⁻¹	k_{US}^A	0.0532	h ⁻¹
k_{TU}^0	0.21	h ⁻¹	k_{TU}^A	0.0798	h ⁻¹
k_{DT}^0	0	h ⁻¹	k_{DT}^A	0.173	h ⁻¹
k_{DS}^0	0.31	h ⁻¹	k_{DS}^A	-0.319	h ⁻¹
k_{SU}^0	0.11	h ⁻¹	k_{SU}^A	-0.133	h ⁻¹

Modeling the Effects of Contaminants at Molecular Level

To simplify the case study let us assume that there are three generic types of contaminants that separately affect each protein, i.e. KaiA, KaiB and KaiC. Therefore the effects will be modeled by reducing the concentrations of KaiA and KaiC or by modifying the constant k_{DS}^A for the case of KaiB.

It is assumed that the contaminant interacts with each protein reducing their “effective” concentration in the organisms. There are several other possibilities to introduce contaminant effects such as the inhibition of *S* or *T* dephosphorylation, but a more detailed/realistic model would require experimental work on the real interactions as well as theoretical work to analyze the contaminant-docking site interactions.

Cyanobacteria Growth Model

Let us assume a simple model without zooplankton grazing. Therefore, the variations of the phytoplankton, *P* (mmol N m⁻³), is described in terms of *growth* (h⁻¹) and mortality *m_P* (h⁻¹) as:

$$\frac{dP}{dt} = growth_P \cdot P - m_P \cdot P
 \tag{6}$$

Phytoplankton growth is modelled as the product of the maximum specific growth rate μ_{max} multiplied with an overall limitation function, representing light limitation *f(I)*,

temperature limitation *f(T)* and nutrient limitation *f(NO₃⁻, NH₄⁺)*, as:

$$growth_P = \mu_{max} \cdot \min[f_1(I), f_2(T), f_3(NO_3^-, NH_4^+)]
 \tag{7}$$

The light limitation is parameterized according to [10] by

$$f_1(I) = \tanh[a_p \cdot I(z, t)]
 \tag{8}$$

$$I(z, t) = I_s \cdot \exp[-(k_{water} + k_{phy}P) \cdot z]
 \tag{9}$$

where *a_p* (m² W⁻¹) denotes the photosynthetic quantum efficiency parameter controlling the slope of *f(I)* versus the irradiance curve and *I_s* denotes the surface intensity of the PAR (photosynthetically active irradiance) taken as half of the incoming solar radiation. *k_{water}* (m⁻¹) is the extinction coefficient of the sea water and *k_{phy}* (m² mmol N⁻¹) is the phytoplankton self-shading coefficient.

The temperature limitation function for phytoplankton is based on [11]

$$f_2(T) = \exp\left[-\left(\frac{T - T_{opt}}{T_{width}}\right)^2\right]
 \tag{10}$$

with *T_{opt}* and *T_{width}* being the optimal temperature in °C and the range of suitable temperatures, respectively.

The nutrient limitation is the sum of ammonium and nitrate limitation:

$$f_3(NO_3^-, NH_4^+) = f_a(NO_3^-) + f_b(NH_4^+)
 \tag{11}$$

where the limitations are expressed by the Michaelis-Menten uptake formulation:

$$f_a(NO_3^-) = \frac{[NO_3^-]}{K_{no} + [NO_3^-]} \cdot \exp(-\psi[NH_4^+])
 \tag{12}$$

$$f_b(NH_4^+) = \frac{[NH_4^+]}{K_{nh} + [NH_4^+]}
 \tag{13}$$

where *K_{no}* and *K_{nh}* are half saturation constants (mmol N m⁻³) for nitrate and ammonium uptake, respectively, and the exponent in Eq. (12) represents the inhibiting effect of ammonium concentration on nitrate uptake with $\psi=3$ m³ mmol N⁻¹ [12].

The mortality of phytoplankton is expressed as a linear function of its biomass.

In all the runs, the model is forced by imposing temperature and solar radiation sinusoidal forcing, which have the following form:

$$T = 9.76 \cdot \sin\left(2 \cdot \pi \left(\frac{t - 114.74}{365}\right)\right) + 12.3
 \tag{14}$$

$$I = 140.0 \cdot \left[\sin\left(2 \cdot \pi \left(\frac{t - 120}{365}\right)\right) + 1\right] + 20.0
 \tag{15}$$

These functions are typical of shallow systems in Europe. Furthermore, we have also included the duration of light during the year and the mean duration value using also two

sinusoidal functions that gives the number of light minutes per day during the year (obtained also for European latitude).

$$L = 70.7 \cdot \sin\left(2 \cdot \pi \left(\frac{t - 81.89}{365}\right)\right) + 729.1 \quad (16)$$

With $L_h = L/60$. The mean value for this duration, normally between 11.7 and 12.3 h is given by:

$$\begin{aligned} \mu_L = & 11.96 + 0.15 \cdot \sin\left(2 \cdot \pi \left(\frac{t + 3.12}{365}\right)\right) + \\ & 0.16 \cdot \sin\left(2 \cdot \pi \left(\frac{t + 6.068}{182.5}\right)\right) \\ & + 0.02 \cdot \sin\left(2 \cdot \pi \left(\frac{t - 3.21}{91.25}\right)\right) \end{aligned} \quad (17)$$

Then I_s in Eq. (8) is calculated as:

$$I_s = I \cdot \exp\left[-\left(\frac{t_{day} - \mu_L}{0.5 \cdot L_h}\right)^2\right] \quad (18)$$

This produces a function that takes into account, when calculating the growth, the day and night cycles as well as their changing duration during the year.

An analysis of the variability of these functions on the effects of competition between phytoplankton, floating macroalgae and rooted macrophytes has been studied in [13] using a similar model, but it is outside the scope in this work to consider here these aspects.

