A Qualitative Framework Dedicated to Toxicology

Benjamin Miraglio[1], Gilles Bernot[1], Jean-Paul Comet[1] and Christine Risso-de Faverney[2]
[1]*Université Côte d'Azur, CNRS, I3S, Sophia Antipolis, France*
[2]*Université Côte d'Azur, CNRS, ECOMERS, Nice, France*

Keywords: Computational Toxicology, Discrete Dynamic Systems, Rule-Based Modelling, Temporal Logic.

Abstract: Emerging constraints have led the toxicology community to complete the classical paradigm of toxicology with the study of molecular events underlying the toxicity of a chemical substance. This evolution motivates the emergence of new modelling approaches for toxicology. In this article, we introduce a qualitative rule-based formalism dedicated to the domain of toxicology. This new formalism departs from other rule-based formalisms such as BioChAM because it directly encodes possible alterations of equilibrium, instead of making equilibriums emerge from the dynamics of the model. Using a simple example of the energy metabolism, we show that this formalism is able to describe both the normal evolution of a biological system and its possible toxic disruptions.

1 INTRODUCTION

Toxicology can be defined as the study of adverse effects caused by exogenous chemical substances to biological systems. The classical paradigm assumes that the more an organism is exposed (in dose and/or time) to a compound, the greater the compound effects will be. In these conditions, any chemical substance can therefore cause harmful effects to an organism if this organism is exposed during a long enough time to a high enough dose of chemical.

That concept serves as a basis to the dose-response relationship, which enables toxicologists to establish a causality between the exposure to a chemical and its induced observed effects. It also allows toxicologists to determine the threshold of toxicity, namely the lowest exposure (in dose and/or time) where an induced effect is observed.

Many experiments carried out recently have pointed out the limitations of this paradigm. Indeed, toxicity assessment is quite complex and, besides the dose and time of exposure, a lot of other factors can affect the results of toxicity tests. In particular, these factors include temperature, food, light, the route of exposure and the chemical interactions of the tested substance with other chemical compounds. Other factors related to the test subject itself, including age, sex, genetics, health status, hormonal status or window of exposure may also greatly influence the vulnerability of an organism to a chemical substance.

To answer these limitations, an increasing trend in toxicology is to focus on the causal sequence of key events occuring during the toxic response and leading to an observable effect. These sequences, called pathways of toxicity, lay the basis of the *mechanistic toxicology* and include events from molecular, cellular and even organ scales.

As mechanistic toxicology allows a better understanding of molecular mechanisms leading to adverse effects, it can cope with many difficulties mentioned earlier, such as the extrapolation of toxicity findings obtained from laboratory animals to humans or the consideration of additional factors in toxicity assessments. Moreover, as distinct pathways of toxicity can share the same key events, knowledge obtained when studying one chemical could be reused when assessing other chemicals.

Concurrently, as the potential toxicity of chemical exposure became an area of great concern to both the public (Colborn et al., 1996; Kepner, 2004) and the regulatory authorities (Backhaus et al., 2010), the production of chemical compounds is increasingly regulated worldwide. Manufacturers must now conduct extensive studies to demonstrate the innocuity of their products, considerably increasing the cost of development of such products.

This context favours the emergence of different mathematical modelling approaches, and so far, most of these approaches are quantitative and aim at either inferring the toxic threshold of a chemical substance

Miraglio B., Bernot G., Comet J. and Risso-de Faverney C.
A Qualitative Framework Dedicated to Toxicology.
DOI: 10.5220/0006168200930103
In *Proceedings of the 10th International Joint Conference on Biomedical Engineering Systems and Technologies (BIOSTEC 2017)*, pages 93-103
ISBN: 978-989-758-214-1

or confirming its specific pathway of toxicity. These objectives require a lot of toxicological data. This can be restrictive given the current acquisition cost of new biological data. There is thus an incentive to develop methods that do not focus on toxic thresholds but instead, aim at describing pathways of toxicity in a *qualitative* manner. Such an approach would focus on equilibrium shifts and would therefore require comparatively less toxicological data.

In this article, we present a new qualitative formalism allowing to enumerate all the conceivable pathways of toxicity linked to a compound present in a given biological system. As a lot of these modelled pathways are biologically improbable, it is then possible to encode into temporal logic basic toxicological knowledge and filter out the less relevant ones. The remaining modelled pathways can finally serve as a basis to design more informative experiments and help toxicologists in their search of new pathways of toxicity.

The next section of this article is dedicated to the brief description of related work focusing on formal frameworks dedicated to model biological systems. As our formalism is presented alongside examples inspired from the energy metabolism, section 3 sketches an overview of the energy metabolism and its key components. In section 4, we explain how to use the new formalism to describe the equilibrium changes of a system. In section 5, we show how to integrate toxicological knowledge in the system using temporal logic. Finally, this formalism is applied to a simplified model of the energy metabolism in section 6.

2 RELATED WORK

The rule-based formalism presented throughout this article was originally inspired from the Boolean semantics of BioChAM (Calzone et al., 2006), an environment able to model biological systems as networks of chemical reactions. However, several specificities of toxicology make this environment not optimal when handling toxicological models, motivating the development of a more domain-oriented formalism. For instance, the notion of abnormal concentrations required us to adopt multivalued semantics. Furthermore, the presence of modulations of reactions, crucial in toxicology, is difficult to handle with BioChAM. This motivated us to directly describe equilibrium changes in a biological system, abstracting any quantitative computing steps.

Pathway logic (Talcott, 2008) is another framework dedicated to the description of biological systems while highlighting structural aspects of the cell. Unfortunately, this framework is also not adapted to deal with specificities of toxicology such as abnormal concentrations or modulations of reactions.

Finally, Bio-PEPA (Ciocchetta and Hillston, 2009) is a modelling approach where biological systems are formalised as discrete models. These models include precise biological information such as kinetic laws and stoechiometry. Different analysis can then be performed on these models, ranging from the construction of continuous time Markov chains to the translation of models into ordinary differential equations. However, the discretisation required for continous time Markov chains and probabilistic model checking approaches is based on a precise knowledge of kinetic parameters. Unfortunately, this precise knowledge is often unavailable, leaving room for a framework where reaction rates are abstracted. Of course, such a framework should maintain the possibility for toxicologists to easily express concurrency between equilibrium changes.

3 A QUICK INTRODUCTION TO METABOLISM

The metabolism can be described as the set of chemical reactions allowing cells to survive. A major distinction in metabolism is made between catabolism and anabolism. Catabolism refers to the set of reactions degrading molecules in smaller parts. Catabolic reactions are mainly oxidations and tend to produce energy for the cell. On the contrary, anabolism gathers synthesis reactions producing key molecules for the cell and tend to require energy.

