Towards a Computer Aided Toxicology

Benjamin MIRAGLIO[1], Gilles BERNOT[1], Jean-Paul COMET[1], and Christine RISSO-DE FAVERNEY[2]

[1]Université Nice Sophia-Antipolis, CNRS UMR 7271, Laboratoire I3S, France
[2]Université Nice Sophia-Antipolis, CNRS FRE 3729 ECOMERS, Parc Valrose, 28 Avenue Valrose, 06108 Nice, France

March 2, 2016

Abstract

If the classical paradigm of toxicology has been used for centuries, recent toxicological findings, soaring experimental costs and an increasing regulatory pressure have led the toxicology community towards mechanistic toxicology. This new area of research, focused on molecular envents underlying the toxicity of a chemical substance, motivates the emergence of new modelling approaches for toxicology. In this chapter, we introduce a qualitative rule-based formalism inspired from BioChAM with semantics adapted to the specificities of toxicology. Using a simple example of thyroid hormone system, we then show that this formalism is able to describe the possible toxic disruptions of a biological system. We finally introduce ToxBioNet, a software platform dedicated to toxicology currently under development and we present its already implemented simulator.

1 Introduction

The study of adverse effects caused by an exogenous chemical substance (known as a xenobiotic) in biological systems is called toxicology. The classical toxicology is based on the principle established by Paracelsus in the XVIth century : "All things are poison and nothing is without poison; only the dose makes a thing not a poison." [16] This means that any chemical substance can cause harmful effects to an organism if the system is exposed during a long enough time to a high enough dose of chemical. In modern toxicology, this concept still holds as the basis of the dose-response relationship. In addition, there is almost always a dose below which no response can be measured and conversely, once a maximum response is reached, any increase in the dose will not result in any increased effect . This relationship enables toxicologists to establish a causality between the exposure to a chemical and its induced observed effects. It also allows to determine the threshold of toxicity, namely the lowest exposure (in dose and/or time) where an induced effect occurs.

Many experiments carried out recently have questioned the legitimacy of this paradigm. Indeed, toxicity assessment is quite complex since many factors can affect the results of toxicity tests. Some of these factors include variables like temperature, food, light, stressful environmental conditions and exposure to other chemical compounds. Other factors related to the test subject itself, including age, sex, health, hormonal status or window of exposure may also greatly influence the vulnerability of an organism to a xenobiotic.

Although lethality is often used to measure toxicity, an increasing trend in toxicology is to focus on the sequence of molecular events occuring during the toxic response and leading to an observable effect. This approach, called mechanistic toxicology, aims to explain the whole causal chain of key events occurring in an organism, from the administration of the compound to its observed adverse effects. In this context, the notion of key event encompasses events occurring at the molecular, cellular and even at the organ scale.

Two almost identical notions concurrently appeared among toxicologists, trying to formalise the chains of key events: the Adverse Outcome Pathways (AOP) [2] and the Modes of Action (MoA) [13]. While only minor parts of their definitions differ, the main distinction between these two notions lies in the context of their use. Indeed, the notion of AOP tends to be used preferentially in ecotoxicology while the MoA notion is mainly used in human toxicology. In this chapter, we only refer to a chain of key events as a pathway of toxicity for the sake of simplicity.

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, data obtained when studying one chemical could be reused when assessing other chemicals. By taking all these facts into account, it is very likely that mechanistic bottom-up approaches will complete classical top-down approaches in the near future.

Concurrently, as the potential toxicity of chemical exposure became an area of great concern to both the public [6, 8] and the regulatory authorities [3], the production of chemical compounds is increasingly regulated in the U.S. and in Europe. Manufacturers must now conduct more 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 modelling approaches, and so far, most of these approaches are quantitative and enable either to infer the toxic threshold of a chemical substance or to confirm its specific pathway of toxicity. To reach these objectives, quantitative approaches need a lot of *in vitro* or *in vivo* toxicological data gathered during the early stages of the development process of the chemical substance. This necessity can be restrictive given the current cost of acquiring new biological data. There is therefore an incentive to develop new approaches that do not focus on toxic thresholds. Instead, they aim to describe pathways of toxicity at the *qualitative* level, namely by discretising continuous concentrations into intervals of interest. These approaches try to enumerate all the possible pathways of toxic-

ity included in a biological system and then check the biological plausibility of these pathways. Their final goal is to highlight the most probable pathways involved in a given toxicity.