Table 2. Parameters Used for the Simulation of the Phytoplankton Model, from [4]

Parameter	Definition	Value	Unit
a_p	Photosynthetic efficiency	0.01	$\text{m}^2 \text{W}^{-1}$
k_{water}	Light extinction coefficient in water	0.08	m^{-1}
k_{phy}	Phytoplankton self shading coefficient	0.07	mmol N^{-1}
μ_{max}	Maximum growth rate	0.03	h^{-1}
T_{opt}	Optimal temperature	15.0	$^{\circ}\text{C}$
T_{width}	Range of temperatures for cyanobacteria	7.0	$^{\circ}\text{C}$
K_{no}	Half saturation for nitrate uptake	0.5	mmol N m^{-3}
K_{nh}	Half saturation for ammonium uptake	0.2	mmol N m^{-3}
Ψ	Ammonium inhibition parameter	3.0	$\text{m}^3 \text{mmol N}^{-1}$
m_p	mortality rate	0.006	h^{-1}

Coupling the Circadian Clock with the Growth Model

According to the latest results in [6], it seems that *Synechococcus elongatus* clock senses the cellular redox state rather than the light intensity. However, the exact mechanism is not clear. What it is known is that LdpA and CikA sense the cellular redox state and that both repress KaiA. In addition, Arita *et al.* [14] and Kutsuna *et al.* [15] have shown that Pex, a transcriptional repressor of KaiA, increases during the dark period having abundance sensitive

to light, but the exact pathway is not clear. Furthermore, Smith and Williams [16] have reported that KaiC is responsible for the slow compactation of *Synechococcus elongatus* chromosome during day and decompactation during night which seems to suggest that the circadian clock controls the global transcription rhythm by modifying the DNA topology.

Experiments by inactivation of the cikA (circadian input kinase) gene performed in [17] have shown that the circadian period was shortened by two hours with changes in the phasing of several rhythms, and abolishes resetting of phase due to a pulse of several hours of darkness.

Let us formulate the following hypothesis: the correct functioning of *Synechococcus elongatus* requires that the organism synchronizes with the external light-dark conditions to optimize their growth and that this synchronization is carried out by a control mechanism that changes the KaiA concentration, between certain limits. Therefore, we will base our approach on the case of unidirectional coupling between two oscillators [18].

The hypothesis is in agreement with the origin of the internal biological clock that some prokaryotes possess for measuring daily time. According to Dong and Golden [6], this constitutes an evolutive advantage for the organisms since they can anticipate and adjust for the dark-light effects in terms of temperature and light intensity rather than respond each time during the whole life cycle. In addition, spontaneous synchronization is a well-known characteristic of circadian oscillators in other species such as mammals [19]. A further confirmation of our hypothesis is the finding of Destici *et al.* [20] that DNA damaging agents, such as ionizing radiation, are able to reset, shift in phase and synchronize the circadian clock in mammalian cells.

Therefore, we will assume that the concentration of KaiA can be expressed as:

$$[KaiA]_{total} = [KaiA]_i + k_{sync} \exp\left[-\left(\frac{t_{day} - \mu_L}{0.5 \cdot L_h}\right)^2\right] \quad (19)$$

where $[KaiA]_i$ is the initial concentration of KaiA in the simulation, k_{sync} in the coupling strength coefficient and the light-dark cycle given by Eqs. (16)-(17).

To scale up the toxic effects from molecular level to population level, we will assume a baseline scenario and compare the differences with increasing doses of contaminants. We assume that for the correct functioning of the organism, external and internal clocks should be synchronized and the departure from this situation will, negatively, affect the growth of the organism. Therefore, we will measure the response, r_i , to a certain dose, d_i , as:

$$r_i = 1 - \frac{\sum_{i=1}^{365} ([KaiC]_p - [KaiC]_p^{baseline})^2}{\max(r_i)} \quad (20)$$

As before, this is an assumption that should be experimentally validated in vivo when the other hypothesis has been assessed in vitro or using molecular modeling approaches. In addition, the baseline scenario has been chosen as the one close to the modeling parameters used in

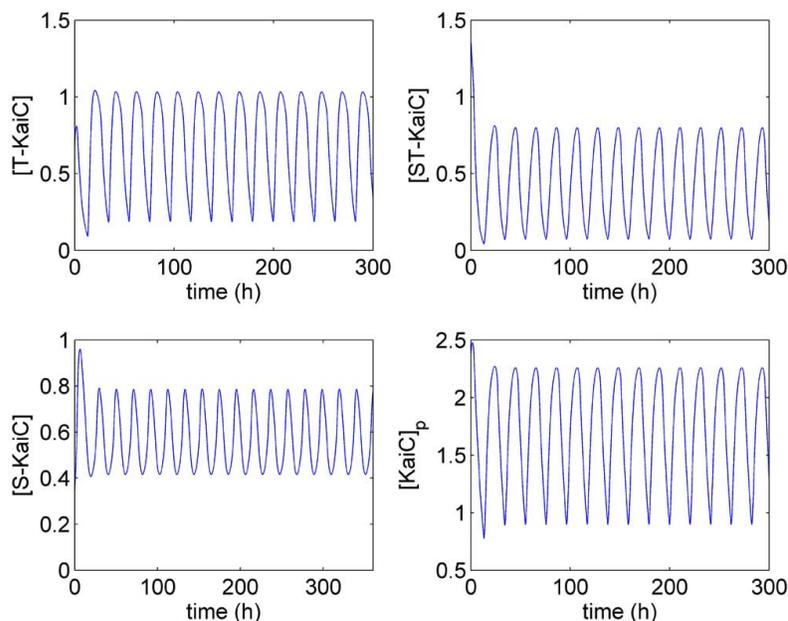


Fig. (3). Simulation of the circadian oscillator with the parameters provided in Table 1 with initial conditions: $[T\text{-KaiC}] = 20\%[KaiC]_{total}$; $[ST\text{-KaiC}] = 40\%[KaiC]_{total}$; $[S\text{-KaiC}] = 10\%[KaiC]_{total}$.

the literature, but that it produces cycles of ~24 hours, i.e. $[KaiC]_{total} = 3.9 \mu\text{M}$, $[KaiA] = 1.26 \mu\text{M}$; $k_{DS}^A = -0.32 \text{ h}^{-1}$ and $k_{sync} = 0.5$ (however, the approach and methodology will not change if the baseline scenario is modified).

RESULTS AND DISCUSSION

Unstressed and Stressed Circadian Oscillation Model

The numerical integration of the model given by Eqs. (1)-(4) using the parameters provided in Table 1 reproduces the circadian oscillation of KaiC phosphorylation, see Figs. (3-4). In Fig. (3) the concentrations of the three phosphory-

lated species and the sum of all phosphorylated species are displayed versus time, whereas in Fig. (4) the state space is shown. As it can be seen, the system, after a transient period, converges to a limit cycle, in this case with a period of about 21 hours.