In addition to the catabolism/anabolism separation, metabolic reactions can also be clustered depending on the type of molecules they handle. Hence, carbohydrate metabolism refers to pathways managing glucose and other carbohydrates and the lipid metabolism regroups reactions that both break down and synthesise lipids. Obviously, these different clusters of reactions are heavily interconnected, and the most notable intermediary molecules are called metabolic crossroads.

The formalism developed in this article will be illustrated by a simple model of the energy metabolism of a mammalian hepatic cell in aerobic conditions. Here, the energy metabolism can be understood as a set of reactions including pathways belonging to carbohydrate and lipid metabolisms as well as additional chemical reactions occuring in mitochondria. This set of reactions is summarised in Figure 1 and frequent references to this figure will be made during the description of the reactions. Note that the subcellular lo-

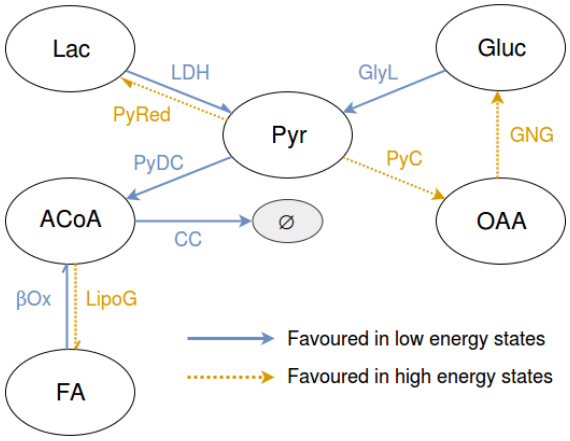

Figure 1: Representation of a simplified energy metabolism model. Arrow captions correspond to rule identifiers developed in Section 6.

cation of the reactions (cytosol or mitochondria) is abstracted in this figure. Moreover, reactions favoured in low energy situations are depicted by using plain arrows while reactions promoted in high energy cases are shown with dotted arrows.

Carbohydrate Metabolism. Carbohydrates constitute one of the main energy supply for cells and their metabolism is essentially located in the cell cytosol.

The main catabolic pathway is glycolysis (GlyL), which converts glucose (Gluc) and several other carbohydrates into pyruvate (Pyr) (Pilkis and Granner, 1992). Pyr then migrates to mitochondria where it is decarboxylated (PyDC) into acetyl-CoA (ACoA), an important metabolic crossroads. Note that the decarboxylation of Pyr into ACoA is performed by the pyruvate dehydrogenase complex (PDH) (Pettit et al., 1975). This enzymatic complex will not be included in the application model developed in Section 6 and is not represented in figure 1. However, it is used in the running example illustrating Section 4.

The anabolic counterpart of glycolysis, the gluconeogenesis (GNG), uses energy to create new molecules of Gluc (Pilkis and Granner, 1992). It should be noted that Pyr carboxylation (PyC) into oxaloacetatic acid (OAA) - the first step of gluconeogenesis - directly competes with Pyr decarboxylation (PyDC) to ACoA. The competition outcome depends on the quantities of available ACoA: if there is a shortage, Pyr is decarboxylated to ACoA, otherwise, it is carboxylated in OAA (Owen et al., 2002). The other reactions composing gluconeogenesis are mainly reversed glycolysis reactions.

Lipid Metabolism. Lipids are another important source of energy for cells. The β-oxidation (βOx) is the main catabolic pathway and degrades fatty acids (FA) into ACoA in mitochondria (Schulz, 1991). Conversely, lipogenesis (LipoG) occurs in the cytosol and converts ACoA into FA (Hellerstein et al., 1991).

It is interesting to note that both lipid and carbohydrate catabolic pathways result in the production of ACoA. Indeed, ACoA is actually an important metabolic crossroads and can be involved in the production of either new molecules - notably lipids - or energy, through its inclusion in the citrate cycle (CC). The inclusion of ACoA in the citrate cycle results in the destruction of ACoA, represented in figure 1 by the symbol "∅".

Citrate Cycle. The citrate cycle (CC), also known as tricarboxylic acid cycle, is a key component of energy production (Owen et al., 2002). It starts with the conjugation of ACoA and OAA to form citrate (Cit) and is then composed of a sequence of reactions resulting in the regeneration of OAA and the production of energy (Owen et al., 2002). Hence, the overall cycle only decreases ACoA levels to produce energy, therefore, only ACoA is consumed in Figure 1. On top of the production of energy, the citrate cycle also produce reduced compounds. These reduced compounds are then used to fuel the mitochondrial respiratory chain (MRC, not represented in Figure 1), generating even more energy (Chance and Williams, 1956).

Reduction-oxidation Reactions. The reduced compounds are actually coenzymes such as nicotinamide adenine dinucleotide or flavine adenine dinucleotide that act as electron transporters in the cell and can have either an oxidised or a reduced form. For the sake of simplicity, we will summarise the ratio between reduced and oxidised coenzymes as the reducing potential of a cell (pRed). A high (resp. low) reducing potential therefore means high (resp. low) concentrations in reduced coenzymes and subsequent low (resp. high) levels of oxidised coenzymes.

As a matter of fact, the whole metabolism can be seen as a network of reduction-oxidation (redox) reactions and the reducing power of a cell can inform on the state of the cell metabolism. For instance, both carbohydrate and lipid catabolic pathways reduce oxidised coenzymes in their reduced form (increasing the cell reducing potential), while conversely, both anabolic pathways oxidise reduced coenzymes in their oxidised form (decreasing the reducing potential).

For the sake of clarity, modifications of the reducing potential are not directly shown in Figure 1 but can be deduced from the arrows nature. Indeed, reactions favoured in low energy states tend to produce reducing potential (solid arrows in figure 1) while reactions favoured in high energy states tend to decrease the reducing potential of a cell (dotted arrows in figure 1).

As previously said, mitochondria are able to create energy from the reduced coenzymes. This process decreases the reducing power, regenerating the oxidised coenzymes supplies of the cell, allowing the continuation of catabolic reactions.

Another important source of coenzymes oxidation lays in the cytosolic reduction of Pyr into lactate (Lac) (Brooks, 1998). If mitochondria functioning falters, the pyruvate reduction (PyRed) is still able to resupply the cell in oxidised coenzymes, preventing the interruption of catabolic pathways. Since pyruvate reduction is reversible, its reversed reaction (lactate dehydrogenation or LDH), can also generate reducing potential while converting Lac in Pyr. The newly created Pyr can, in turn, either generate a lot of energy through its decarboxylation in ACoA or participate to the gluconeogenesis through carboxylation in OAA (see Carbohydrate metabolism paragraph).