In this chapter, we present a new qualitative formalism allowing to describe a biological system with its possible toxicological disruptions. This formalism was originally inspired from the boolean semantics of BioChAM [5], an environment able to model biological systems as networks of chemical reactions. However, these semantics are somehow too rough for toxicology due to particular features present in the toxicological models. Our new formalism therefore extends the boolean semantics of BioChAM to take into account these specificities, such as the notion of abnormal concentrations or the presence of modulating interactions impossible to manage similarly to classical chemical reactions. The purpose of this new formalism is to help toxicologists in their search for new pathways of toxicity.

Throughout this chapter, the presentation of the formalism will be illustrated by the thyroid hormone system. Indeed, this system is one of the least sex hormone dependent system and its mechanisms are well described in the literature. The next section is thus dedicated to the description of the thyroid hormone system and the various mechanisms ensuring its homeostasis. In Section 3, we explain how to use the new formalism to construct a toxicological system and the associated semantics. This formalism is then applied to a simplified thyroid hormone system in Section 4 and finally, we describe the aim of the ToxBioNet software platform and its first component, a simulator dedicated to our formalism, in Section 5.

2 Thyroid Hormone Homeostasis

The underlying biological network ensuring the homeostasis of the thyroid system is complex and results in a finely regulated system where thyroid hormone levels only vary subtly during the day [19]. The homeostasis of thyroid system is necessary since any perturbation of this system can have major effects on the health of individuals, especially when it occurs in the earliest stages of development of an organism [1].

The hypothalamo-pituitary-thyroid axis (HPT axis) is part of the neuroendocrine system involved in the regulation of metabolism and in the thyroid homeostasis in particular. As suggested by its name, this axis is composed of three compartments: the hypothalamus, the pituitary and the thyroid gland. The hypothalamus is a brain structure that controls endocrine glands. Part of this region secretes a neuropeptide, Thyrotropin-Releasing-Hormone (TRH). TRH is transported in axonal fluid to stimulate thyrotrophic cells in the anterior pituitary gland, stimulation that triggers the synthesis and secretion of Thyroid Stimulating Hormone (TSH). TSH is released into blood circulation and stimulates the follicular cells of the thyroid gland, leading to the synthesis of thyroid hormones (TH) and their secretion into the blood circulation [15].

Thyroid hormones (TH) are derived from the tyrosine amino acid and can be iodinated at different levels. For example, tri-iodo-thyronine (T3) and tetra-iodo-thyronine, also known as thyroxine (T4), are respectively iodinated three and four times. Moreover, the position of iodine residues in the chemical structure is important for the function of the hormone. Indeed, the reverse tri-iodo-thyronine (rT3) is as iodinated as T3 but does not have the same effects since its iodine residues are not located in the same places.

Historically, T3 is considered as the sole active form of thyroid hormone, T4 only being a pro-hormone that can be activated into T3 by deiodination [9]. Most of the T4 is converted into T3 in the liver. In this classic view, the action of TH on target genes is mediated by Thyroid hormone Receptors (TR). These receptors are constitutively located in the cell *nucleus* of any cell targeted by the thyroid hormone. TR can bind to T3 and more marginally to T4 [23]. While TR-T4 complexes are ineffective [23], TR-T3 complexes present the ability to bind to precise regions of DNA called thyroid hormone response elements. Once binded to these elements, TR-T3 complexes can then influence the transcription of target genes, either in a positive or a negative manner depending on the gene [23].

Recent studies have shown that T4, rT3 and other products of TH deiodination also have a biological activity that does not involve TR [14, 20]. These actions are currently under further investigations by the endocrinology community and will not be developed in this chapter.