To analyze the effects of contaminants in the circadian oscillator we have changed, as discussed in Section 2, the concentrations of KaiA, the total concentration of KaiC and the constant k_{DS}^A related to the inhibition from KaiB on KaiA. The simulation results, in terms of oscillations amplitude and period are summarized in Figs. (5-6). As it can be observed, there exists a region in parameter space where there is a continuous change in the amplitude and

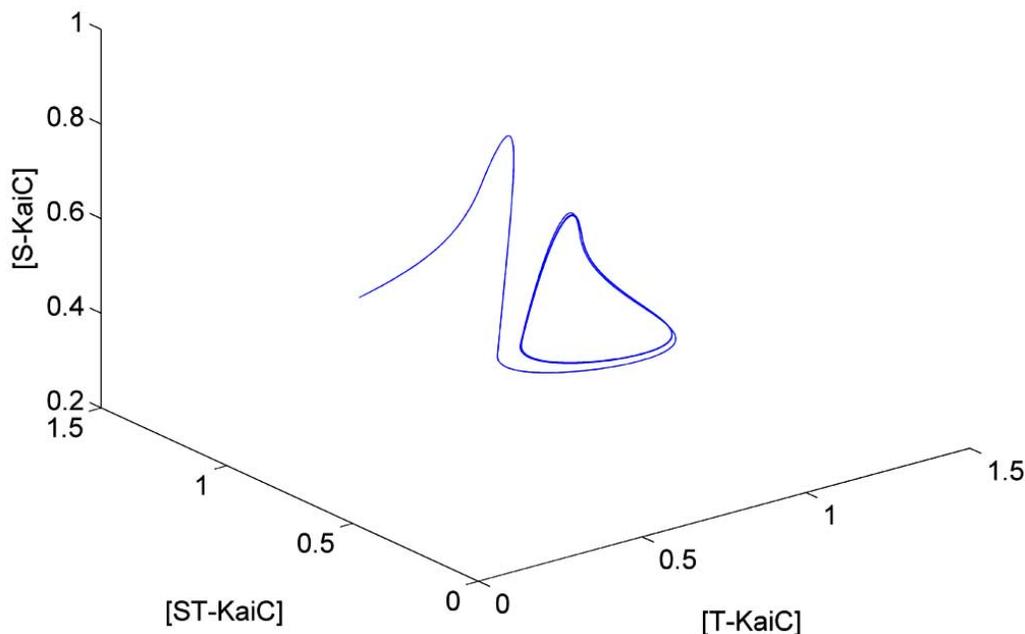


Fig. (4). Limit cycle in the circadian oscillator. Parameters as in Table 1.

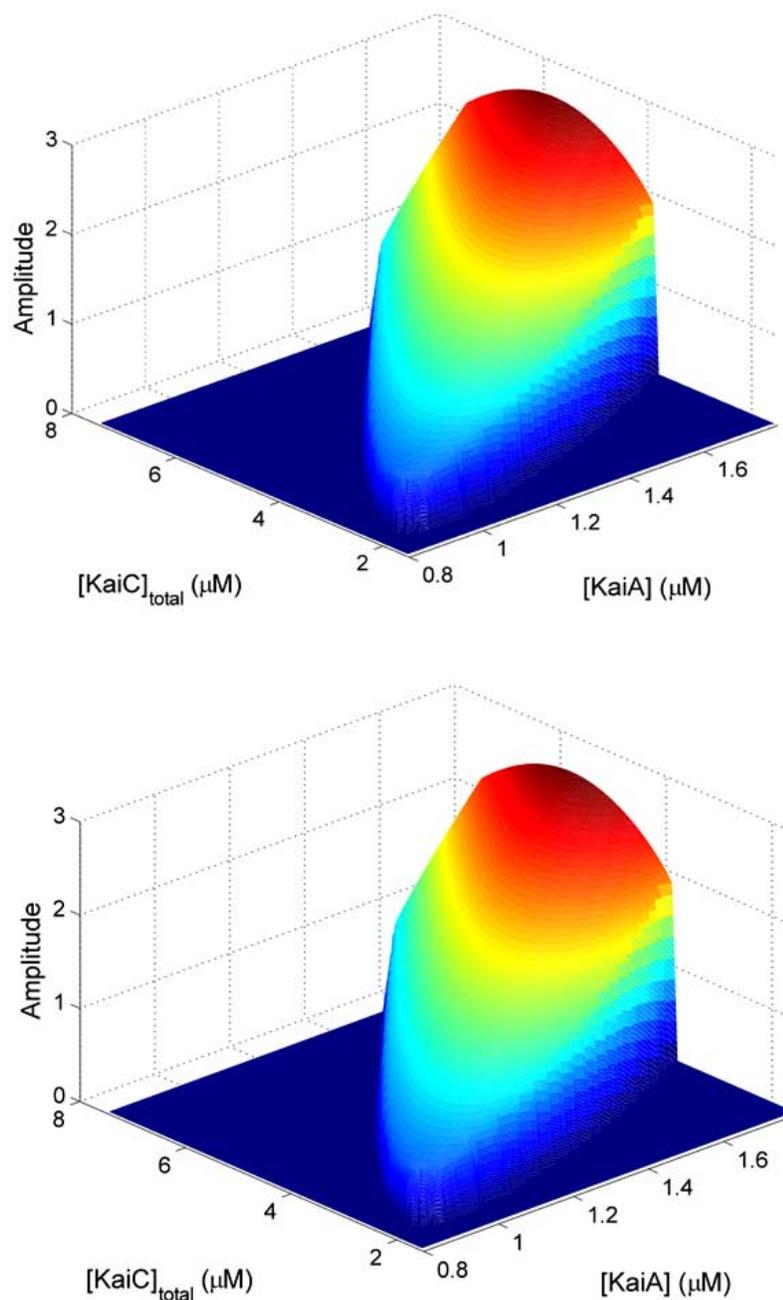


Fig. (5). Amplitude and period (hours, limit cycle) of the phosphorylated KaiC oscillations ($[\text{KaiC}]_p = [\text{T-KaiC}] + [\text{S-KaiC}] + [\text{ST-KaiC}]$) as a function of the total concentrations of KaiA and total concentration of KaiC.

period of the oscillations. In this region, the system converges to a limit cycle. However, for certain parameter's values, the oscillations stop and the system then converges to a fixed point. Between these two types of attractors there is a bifurcation point. To gain a better insight on the nonlinear dynamics of this system, a bifurcation analysis study, similar to the one carried out in [21], is being conducted.