Disruptions of the Cell Reducing Potential. As the cell reducing potential is central to a lot of metabolic reactions, disruptions of the redox equilibrium can have great impacts on the organism. For instance, alcohol dehydrogenase is able to convert ethanol (Eth, not represented in Figure 1) to acetaldehyde while greatly increasing the cell reducing potential. If this increase is strong enough, it can saturate the mitochondrial respiratory chain and trigger the reduction of Pyr into Lac. The subsequent accumulation of Lac in cells can then lead to troubles such as metabolic acidosis.

Furthermore, as the presence of Eth triggers Pyr reduction into Lac, the amount of Pyr available to carboxylation in OAA is decreased. The subsequent production of Gluc from OAA through the gluconeogenesis is then strongly impacted. Since gluconeogenesis is an important pathway to address hypoglycemia, a fasting organism can thus see its capacity to recover from hypoglycemia strongly damaged after its exposition to ethanol (Field et al., 1963).

4 DESCRIBING EQUILIBRIUM CHANGES

A biological system can be described as a set of biological entities interacting with each other at different concentrations. In a given organism, each entity has a concentration regarded as normal in standard conditions. For instance, the normal blood concentration of glucose is about 1 g/L in an adult human.

Our domain-oriented formalism allow us to represent the evolution of the concentration of each entity and to depict abnormal concentrations from which toxicity can arise. Indeed, we introduce four qualitative abstract levels, which are listed here in increasing order:

- ε reflects a negligible concentration of a given entity, that is to say a concentration too low to trigger any reaction in the biological system.
- ι conveys an abnormally low concentration, *i.e.* a relative lack of this entity that can affect some mechanisms in the biological system.
- Δ indicates a normal concentration.
- θ shows an abnormally high concentration, namely an excess of this entity.

Notation 1. [Concentration levels] *We note* $\mathbb{L}$ *the set* $\{\varepsilon, \iota, \Delta, \theta\}$ *equipped with the total order relation such that:* $\varepsilon < \iota < \Delta < \theta$. *The elements of* $\mathbb{L}$ *are called* concentration levels.

In a given biological system and depending on the studied issue, not all entities have concentrations regarded as abnormally low or high. Therefore, only the levels ε and Δ are mandatory for each entity, ι and θ being optional.

Taking this variation in consideration, the signature of a biological system allows the definition of the set of biological entities considered in the system and, for each entity, its admissible concentration levels.

Definition 1. [Signature] *A* signature *is an application* $\mathcal{E} : E \to \mathcal{P}(\mathbb{L})$ *where E is a finite set and for all* $e \in E$, $\{\varepsilon, \Delta\} \subset \mathcal{E}(e)$. *Elements of E are called* entities *and for each entity e,* $\mathcal{E}(e)$ *is called the set of* admissible levels *of e.*

For instance, the signature of a basic energy metabolism model may involve $E = \{\text{PDH}, \text{Pyr}, \text{ACoA}, \text{OAA}, \text{Cit}\}$ can correspond to the set of five entities where each entity has its own set of admissible levels. For example, we may have $\mathcal{E}(\text{Pyr}) = \{\varepsilon, \iota, \Delta, \theta\}$.

After defining the system signature, the state of the system can be defined as the qualitative level of each entity present in the system. The previous example model can be at a state η_0 where PDH

is at the level Δ, noted $\eta_0(\text{PDH}) = \Delta$ and where $\eta_0(\text{Pyr}) = \theta$, $\eta_0(\text{ACoA}) = \iota$, $\eta_0(\text{OAA}) = \Delta$ and $\eta_0(\text{Cit}) = \varepsilon$. This state can also be written:

$$\eta_0 = (\Delta, \theta, \iota, \Delta, \varepsilon) \quad (1)$$

where the order of variable is (PDH, Pyr, ACoA, OAA, Cit).

Definition 2. [State] *A signature $\mathcal{E}$ being given, the set of* states ζ *is the set of functions $\eta : E \to \mathbb{L}$ such that for all $e \in E$, $\eta(e) \in \mathcal{E}(e)$.*

In this formalism, the evolution of the system is represented by two functions: the incrementation, noted *incr*, and the decrementation, noted *decr*. These functions apply to one entity at a time and return the level of this entity just above (resp. below) its current level. Because all entities do not have the same set of admissible levels, there is one function defined for each entity. For instance, if $\mathcal{E}(\text{OAA}) = \{\varepsilon, \Delta, \theta\}$ and $\eta_0(\text{OAA}) = \Delta$, then $incr_{\text{OAA}}(\eta_0(\text{OAA})) = \theta$ and $decr_{\text{OAA}}(\eta_0(\text{OAA})) = \varepsilon$. It should be noted that the incrementation (resp. decrementation) function is not defined on the maximal (resp. minimal) level of the admissible levels. Therefore, in our previous example, $incr_{\text{OAA}}(\eta(\text{OAA}))$ is not defined if $\eta(\text{OAA}) = \theta$.

Besides these functions, the formalism also makes use of formulas to describe properties about the entities concentration levels.

Definition 3. [Formula] *The set $\mathcal{A}$ of* atomic formulas *on a signature $\mathcal{E}$ is the set of expressions of the form $a \leqslant b$ where a and b can be any element of $E \cup \mathbb{L}$.*

The set $\mathcal{F}$ of formulas *on a signature $\mathcal{E}$ is inductively defined by:*

- $\mathcal{A} \subset \mathcal{F}$.
- *if φ and ψ are elements of $\mathcal{F}$, then $\neg\varphi$, $\varphi \wedge \psi$, $\varphi \vee \psi$, $\varphi \Rightarrow \psi$ are also elements of $\mathcal{F}$.*

Definition 4. [Satisfaction relation] *A state η and a formula $\varphi \in \mathcal{F}$ on a signature $\mathcal{E}$ being given, the* satisfaction relation $\eta \vDash \varphi$ *is inductively defined by:*

- *if φ is an atom of the form $a \leqslant b$, then $\eta \vDash \varphi$ if and only if $\overline{\eta}(a) \leqslant \overline{\eta}(b)$ where $\overline{\eta}$ is the extension of η to $E \cup \mathbb{L}$ by the identity on $\mathbb{L}$.*
- *if φ is of the form $\varphi_1 \wedge \varphi_2$ then $\eta \vDash (\varphi_1 \wedge \varphi_2)$ if and only if $\eta \vDash \varphi_1$ and $\eta \vDash \varphi_2$. We proceed similarly for the other connectives.*

Moreover, "$\eta \vDash \varphi$" is read "η satisfies φ".