Several negative feedbacks are present in the HPT axis in order to ensure a proper regulation of the thyroid system. Actually, the production of TRH and TSH are repressed by the negative feedback effects of T3 over respectively the hypothalamus and the anterior pituitary [10, 7]. T3 also stimulates the production of Pyroglutamyl Peptidase 2 (PP2), an enzyme known to destroy the TRH before it can reach the pituitary gland [18]. Those regulatory effects are mediated by the binding of T3 to TR located in target cells.

Degradation of TH is ensured by two types of enzymes. Specific enzymes such as the deiodinases (Dio), present in different tissues such as the liver, the kidney or the brain, inactivate TH by removing one or more iodine residues [11]. In contrast, the non-specific hepatic enzymes such as the glucuronosyltransferases (UGT) conjugate TH with a residue leading to the biliary excretion of TH [17]. It should be noted that these non-specific hepatic enzymes are also generally activated when the organism is exposed to some foreign chemicals such as drugs or poisons considered as *indirect Endocrine Disruptors (iED)*. In this context, an over-activation of UGT can cause a major decrease in blood TH and disrupt indirectly the thyroid hormone homeostasis [17].

Other foreign chemicals, considered as *direct Endocrine Disruptors (dED)* can also disrupt the thyroid hormone homeostasis by acting directly on the production of TH in the thyroid gland [12]. Thus, a direct or indirect disruption leading to the decrease of TH blood levels will result in the compensatory increase of both TRH and/or TSH

levels. This increase, when it is lasting, may raise the risk of developping some thyroid cancers [4, 12].

We propose at first a simple representation of the thyroid hormone system that can summarise this knowledge about the physiology of the thyroid hormone system. This representation comprises TRH, TSH, TH, TR, PP2, Dio, UGT and we can also add a direct endocrine disruptor (abbreviated as ED for the sake of simplicity) that will interfere with the production of TH (see Figure 1).

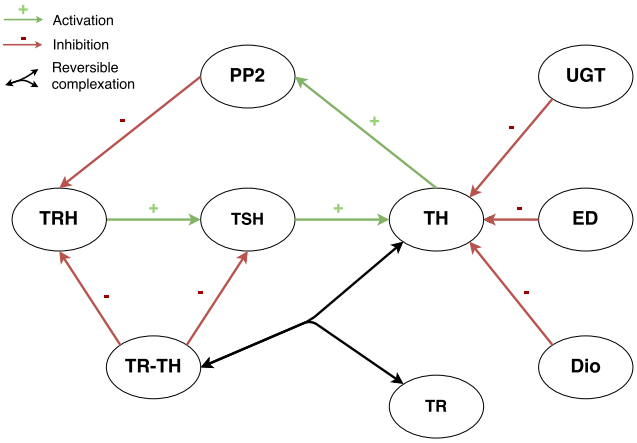

Figure 1: Simple representation of the thyroid hormone system.

For pedagogical purposes, we will over-simplify this representation. Here, since TRH only stimulates TSH production, we can abstract it and assume that PP2 and TR-TH negative interactions directly concern TSH. Since PP2 and TR-TH negative influences both originate from TH and concern both TSH, we can also abstract PP2. Moreover, since the ED only disrupts directly TH levels, we can put aside the TH degradation processes and pull out Dio and UGT from the representation. In the end, the resulting simplified representation only includes TH, TSH, TR and the ED (see Figure 2).

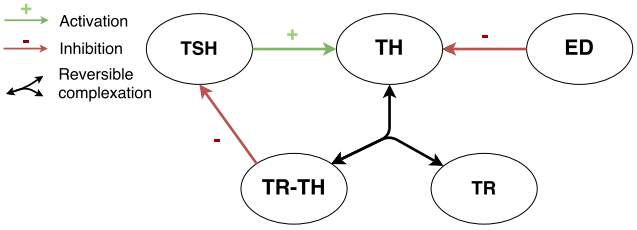

Figure 2: Over-simplified representation of the thyroid hormone system.