From this preliminary analysis we can conclude that the effects of contaminants able to interact with the three proteins that compose the system will change the amplitude

and period of the circadian oscillator; being able, for certain values, to stop completely the oscillations.

Of course, these results are only a modeling hypothesis that should be confirmed with experiments. In addition, we have assumed that the model works properly outside of the domain in which was validated [4]. However, similar effects – short circadian periods- have already been observed *in vivo* by inactivation of the gene *cikA* (circadian input kinase) [17] and in mammalian cells under the effect of ionizing radiation [20].

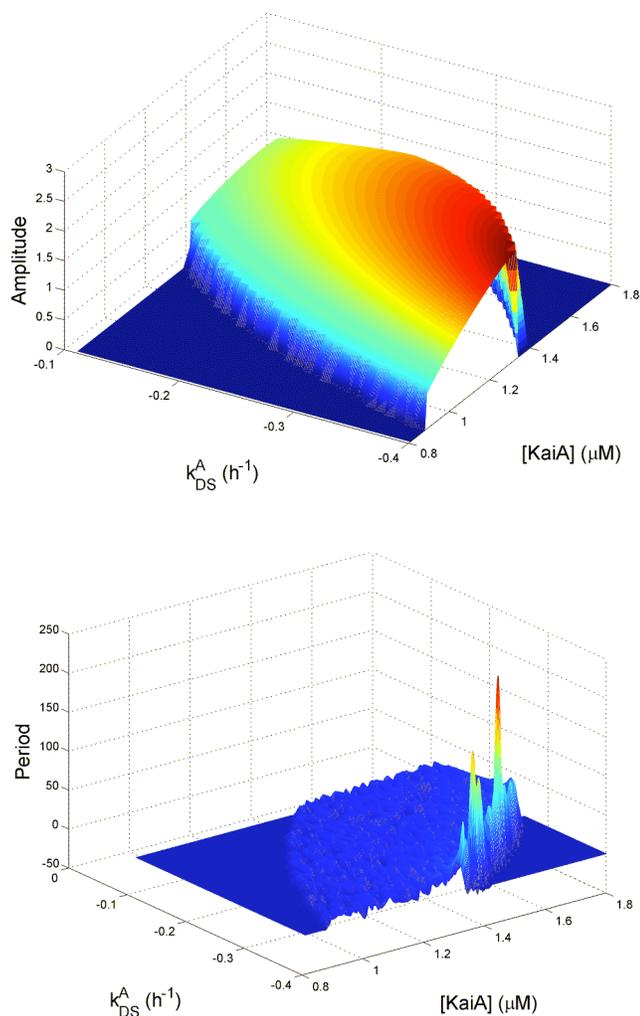


Fig. (6). a) Amplitude and b) period (hours, limit cycle) of the phosphorylated KaiC oscillations ($[KaiC]_p = [T-KaiC] + [S-KaiC] + [ST-KaiC]$) as a function of the total concentrations of KaiA and k_{DS}^A which measures the effect of KaiB.

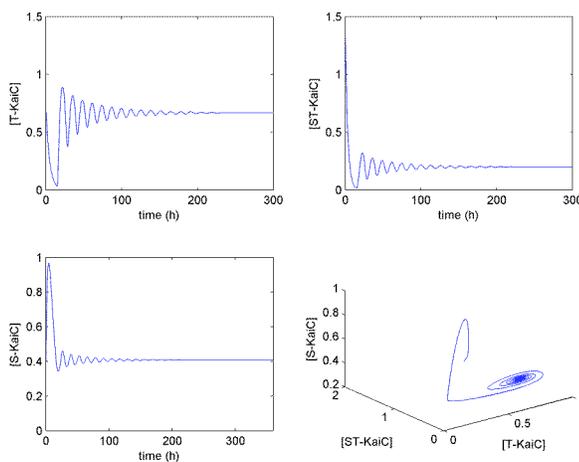


Fig. (7). Simulation of the circadian oscillator with the parameters as in Fig. (3), but with $[KaiA] = 0.9 \mu M$.

Figs. (7-10) illustrate some particular cases of the dynamic behaviour of the circadian oscillator when $[KaiA]$ is changed.

Cyanobacteria Growth Model

The cyanobacteria model was run under constant nutrient conditions using the temperature, light intensity, light-dark

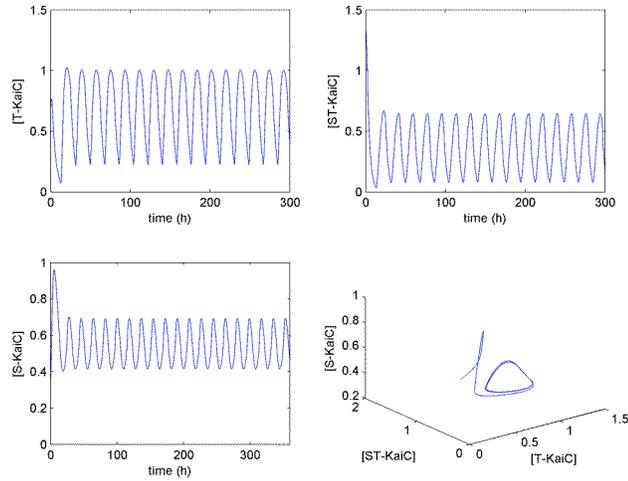


Fig. (8). Simulation of the circadian oscillator with the parameters as in Fig. (3), but with $[KaiA] = 1.2 \mu M$.

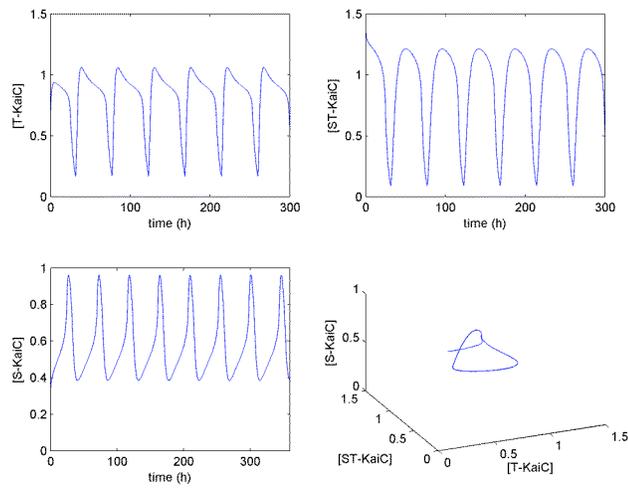


Fig. (9). Simulation of the circadian oscillator with the parameters as in Fig. (3), but with $[KaiA] = 1.6 \mu M$.