We may also use the abbreviation $a = b$ as a shortcut for $(a \leqslant b) \wedge (b \leqslant a)$. We proceed similarly for $a < b$, $a > b$ and $a \geqslant b$.

Examples of formulas can be $\varphi \equiv (\text{Pyr} = \theta)$, stating an excessive presence of Pyr or $\psi \equiv (\text{Cit} > \text{ACoA})$, stating that the qualitative level of Cit is strictly superior to the one of ACoA. The state η_0, previously described in eq. 1, satisfies φ but not ψ.

To determine the evolution of the system, a set of rules is then used. A rule can be interpreted as possible modifications in the state which can be abstracted by the following representation:

$$r : A_1 + \cdots + A_m \Rightarrow A_{m+1} + \cdots + A_n \;\; boost(\varphi) \;\; block(\psi)$$

Beside its identifier r, each rule includes two sets of entities. The first one, for all i in $[1, m]$, constitutes the set of "reactants" whose level can be reduced by the application of the rule. The other one, for all i in $[m+1, n]$, represents the set of "products" whose level can be increased by the application of the rule. A rule also includes two modulating conditions $boost(\varphi)$ and $block(\psi)$ (φ and ψ being formulas) representing respectively a possible positive and negative modulation of the rule. The $boost(\varphi)$ (resp. $block(\psi)$) modulation takes only effect if φ (resp. ψ) is satisfied and its effects are further detailed later on. Of course, if no modulation is known for a given rule, *boost* and *block* regulations are not displayed in the rule representation.

Definition 5. [Biological action network] *A biological action network on a signature $\mathcal{E}$, or $\mathcal{E}$-action network, is a set R of rules of the form:*

(1) $r : A_1 + \cdots + A_m \Rightarrow A_{m+1} + \cdots + A_n \;\; boost(\varphi) \;\; block(\psi)$ *where:*

- *r is an identifier such that there are not two rules in N with the same r.*
- *$\forall i = 1 \ldots n, A_i \in E$.*
- *$\{A_1 \ldots A_m\} \cap \{A_{m+1} \ldots A_n\} = \varnothing$.*
- *φ and ψ are elements of $\mathcal{F}$.*

For short, we will call such rules $\mathcal{E}$-rules and we will call state of *R a state on the signature of R.*

Let us note that a rule representing possible alterations of a biological state, it makes no sense to have an entity being part of both reactants and products of a same rule.

Moreover, please notice that a rule can be devoid of any reactant or product. In the previous definition, the index m can be equal to zero (the rule does not need any reactant) or m can be equal to n (the rule has no product). A rule without reactant can be considered as the constitutive production of an entity in a given model and a rule without product can be interpreted as the degradation of an entity. In either cases, the empty solution is depicted using the _ symbol.

It is worth mentioning that despite the strong resemblance between a rule and a chemical reaction, a rule must *not* be interpreted as quanta of reactants converted into quanta of products but as a possible evolution of the levels of entities present in the rule.

As a basic example of rule, the condensation of acetyl-CoA and oxaloacetate to form Cit can be represented by the following rule:

$$r_A : \text{ACoA} + \text{OAA} \Rightarrow \text{Cit}$$

Since neither positive nor negative modulating conditions are considered here, only reactants and products are displayed.

In order to be applicable at a given state, a rule must meet basic criteria inspired from biology. First, since the level ε is interpreted as a negligible concentration, a rule is applicable only if all its reactants are present at least at the level ι. In addition, a rule cannot be applied if the negative modulating condition $block()$ applies, namely if the corresponding formula is satisfied.

Definition 6. [Applicable rule] *Let us consider a state η on a signature $\mathcal{E}$. An $\mathcal{E}$-rule $r \in R$ of the form (1) is said* applicable *at the state η if and only if:*

- $\forall i = 1 \dots m,\ \eta(A_i) \neq \varepsilon$.
- $\eta \not\models \psi$.

For instance, let us consider the conversion of Pyr into acetyl-CoA by the enzyme name Pyr dehydrogenase (PDH). If we assume that $\mathcal{E}(\text{PDH}) = \{\varepsilon, \iota, \Delta, \theta\}$, the conversion can be written as:

$$r_B : \text{Pyr} \Rightarrow \text{OAA} \quad block(\text{PDH} < \Delta)$$

This rule is applicable if and only if the level of Pyr is strictly greater than ε and the level of PDH is at least Δ, namely if there is Pyr in the system and a normal concentration of PDH. Note that the catalysis, namely the necessary presence of an enzyme to the proper conduct of a reaction, can be expressed using the $block()$ condition as in the previous example.

Considering rules as possible alterations of the equilibrium led us to a difficult choice on the way of handling the emergence of abnormal levels of products.

On one hand, we can consider that every reactant of a rule has to be in excess to shift the product(s) equilibrium(s) to excessive levels. This vision is labelled optimistic since we suppose the system less prone to drift towards excessive states. On the other hand, with a more pessimistic perspective, only one reactant in excess is enough to propagate the excess to the product(s).

Both the optimistic and the pessimistic approaches need an exception system to take into account particular biological cases. In the optimistic approach, the exception is a $boost()$ statement that relaxes the conditions for a product to reach excessive levels. In the pessimistic approach, the exception would be a $brake()$ statement selectively preventing excesses to propagate.

As the optimistic approach is predominant in toxicological data, our formalism implements it thanks to stricter conditions for a product level to increase. These constraints can be found in the way of computing the potential next level of products.

Definition 7. [Potential next level] *Let R be an $\mathcal{E}$-action network, let η be a state of R, let $r \in R$. We note $\eta_r^{\triangleright} : E \to \mathbb{L}$ the partial function such that $\eta_r^{\triangleright}(e)$ is defined if and only if r is applicable and if one of the following conditions is satisfied:*

- *$e \in \{A_1 \dots A_m\}$ and in this case $\eta_r^{\triangleright}(e) = decr_e(\eta(e))$.*
- *$e \in \{A_{m+1} \dots A_n\}$, $\eta(e) < max(\mathcal{E}(e))$, and in this case:*
  - *if $\eta \not\models \varphi$ and $\eta(e) < \min_{i \in \{1 \dots m\}} (\eta(A_i))$ then $\eta_r^{\triangleright}(e) = incr_e(\eta(e))$.*
  - *if $\eta \models \varphi$ then $\eta_r^{\triangleright}(e) = incr_e(\eta(e))$.*

The potential next level of an entity through an applicable rule refers to the next level of the entity after the application of that rule. If the entity acts as a reactant, its potential next level is the one returned by the decrementation function applied to that entity.