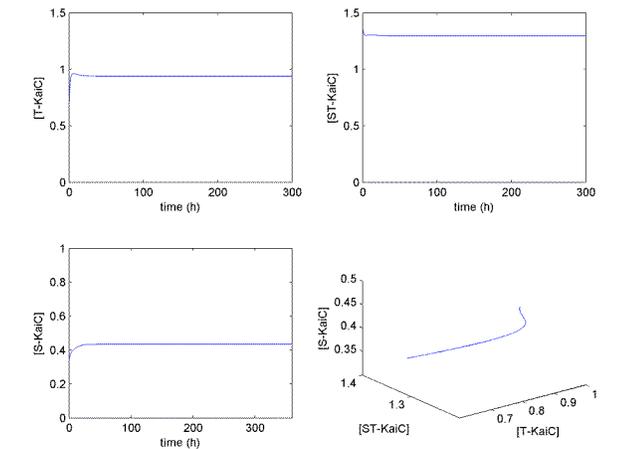


Fig. (10). Simulation of the circadian oscillator with the parameters as in Fig. (3), but with $[KaiA] = 1.7 \mu M$.

and duration, Eqs. (14)-(18), as forcing functions. An annual cycle of the cyanobacteria model is shown in Fig. (11a) whereas a detail of the daily oscillations in phytoplankton mass due to the light-dark cycle are shown in Fig. (11b). The

model results show the spring bloom biomass in spring and the decrease in autumn and winter. The shape of the curve is also typical of models where zooplankton grazing is not considered.

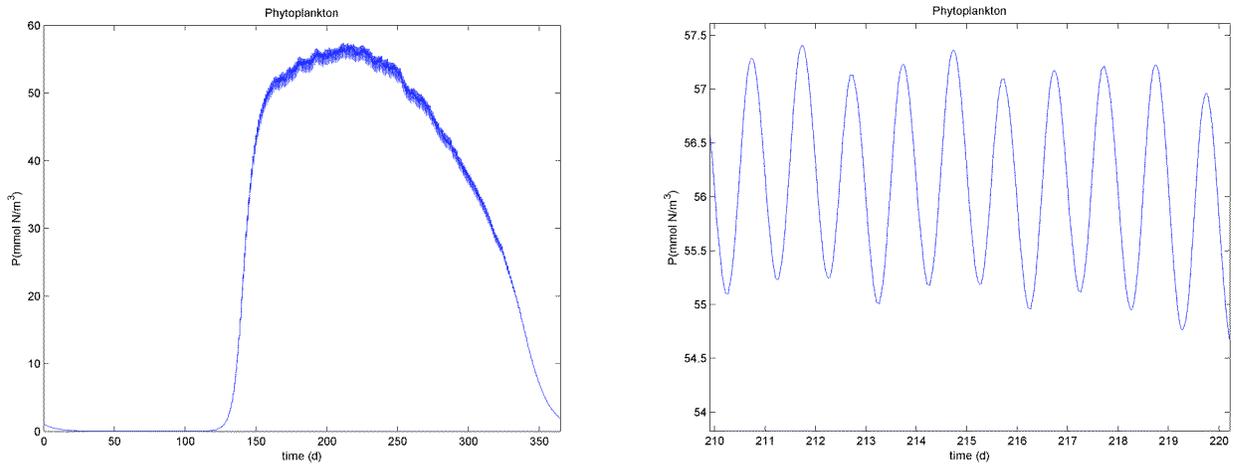


Fig. (11). (a) One year simulation of phytoplankton growth with forcing conditions provided by Eqs. (14)-(18) and constant nutrient concentrations: $[\text{NO}_3^-] = 0.8$, $[\text{NH}_4^+] = 0.3 \text{ mmol m}^{-3}$; (b) detail from a/ showing the daily oscillations of phytoplankton biomass.

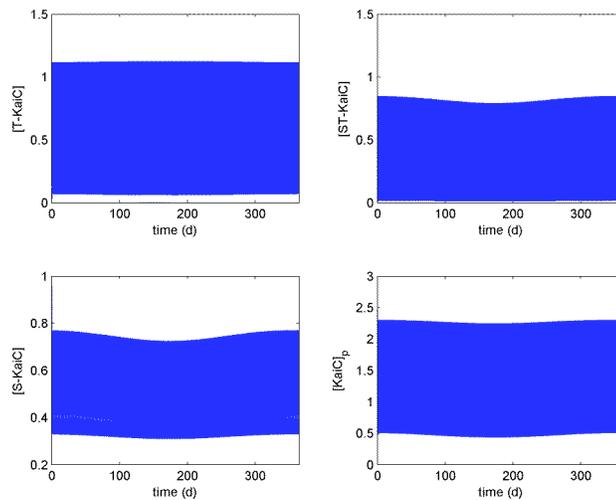


Fig. (12). One year simulation of the forced circadian oscillator with the parameters provided in Table 1 with and $k_{sync}=1$.

In real situations, a phytoplankton bloom is followed by a zooplankton bloom that depletes phytoplankton biomass. The phytoplankton oscillations are in agreement with more detailed models that use meteorological data as forcing [22, 23]. However, in that case, the oscillations are less evident since cloud coverage is also taken into account.

The fact that phytoplankton growth depends on light intensity and duration provides the coupling condition with the circadian oscillator model.

Synchronization of the Coupled Model

As a first step, we introduced Eq. (19) in the circadian oscillator model and tested the values of k_{sync} assuming that during darkness the concentration of KaiA decreases, i.e. $k_{sync} > 0$ [15]. Depending on the coupling strength the system synchronizes to the light-dark oscillations. Fig. (12) shows an example of one year simulation of the circadian oscillator when coupling is acting. It is also possible to observe the annual modulation of $[\text{ST-KaiC}]$ and $[\text{S-KaiC}]$ in the daily oscillations. In this case the system dynamics in phase space

is not a limit cycle but changes as the light-dark conditions change, see Fig. (13).