If the entity acts as a product, its potential next level depends on the $boost()$ statement:

- if the $boost()$ statement is not satisfied, a product level can increase *only if all the reactants levels are strictly greater* (this is due to the optimistic vision explained previously). In this case, the potential next level of a product is thus the one returned by the incrementation function applied to the product.
- if the $boost()$ statement is satisfied, the previous restriction no longer applies. In such cases, the potential next level of a product is returned by the incrementation function applied to it, independently of the reactant levels.

Let us note that the potential next level is returned either by the incrementation or decrementation function. Therefore, when these functions are not defined, the potential next level of an entity is also not defined.

Keeping the conversion of Pyr as an example, we can also specify that an excess of Pyr dehydrogenase can cause trouble in oxaloacetate levels by adding a $boost()$ condition to the rule r_B:

$r_C : \text{Pyr} \Rightarrow \text{OAA} \quad block(\text{PDH} < \Delta) \quad boost(\text{PDH} > \Delta)$

Here, assuming that the rule is applicable at the state η_0 and that $\eta_0(\text{OAA}) = \Delta$, the potential next level of oxaloacetate by this rule can be θ only if $\eta_0(\text{Pyr}) = \theta$ or if $\eta_0(\text{PDH}) > \Delta$ (so, $\eta_0(\text{PDH}) = \theta$).

Among all the applicable rules at a given state, only one is applied at a time. When a rule is applied, one and only one of its entities sees its level changing to its potential next level. This means that the

level of an entity has to change in order to consider that the rule was applied. Importantly, this also means that a product cannot be updated simultaneously with a reactant, and conversely. Similar ideas have been firstly developed for discrete gene models by Thomas and Snoussi (Snoussi, 1989; Thomas, 1991). This behaviour reflects the possibility for an entity to cross a threshold without all the other entities levels doing likewise.

In brief, starting from a given state, it is possible to determine which rules of the system are applicable at that state. The application of one of these rules then changes the level of one entity, modifying the system state. However, it is possible to stay indefinitely at a same system state thanks to the rule *Id* (whose application does not change the levels of the system entities and that is always applicable).

It is then possible to establish a transition graph, mapping all the possible transitions between the states of a system. An infinite succession of transitions such that the output state of a transition is the input state of the next one is here called a path of the transition graph.

Definition 8. [Transition graph] *The* transition graph *of an $\mathcal{E}$-action network R is the labelled graph whose set of vertices is the set of states ζ and the set of edges T is the set of transitions of the form $\eta \xrightarrow{r} \eta'$ such that one of the following condition is satisfied:*

- $r = Id$ *and* $\eta' = \eta$
- $r \in R$ *and there exists an entity* $e \in E$ *such that* $\eta_r^{\triangleright}(e)$ *is defined and:*
  - $\eta'(e) = \eta_r^{\triangleright}(e)$
  - $\forall\, e' \in E \setminus \{e\},\ \eta'(e') = \eta(e')$.

Remark: The transition graph of an $\mathcal{E}$-action network R canonically defines a labelled Kripke structure $L = (\mathcal{L}, \Sigma, T)$ as follows:

- $\mathcal{L}(\eta) = \{\alpha \in \mathcal{A} \mid \eta \vDash \alpha\}$.
- $\Sigma = R \cup \{Id\}$.
- *T can obviously be seen as the set of triplets (η, r, η') such that $(\eta \xrightarrow{r} \eta')$ is a transition of T.*

A path $(\pi \equiv \eta_0 \xrightarrow{r_0} \eta_1 \xrightarrow{r_1} \ldots \xrightarrow{r_{i-1}} \eta_i \xrightarrow{r_i} \ldots)$ is then an infinite sequence of labelled transitions such that the input state of r_i is equal to the output state of r_{i-1} for all $i > 0$. The set of paths is called Π_R.

5 INTEGRATING TOXICOLOGICAL KNOWLEDGE

As the transition graph of a biological system includes many biologically improbable paths, it is necessary to filter out the irrelevant ones and only characterise the interesting paths for toxicologists. Temporal logic and model checking tools have been successfully applied to biological systems, either using Linear Temporal Logic (Ito et al., 2014) or Computation Tree Logic (Bernot et al., 2004). Here, since we seek to filter paths, we need a logic able to express both state and transition properties. We thus use the state/event linear temporal logic (SE-LTL) developed by Chaki (Chaki et al., 2004).

Since a path can be seen as an infinite alternance between states and transitions, atomic temporal formulas concern either a state or a transition. For states, atomic temporal formulas are similar to atomic formulas exposed in Definition 3. For transitions, atomic temporal formulas only involve a rule identifier or the identity operator.

Definition 9. [Temporal formula] *Given an $\mathcal{E}$-action network R, the set $\mathcal{T}_R$ of* temporal formulas *on R is inductively defined by:*

- $(\mathcal{A} \cup R \cup \{Id\}) \subset \mathcal{T}_R$
- *if φ and ψ are formulas of $\mathcal{T}_R$, then $\neg\varphi$, $\varphi \wedge \psi$, $\varphi \vee \psi$, $\varphi \Rightarrow \psi$, $X\varphi$, $F\varphi$, $G\varphi$, $\varphi U \psi$ are formulas of $\mathcal{T}_R$.*

Definition 10. [Temporal formula satisfaction] *Given an $\mathcal{E}$-action network R and a path $(\pi \equiv \eta_0 \xrightarrow{r_0} \eta_1 \xrightarrow{r_1} \ldots) \in \Pi_R$, the* satisfaction relation $\vDash \subset \Pi_R \times \mathcal{T}_R$ *is inductively defined on the temporal formulas of $\mathcal{T}_R$ by :*

- $\pi \vDash \alpha$ *where* $\alpha \in \mathcal{A}$ *if and only if* $\eta_0 \vDash \alpha$,
- $\pi \vDash r$ *where* $r \in R \cup \{Id\}$ *if and only if* $r = r_0$,
- $\pi \vDash \varphi \wedge \psi$ *where* $(\varphi, \psi) \in {\mathcal{T}_R}^2$ *if and only if* $\pi \vDash \varphi$ *and* $\pi \vDash \psi$, *other propositional logic connectives are treated similarly,*
- $\pi \vDash X\varphi$ *where* $\varphi \in \mathcal{T}_R$ *if and only if* $(\eta_1 \xrightarrow{r_1} \eta_2 \xrightarrow{r_2} \ldots) \vDash \varphi$,
- $\pi \vDash G\varphi$ *where* $\varphi \in \mathcal{T}_R$ *if and only if for all* $i \in \mathbb{N}$, $(\eta_i \xrightarrow{r_i} \eta_{i+1} \xrightarrow{r_{i+1}} \ldots) \vDash \varphi$,
- $\pi \vDash F\varphi$ *where* $\varphi \in \mathcal{T}_R$ *if and only if there exists* $i \in \mathbb{N}$, $(\eta_i \xrightarrow{r_i} \eta_{i+1} \xrightarrow{r_{i+1}} \ldots) \vDash \varphi$,
- $\pi \vDash \varphi\ U\ \psi$ *where* $(\varphi, \psi) \in {\mathcal{T}_R}^2$ *if and only if there exists* $j \in \mathbb{N}$, $(\eta_j \xrightarrow{r_j} \ldots) \vDash \psi$ *and for all* $0 \leqslant i < j$, $(\eta_i \xrightarrow{r_i} \ldots) \vDash \varphi$.