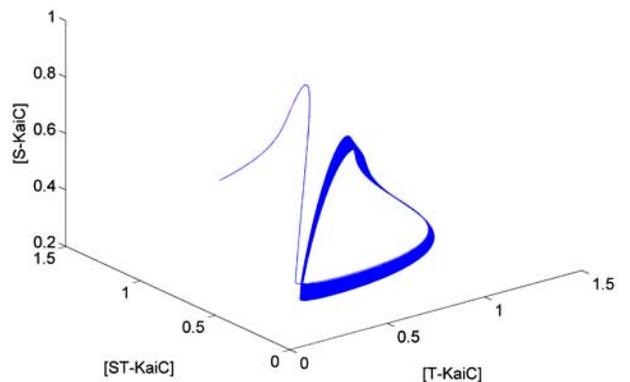


Fig. (13). State-space representation of the circadian oscillator when forced with dark-light conditions during one year, $k_{sync}=1$.

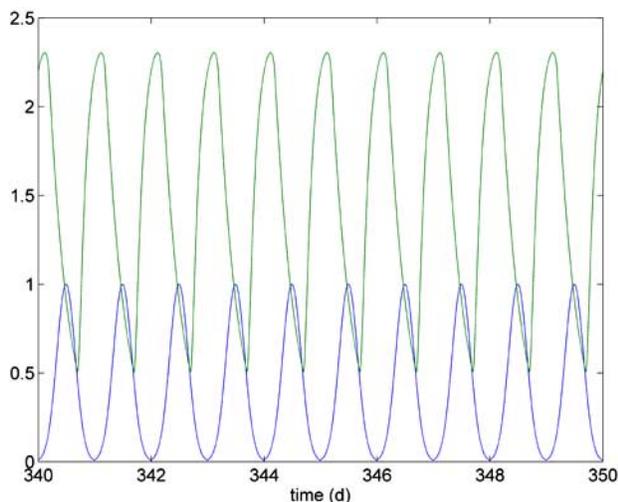


Fig. (14). Simulated phosphorylated KaiC concentration, $[KaiC]_p = [S-KaiC] + [T-KaiC] + [ST-KaiC]$ - green line- and light-dark cycles for one period of the year - blue line-. Parameters: $[KaiC]_{total} = 3.4 \mu M$, $[KaiA] = 1.3 \mu M$ and $k_{sync} = 1$.

The coupling takes place approximately after the first eighteen days; examples of the coupling in a different period during the year is shown in Fig. (14). Similar behaviour can be observed with different initial parameters and coupling strengths, but several types of synchronization behaviour may occur. For example, Fig. (15) shows a 48 h synchronization for $k_{sync} = 0.1$ and $[KaiC]_{total} = 3.9$ and $[KaiA] = 1.26 \mu M$, respectively. A detailed analysis of synchronization mechanisms is outside the scope of this theoretical work, since it would require *in vivo* experiments and a metabolic approach to measure the change in concentrations of several compounds responsible for the functioning of the system.

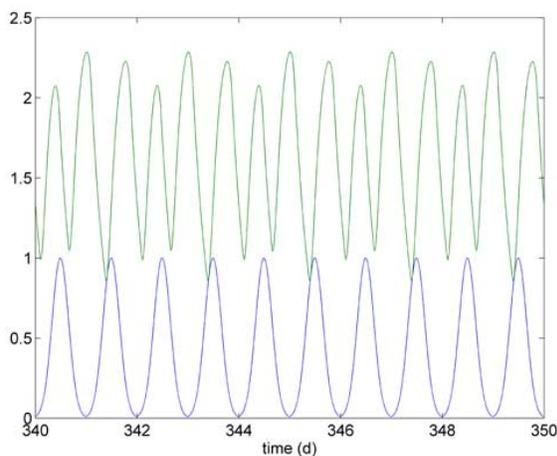


Fig. (15). Simulated phosphorylated KaiC concentration, $[KaiC]_p = [S-KaiC] + [T-KaiC] + [ST-KaiC]$ - green line- and light-dark cycles at the end of the year- blue line-, notice the 48h synchronization period. Parameters: $[KaiC]_{total} = 3.9 \mu M$, $[KaiA] = 1.26 \mu M$ and $k_{sync} = 0.1$.

Simulated Dose-Response Curves in the Coupled Model

As discussed above, let us analyze the case of three contaminants that interact separately with the three proteins,

KaiC, KaiA, KaiB, that form the circadian oscillator. Therefore we will measure the dose as the decrease of the amount of concentration with respect to our baseline, and the effect as the normalized distance from the baseline, represented by the oscillations in concentration of the phosphorylated KaiC compounds, during one year. In this example, the baseline scenario has been defined as (see Section 2.4): $[KaiC]_{total} = 3.9 \mu M$, $[KaiA] = 1.26 \mu M$; and $k_{DS}^A = -0.32 h^{-1}$; $k_{sync} = 0.5$.

Figs. (16-18) represent the dose-response curve for three hypothetical contaminants that interact with the three proteins. Strikingly, the shape of the curves is quite similar to those obtained experimentally. In addition, even though all contaminants interact with the circadian oscillator, the shape of the dose-response curves is also different depending on the mode of interaction. Similar results should be also hypothesized for other molecular machinery and, therefore, this seems a plausible explanation for the fact that different chemical compounds can act of the same system even if there are no structural similarities between them.

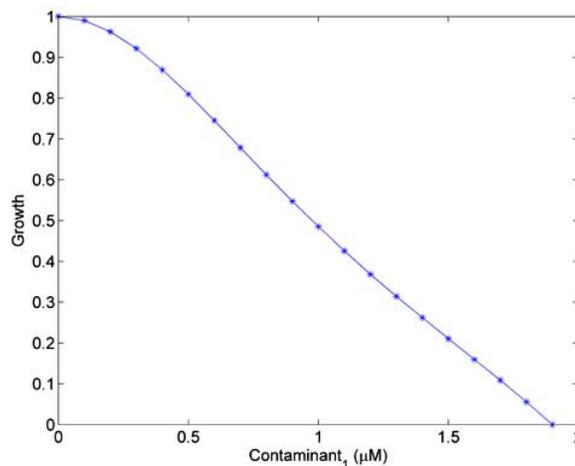


Fig. (16). Simulated dose-response curve for a contaminant that interacts with $[KaiC]_{total}$.