Furthermore, for all $r \in R$ of the form $r : A_1 + \cdots + A_m \Rightarrow A_{m+1} + \cdots + A_n$ $boost(\varphi)$ $block(\psi)$, we note $app(r)$ the temporal formula $(\bigwedge_{i=1}^{m} A_i > \varepsilon) \wedge \neg\psi$ stating that r is applicable at the current state (see Definition 6).

In addition, for all $e \in \mathcal{E}$, we note $\downarrow e$ the temporal formula stating that the level of the entity e decreases in the next state:

$$\bigvee_{l \in \mathcal{E}(e) \setminus \{\varepsilon\}} \Big(e = l \wedge X\big(e = decr_e(l)\big)\Big).$$

We proceed similarly for $\uparrow e$.

For instance in our running example, the property χ characterising paths where an excess of Pyr leads to a future excess of oxaloacetate can be written as: $G(Pyr > \Delta \Rightarrow F(OAA > \Delta))$ and the formula ξ stating that the rule r_A is the first applied when Cit is absent from the system can be written as: $G(Cit = \varepsilon \Rightarrow r_A)$. In this situation, the path beginning with $(\eta_0 \xrightarrow{r_B} \eta_1)$, where $\eta_0 = (\Delta, \theta, \iota, \Delta, \varepsilon)$ and $\eta_1 = (\Delta, \theta, \iota, \theta, \varepsilon)$ satisfies χ but not ξ.

Finally, the association of the transition graph of a system with a set of properties representing the relevant biological pathways is called a *constrained network*. This constrained network is actually a subset of paths from the transition graph, with each path in this subset satisfying all the expressed biological properties.

Definition 11. [Constrained network] *An* $\mathcal{E}$-constrained network *is a couple* $N = (R, Ax)$ *where* R *is an* $\mathcal{E}$*-action network and* Ax *is a set of temporal formulas.*

Definition 12. [Dynamics of a constrained network] *Given an* $\mathcal{E}$*-constrained network* $N = (R, Ax)$*, the* dynamics of N *is the subset* Π_N *of* Π_R *such that* $\pi \in \Pi_R$ *belongs to* Π_N *if and only if* $\pi \vDash Ax$.

Since properties filter out irrelevant paths from the transition graph, it is thus possible to use them in conjunction to formal methods to insure that the final constrained network respects basic biological and toxicological properties as well as specific properties related to the studied issue.

6 APPLICATION TO THE ENERGY METABOLISM

To illustrate the formalism previously described, we continue with the simplified energy metabolism developed in Section 3. The set of elements introduced in Figure 1 is thus completed with the cell reducing potential (pRed) and ethanol (Eth). The resulting system signature is $\mathcal{E}_0 = \{$Gluc, Lac, Pyr, ACoA, OAA, FA, pRed, Eth$\}$.

In order to maximise the amount of possible paths, let us consider that the set of admissible levels of the endogenous entities (namely all entities except Eth) is $\{\varepsilon, \iota, \Delta, \theta\}$. In parallel, we will consider the ethanol either absent, present moderately or present in excess in the cell. Its set of admissible levels is thus $\{\varepsilon, \Delta, \theta\}$.

The role of each of these entities was developed in Section 3. The reducing potential is here designated as pRed, but as previously described, it is an abstraction of the balance between oxidised and reduced coenzymes. The rule $\_ \Rightarrow$ pRed thus abstracts the rule OxidisedCoenzymes $\Rightarrow$ ReducedCoenzymes.

As the amounts of oxidised and reduced coenzymes are interdependent, an increase in pRed means both an increase in reduced coenzymes and a decrease in oxidised coenzymes. This means that the rule $\_ \Rightarrow$ pRed cannot apply when there is an important lack of oxidised coenzymes, namely when the reducing potential is in excess. This explains the presence of *block* modulations linked to an excess of pRed in some of the rules presented hereunder (GlyL, PyDC, LDH, CC, βOx and EthOx).

The following rules summarise the interactions described in Section 3 and constitute the $\mathcal{E}_0$-action network R_0:

GlyL : Gluc $\Rightarrow$ Pyr + pRed $block(\text{pRed} = \theta)$
PyDC : Pyr $\Rightarrow$ ACoA + pRed $block(\text{pRed} = \theta \vee \text{ACoA} \geqslant \Delta)$
PyC : Pyr $\Rightarrow$ OAA
PyRed : Pyr + pRed $\Rightarrow$ Lac
LDH : Lac $\Rightarrow$ Pyr + pRed $block(\text{pRed} = \theta)$
CC : ACoA $\Rightarrow$ pRed $block(\text{pRed} = \theta \vee \text{OAA} = \varepsilon)$
MRC : pRed $\Rightarrow$ _
GNG : OAA + pRed $\Rightarrow$ Gluc
LipoG : ACoA + pRed $\Rightarrow$ FA
βOx : FA $\Rightarrow$ ACoA + OxPw $block(\text{pRed} = \theta)$
EthOx : Eth $\Rightarrow$ pRed $block(\text{pRed} = \theta)$ $boost(\text{Eth} > \varepsilon)$

Rule *GlyL* abstracts the whole glycolysis with its transformation of Gluc into Pyr concomitant to the production of pRed. The different futures of Pyr are summarised in rules *PyDC*, *PyC* and *PyRed*. It should be noted that the *block* modulation of *PyDC* represents possible conditions in which Pyr decarboxylation is stopped while *LDH* is the reverse of rule *PyRed*.

The rule *CC* represents the citrate cycle and its production of pRed through the consumption of ACoA. As OAA is an integral part of the cycle, this rule is stopped by a lack of OAA. The rule *MRC* represent the ability for mitochondria to regenerate the oxidised coenzyme (and therefore to decrease pRed).