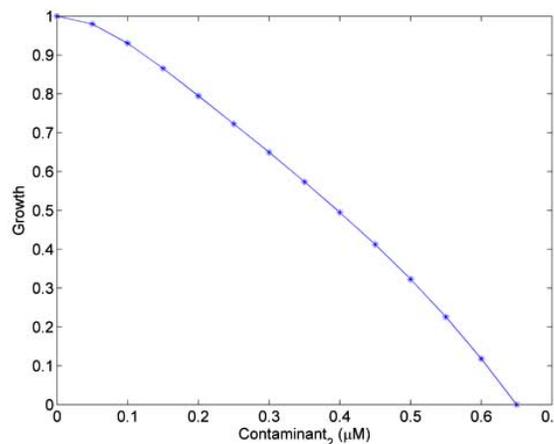


Fig. (17). Simulated dose-response curve for a contaminant that interacts with $[KaiA]$.

Population Response

Finally, as an example, Fig. (19) shows the dynamics of the cyanobacteria population exposed to constant doses of a

contaminant that reduces its growth by 10, 20, 30 and 40% after the first year, respectively. The figure also illustrates the propagation of the effects from individual level to population level, the chronic effects of exposure and the response time of the system.

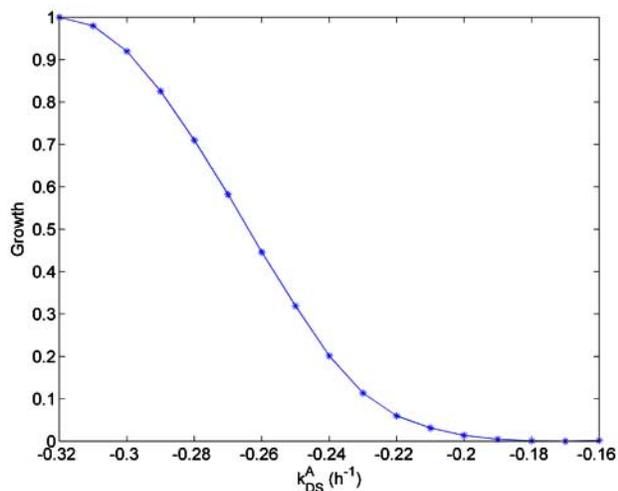


Fig. (18). Simulated dose-response curve for a contaminant that interacts with [KaiB] through k_{DS}^A .

CONCLUSIONS AND FUTURE WORK

A first attempt to analyze the possible toxic effects of several compounds, acting at molecular level, on the population has been carried out using a coupled modeling approach based on a circadian oscillators and a cyanobacteria growth model. To develop further this approach, several assumptions have been made on the linkage between the circadian clock functioning and the population growth as well as on the effects of contaminants at molecular level. Synchronization with light-dark conditions has been introduced as the coupling mechanism and its loss as the influencing mechanism in the growth model. Then dose-response curves have been developed. The obtained curves show a striking resemblance to those obtained experimentally, even though no assumptions have been made in the model about their shape.

The modeling results suggest new experimental approaches to validate the assumptions as well as to understand toxicity effects, the functioning and malfunctioning of molecular machinery and possible consequences. In addition, several modeling hypothesis could be refined when experimental data become available.

The results point to the need that to understand toxic effects we have to focus our study on the interactions that a certain compound may have with proteins, and/or DNA,

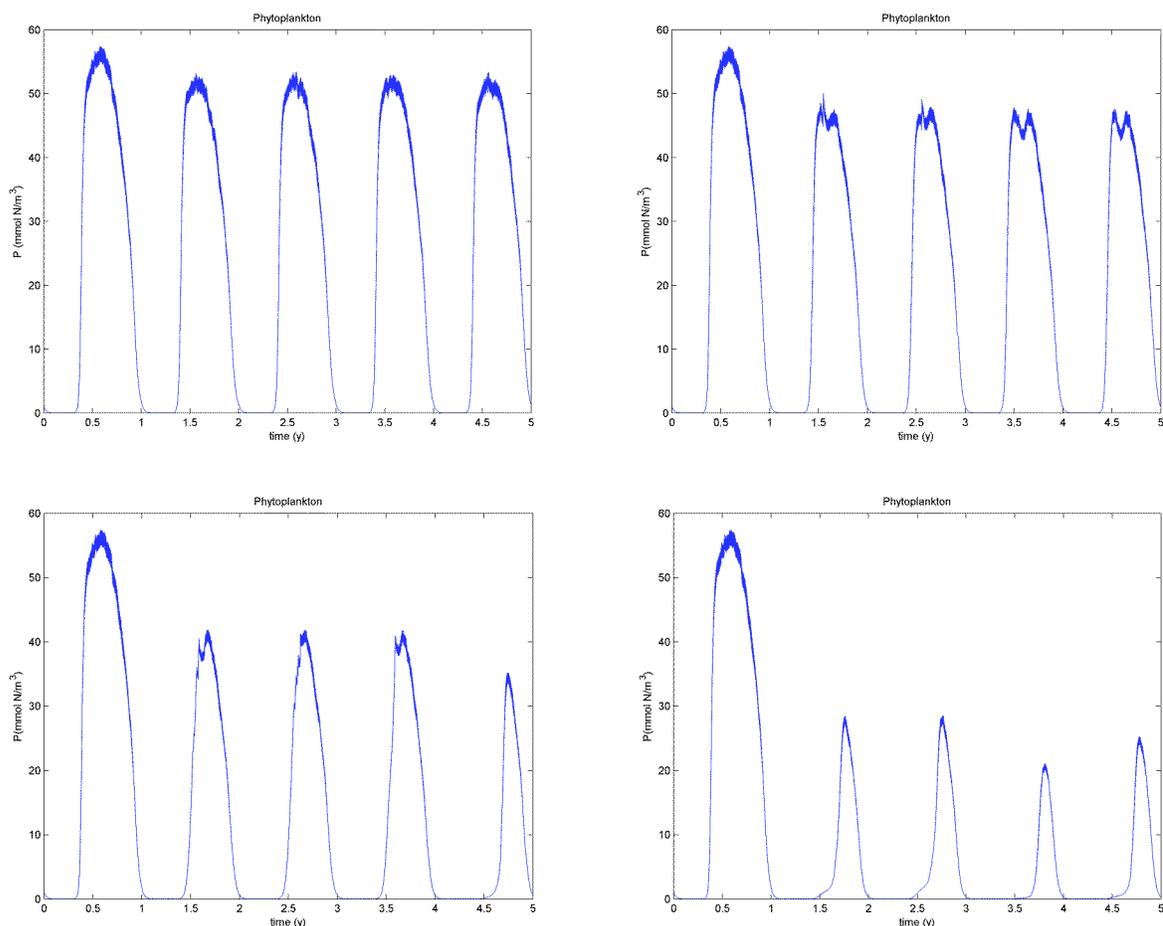


Fig. (19). Simulated cyanobacteria model with a constant contaminant dose that reduces by a) 10%; b) 20%; c) 30%; d) 40% the growth after the first year.

since the result of these interactions will determine the final mode of action at macroscopic level. Therefore an intermediate step for assessing toxic effects, based on the type of interaction at molecular level, is proposed for developing a coherent toxicity framework analysis. This would explain why completely different compounds have the same mode of actions whereas similar ones at the level of structure have sometimes different mode of action.

Since the molecular clock system is quite simple, experiments could be carried out *in vitro* to validate the model results as well as the concentration thresholds. In addition, the structures of the proteins KaiA, KaiB and KaiC are known, so it could be possible, in principle, to select the chemicals that dock (block the functioning) of one but not the others proteins, to compare experimental and modeling results. Finally, *in vivo* experiments with *Synechococcus elongatus* could be performed.

Missing aspects in the model include the possible delays due to the kinetics of the proteing-ligand (contaminant) docking and due to the diffusion of the contaminant from the water to the organism, to take into account internal concentrations.

REFERENCES

- [1] U.S. EPA, A framework for a computational toxicology research program. Washington D.C. EPA600/R-03/65. (2003).
- [2] Palsson BØ. Systems biology: properties of reconstructed networks. Cambridge University Press: UK 2006.
- [3] Van der Oost T, Beyer J, Vermeulen NPE. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. *Environ Toxicol Pharmacol* 2003; 13: 57-149.
- [4] Rust MJ, Markson JS, Lane WS, Fisher DS, O'Shea EK. Ordered phosphorylation governs oscillation of a three-protein circadian clock. *Science* 2007; 318: 809-12.
- [5] Dueri S, Hjorth M, Marinov D, Dallhof I, Zaldivar JM. Modeling the combined effects of nutrients and pyrene on the plankton population: Validation using mesocosm experimental data and scenario analysis. *Ecol Model* 2009; 220: 2060-7.
- [6] Dong G, Golden SS. How a cyanobacterium tells time. *Curr Opin Microbiol* 2008; 11: 541-6.
- [7] Tomita J, Nakajima M, Kondo T, Iwasaki H. No transcription-translation feedback in circadian rhythm of KaiC phosphorylation. *Science* 2005; 307: 251-4.
- [8] Nakajima M, Imai K, Ito H, *et al.* Reconstruction of circadian oscillation of cyanobacterial KaiC phosphorylation *in vitro*. *Science* 2005; 308: 414-5.
- [9] Tsai TY-C, Choi YS, Ma W, Pomerening JR, Tang C, Ferrell Jr JE. Robust, tunable biological oscillations from inlinked positive and negative feedback loops. *Science* 2008; 321: 126-9.
- [10] Jassby AD, Platt T. Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnol Oceanogr* 1976; 21: 540-7.
- [11] Lancelot CJ, Staneva D, Van Eeckhout J, Beckers M, Stanev E. Modeling the Danube-influenced north-western continental shelf of the Black Sea. II. Ecosystem response to changes in nutrient delivery by the Danube river after its damming in 1972. *Estuar Coast Shelf Sci* 2002; 54: 473-99.
- [12] Wroblewski JA. A model of phytoplankton bloom formation during variable Oregon upwelling. *J Mar Res* 1977; 35: 357-94.
- [13] Zaldivar JM, Bacelar F, Dueri S, Marinov D, Viaroli P, Hernández-García E. Modeling approach to regime shifts of primary production in shallow coastal ecosystems. *Ecol Model* 2009; 220: 3100-10.
- [14] Arita K, Hashimoto H, Igari K, *et al.* Structural and biochemical characterization of a cyanobacterium circadian clock-modifier protein. *J Biol Chem* 2007; 282: 1128-35.
- [15] Kutsuna S, Kondo T, Ikegami H, Uzumaki T, Katayama M, Ishiura M. The circadian clock-related gene *pex* regulates a negative *cis* element in the *kaiA* promoter region. *J Bacteriol* 2007; 189: 7690-6.
- [16] Smith RM, Williams SB. Circadian rhythms in gene transcription imparted by chromosome compaction in the cyanobacterium *Synechococcus elongates*. *Proc Nat Acad Sci USA* 2006; 103: 8564-9.
- [17] Schmitz O, Katayama M, Williams SB, Kondo T, Golden SS. CikA, a bacteriophytochrome that resets the cyanobacterial circadian clock. *Science* 2000; 289: 765-8.
- [18] Chen G, Dong X. From chaos to order: Methodologies, perspectives and applications. World Scientific: Singapore 1998.
- [19] Gonze D, Bernard S, Waltermann C, Kramer A, Herzel H. Spontaneous synchronization of coupled circadian oscillators. *Biophys J* 2005; 89: 120-9.
- [20] Destici E, Oklejewicz M, Nijman R, Tamanini F, van der Horst GTJ. Impact of the circadian clock on *in vitro* genotoxic risk assessment assays. *Mutat Res* 2009 ([Epub ahead of print], doi: 10.1016/j.mrgentox.2009.09.001).
- [21] Bacelar FS, Dueri S, Hernández-García E, Zaldivar JM. Joint effects of nutrients and contaminants on the dynamics of a food chain in marine ecosystems. *Math Biosci* 2009; 218: 24-32.
- [22] Zaldivar JM, Cattaneo E, Plu M, Murray CN, Giordani G, Viaroli P. Long-term simulation of main biogeochemical events in a coastal lagoon: Sacca di Goro(Northern Adriatic Coast, Italy). *Continental Shelf Res* 2003; 23: 1847-76.
- [23] Marinov D, Dueri S, Puillat I, *et al.* Integrated modeling of polycyclic aromatic hydrocarbons (PAHs) in the marine ecosystem: coupling of hydrodynamic, fate and transport, bioaccumulation and planktonic food web models. *Mar Pollut Bull* 2009; 58: 1554-61.

Received: May 06, 2009

Revised: June 20, 2009

Accepted: October 28, 2009

© José-Manuel Zaldivar Comenges; Licensee Bentham Open.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>), which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.