Rules *GNG* and *LipoG* represent both carbohydrate (gluconeogenesis) and lipid (lipogenesis) anabolic pathways and their use of pRed. Conversely, rule βOx abstracts β-oxidation and its production of

Table 1: The set of states present in path π_0 with rules applied at each step of the path. In all these states, FA and pRed levels are normal (Δ) while there is a lack of Lac (ι) and a complete absence of Eth (ε). The level updated between each state is shown in bold.

State	Gluc	Pyr	OAA	ACoA
η_0	Δ	ι	ι	ι
η_1	Δ	$\mathbf{\Delta}$	ι	ι
η_2	$\boldsymbol{\iota}$	Δ	ι	ι
η_3	ι	Δ	$\mathbf{\Delta}$	ι
η_4	$\mathbf{\Delta}$	Δ	Δ	ι

pRed.

Finally, the detoxification of Eth by the cell is depicted in *EthOx*, with the *boost* modulation representing the important amount of pRed possibly produced by the detoxification. It should be noted that this detoxification is known to be performed with high priority by the hepatic cell, but this kind of information cannot appear in the rule.

Instead, it will be integrated in the model thanks to temporal formulas as seen in Section 5. For instance, it is known that Pyr can either be carboxylated (*PyC*) or decarboxylated (*PyDC*), depending on the amount of available ACoA. This can be summarized in the temporal formula φ_0:

$$G((ACoA < \Delta \wedge app(PyC) \wedge app(PyDC)) \Rightarrow \neg PyC)$$

This property states that when *PyC* and *PyDC* are applicable, and that there is a lack of ACoA, the rule *PyC* is not applied. Note that the *G* operator surrounding the formula indicates that the property remains true at every step of the path. *PyC* thus never applies when the previous conditions are satisfied.

It is also known that both decarboxylation and carboxylation of pyruvate prevail over pyruvate reduction (*PyRed*), as written in φ_1:

$$G((app(PyRed) \wedge (app(PyC) \vee app(PyDC))) \Rightarrow \neg PyRed)$$

This property is similar to the previous one and litterally means that when *PyRed* and either *PyC* or *PyDC* are applicable, *PyRed* never applies.

As previously said, these properties can be used to characterise interesting paths allowed by R_0. Let then N_0 be the constrained network associating R_0 with the two previous properties (φ_0 and φ_1), and let us consider the path π_0 beginning with the following prefix (see Table 1):

$$\eta_0 \xrightarrow{GlyL} \eta_1 \xrightarrow{GlyL} \eta_2 \xrightarrow{PyC} \eta_3 \xrightarrow{GNG} \eta_4 \xrightarrow{PyC} \dots$$

This prefix starts in state η_0. This state can be assimilated to a fasting state, where there is a normal amount of energy supplies (Gluc, FA) and a lack of every other metabolite. The first applied rule, *GlyL* is the glycolysis, restablishing the normal level of Pyr (η_1). The rule is then triggered again, leading to a decrease in Gluc level. The rule *PyDC* is then applied, leading to an increase in OAA (η_3). Finally, OAA fuels the gluconeogenesis (*GNG*), restablishing normal glucose levels (η_4).

Table 2: The set of states present in path π_1 with rules applied at each step of the path. In all these states, Lac, ACoA and FA levels are normal (Δ). The level updated between each state is shown in bold.

State	Gluc	Pyr	OAA	pRed	Eth
η_{10}	ι	Δ	ι	Δ	ε
η_{11}	ι	Δ	$\mathbf{\Delta}$	Δ	ε
η_{12}	$\mathbf{\Delta}$	Δ	Δ	Δ	ε

Table 3: The set of states present in path π_3 with rules applied at each step of the path. In all these states, Lac, ACoA and FA levels are normal (Δ). The level updated between each state is shown in bold.

State	Gluc	Pyr	OAA	pRed	Eth
η_{20}	ι	Δ	ι	Δ	θ
η_{21}	ι	Δ	ι	Δ	$\mathbf{\Delta}$
η_{22}	ι	Δ	ι	$\boldsymbol{\theta}$	Δ
η_{23}	ι	$\boldsymbol{\iota}$	ι	θ	Δ
η_{24}	ι	ι	ι	$\mathbf{\Delta}$	Δ

Although the prefix of π_0 is allowed by the $\mathcal{E}_0$-action network, it does not satisfy φ_0. Indeed, the priming of Pyr carboxylation (*PyC*) on Pyr decarboxylation (*PyDC*), namely the change from η_2 to η_3, in a fasting situation is very unlikely. As the prefix does not satisfy one of the properties of the constrained network, the entire path is filtered out.

Properties are also important to express toxicological knowledge. For instance, the priming of ethanol detoxification (*EthOx*) over all the other rules can be expressed by ψ_0:

$$G(app(\mathrm{EthOx}) \Rightarrow \mathrm{EthOx})$$

Furthermore, the detoxification reaction produce a lot of reducing potential. This means that whenever an excess of ethanol is detoxified through *EthOx*, the reducing potential of the cell is impacted, hence the formula ψ_1:

$$G((\mathrm{Eth} = \theta \wedge \mathrm{EthOx}) \Rightarrow (\downarrow \mathrm{Eth} \wedge X(\mathrm{EthOx} \wedge \uparrow \mathrm{pRed})))$$

Finally, if the cell struggles to decrease the pRed level in presence of Eth, this means that the mitochondrial respiratory chain is saturated. In such cases, the reduction of Pyr (*PyRed*) is triggered, hence the formula ψ_2:

$$G((\mathrm{Eth} > \varepsilon \wedge \neg app(\mathrm{EthOx}) \wedge app(\mathrm{PyRed})) \Rightarrow \mathrm{PyRed})$$

To illustrate the consequences of such constraints, let us consider N_1, the constrained network associating R_0 with the set of properties $\{\psi_0, \psi_1, \psi_2\}$. Let us also consider the path π_1 beginning with the follow-

ing prefix (see Table 2):

$$\eta_{10} \xrightarrow{PyC} \eta_{11} \xrightarrow{GNG} \eta_{12} \xrightarrow{PyDC} \dots$$

This path starts in the state η_{10}, where there is no ethanol, a lack of Gluc and OAA and a normal amount of every other metabolite. This state corresponds to an hypoglycemic state where the cell has the ability to quickly recover its normal glucose level through its supplies in FA and Pyr. Indeed, π_1 illustrates one of the possible paths leading to the regeneration of glucose, first with the application of *PyC* to restore OAA levels, then with the application of *GNG*.

Let us now consider π_2, a path similar to π_1 except on the beginning state (see Tables 2 and 3):

$$\eta_{20} \xrightarrow{PyC} \eta_{11} \xrightarrow{GNG} \eta_{12} \xrightarrow{PyDC} \dots$$

This path starts from η_{20}, which is identical to η_{10} except for the excessive presence of ethanol (see Table 3). This path does not satisfy ψ_0 since ethanol detoxification is not performed as soon as possible. π_2 is thus not retained in N_1.

Finally, let us take a look at path π_3 (see Table 3):

$$\eta_{20} \xrightarrow{EthOx} \eta_{21} \xrightarrow{EthOx} \eta_{22} \xrightarrow{PyRed} \eta_{23} \xrightarrow{PyRed} \eta_{24} \xrightarrow{EthOx} \dots$$

Also starting from η_{20}, π_3 then leads to the detoxification of a part of Eth through *EthOx* (η_{21}), satisfying ψ_0. The rule *EthOx* is then applied again, leading to the increase in pRed and satisfying ψ_1. Rule *PyRed* is then applied, decreasing the level of Pyr to ι. As pRed is still in excess, *PyRed* is applied a second time, leading to the recovery of normal levels of pRed and satisfying ψ_2. As π_3 satisfies ψ_0, ψ_1 and ψ_2, it is retained in N_1, illustrating the impaired ability for a cell to regenerate normal glucose levels through the gluconeogenesis in presence of ethanol.

7 CONCLUSION

In this article is presented a new formal framework able to handle several specificities of the toxicology domain not taken into account so far. This rule-based modelling framework relies on the direct description of equilibrium changes happening in a biological system. This description does not model the difference of reaction speed between the model rules, which can affect the system equilibrium. It is however possible to integrate biological and toxicological knowledge about rule kinetics through formulas expressed in temporal logic.

As demonstrated on a simple model of the energy metabolism, its expressive power allows us to describe both the equilibrium changes in the biological system and knowledge about the prioritisation of reactions. This knowledge is then used to filter out irrelevant paths from the resulting model.

In the future, our formalism will be coupled with formal methods with the purpose of generating the comprehensive list of pathways of toxicity present in a model. Indeed, through the use of biological properties, it is possible to define pathological states and list all the paths leading to these states. The resulting paths shall finally be sorted thanks to additional toxicological knowledge. Furthermore, filtering the resulting paths could also highlight gaps in the current toxicological knowledge and help toxicologists in their design of new experiments.

Finally, this formalism will serve as a basis to develop a software platform dedicated to toxicology. This platform is currently under development and it is already possible to run simulations on biological action networks. In the future, the platform will also be able to integrate the temporal formulas and to filter out paths from the biological action networks that do not satisfy the formulas. This will be achieved generating all the paths allowed by a system biological action network and directly checking these paths for their biological relevance thanks to expressed biological properties. Finally, by defining states regarded as pathologic, the platform will then be able to compute all the paths leading to pathologic states and thus propose putative pathways of toxicity to toxicologists.

REFERENCES

Backhaus, T., Blanck, H., and Faust, M. (2010). *Hazard and risk assessment of chemical mixtures under REACH: state of the art, gaps and options for improvement*. Swedish chemicals agency.

Bernot, G., Comet, J.-P., Richard, A., and Guespin, J. (2004). Application of formal methods to biological regulatory networks: extending thomas' asynchronous logical approach with temporal logic. *Journal of theoretical biology*, 229(3):339–347.

Brooks, G. A. (1998). Mammalian fuel utilization during sustained exercise. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, 120(1):89–107.

Calzone, L., Fages, F., and Soliman, S. (2006). Biocham: an environment for modeling biological systems and formalizing experimental knowledge. *Bioinformatics*, 22(14):1805–1807.

Chaki, S., Clarke, E. M., Ouaknine, J., Sharygina, N., and Sinha, N. (2004). State/event-based software model checking. In *International Conference on Integrated Formal Methods*, pages 128–147. Springer.

Chance, B. and Williams, G. (1956). The respiratory chain and oxidative phosphorylation. *Adv Enzymol Relat Areas Mol Biol*, 17:65–134.

Ciocchetta, F. and Hillston, J. (2009). Bio-pepa: A framework for the modelling and analysis of biological sys-

tems. *Theoretical Computer Science*, 410(33):3065–3084.

Colborn, T., Dumanoski, D., and Myers, J. P. (1996). *Our stolen future: are we threatening our fertility, intelligence, and survival?: a scientific detective story*. Dutton.

Field, J. B., Williams, H. E., and Mortimore, G. E. (1963). Studies on the mechanism of ethanol-induced hypoglycemia. *Journal of Clinical Investigation*, 42(4):497.

Hellerstein, M., Christiansen, M., Kaempfer, S., Kletke, C., Wu, K., Reid, J., Mulligan, K., Hellerstein, N., and Shackleton, C. (1991). Measurement of de novo hepatic lipogenesis in humans using stable isotopes. *Journal of Clinical Investigation*, 87(5):1841.

Ito, S., Hagihara, S., and Yonezaki, N. (2014). A qualitative framework for analysing homeostasis in gene networks. In *proceedings of 5th International Conference on Bioinformatics Models, Methods and Algorithms (BIOINFORMATICS2014)*, pages 5–16.

Kepner, J. (2004). Synergy: The big unknowns of pesticide exposure. *Pesticides and You*, 23(4):17–20.

Owen, O. E., Kalhan, S. C., and Hanson, R. W. (2002). The key role of anaplerosis and cataplerosis for citric acid cycle function. *Journal of Biological Chemistry*, 277(34):30409–30412.

Pettit, F. H., Pelley, J. W., and Reed, L. J. (1975). Regulation of pyruvate dehydrogenase kinase and phosphatase by acetyl-coa/coa and nadh/nad ratios. *Biochemical and biophysical research communications*, 65(2):575–582.

Pilkis, S. J. and Granner, D. (1992). Molecular physiology of the regulation of hepatic gluconeogenesis and glycolysis. *Annual review of physiology*, 54(1):885–909.

Schulz, H. (1991). Beta oxidation of fatty acids. *Biochimica et Biophysica Acta (BBA)-Lipids and Lipid Metabolism*, 1081(2):109–120.

Snoussi, E. H. (1989). Qualitative dynamics of piecewise-linear differential equations: a discrete mapping approach. *Dynamics and stability of Systems*, 4(3-4):565–583.

Talcott, C. (2008). Pathway logic. In *International School on Formal Methods for the Design of Computer, Communication and Software Systems*, pages 21–53. Springer.

Thomas, R. (1991). Regulatory networks seen as asynchronous automata: a logical description. *Journal of theoretical biology*, 153(1):1–23.