3 A New Discrete Framework for Toxicology

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

In order to represent the evolution of the concentration of each entity and to detect abnormal concentrations, we introduce four qualitative abstract levels, which are enumerated here in increasing order :

- ε reflects a negligible concentration of a given entity, that is to say a concentration too low to trigger any mechanism 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.

In a given biological system, not all entities have abnormally low or high concentrations depending on the studied issue. Therefore in this formalism, only the levels ε and Δ are mandatory for each entity, ι and θ are optional. All these facts are gathered in the signature of a biological system which defines the set of biological entities present in the system and the levels admissible for each entity.

Definition 1 [Signature] *A* signature *is a finite set E whose elements are named entities. Moreover, E is given an application $\tau : E \to \mathcal{P}(\mathbb{L})$ where $\mathbb{L} : \{\varepsilon, \iota, \Delta, \theta\}$, and such that for each entity $e \in E$, $\{\varepsilon, \Delta\} \subset \tau(e)$. $\tau(e)$ is the set of* admissible levels of the entities e. *Moreover, by convention, $\mathbb{L}$ is equipped with the strict total order relation $\varepsilon < \iota < \Delta < \theta$.*

For instance, the signature of the simplified thyroid hormone system corresponds to the set of five entities {TSH, TH, TR, TR-TH, ED} and each entity has its own set of admissible levels. For example the set of admissible levels of TH, $\tau(\text{TH})$, is equal to $\{\varepsilon, \iota, \Delta, \theta\}$ as TH can be in excess or abnormally low in some cases.

Once the system signature is defined, it is then possible to define the state of the system as the qualitative levels of all entities of the system. For example the simplified thyroid hormone system can be at a state η_0 where TSH is at the level θ, noted $\eta_0(\text{TSH}) = \theta$ and where $\eta_0(\text{TH}) = \iota$, $\eta_0(\text{TR}) = \Delta$, $\eta_0(\text{TR-TH}) = \iota$ and $\eta_0(\text{ED}) = \varepsilon$. This state can then be written :

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

where the order of variable is TSH, TH, TR, TR-TH, ED.

Definition 2 [State] *A signature E being given, a* state *η is a function $E \to \mathbb{L}$ such that for all $e \in E$, $\eta(e) \in \tau(e)$.*

In order to represent the evolution of the system, we introduce 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 have not the same set of admissible levels, there is one function defined for each entity. For instance, if $\tau(\text{TSH}) = \{\varepsilon, \Delta, \theta\}$ and $\eta_0(\text{TSH}) = \Delta$, then $incr_{\text{TSH}}(\eta_0(\text{TSH})) = \theta$ and $decr_{\text{TSH}}(\eta_0(\text{TSH})) = \varepsilon$. 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, $incr_{\text{TSH}}(\eta(\text{TSH}))$ is not defined if $\eta(\text{TSH}) = \theta$.

Alongside these functions, some properties on the entity levels can be described by formulas.

Definition 3 [Formula] *A signature E being given, the set of* formulas on E *is inductively defined by :*

- *for all symbols a and b belonging to E or $\mathbb{L}$, the atoms $a = b$, $a > b$, $a \geqslant b$, $a \leqslant b$ and $a < b$ are atomic formulas.*
- *if φ and ψ are well-formed formulas on E, then $\neg\varphi$, $\varphi \wedge \psi$, $\varphi \vee \psi$, $\varphi \Rightarrow \psi$ are also well-formed formulas on E.*

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

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

For instance, the formula φ stating the presence of TR at a normal level can be written as: $\text{TR} = \Delta$ and the formula ψ stating that the level of TH is strictly superior to the one of TSH can be written as: $\text{TH} > \text{TSH}$. The state η_0, previously described in eq. 1, satisfies φ but not ψ.

To determine the evolution of the biological system, a set of rules is given. This set is interpreted as biological transformations. In short, a rule can be resumed by the following representation :

$$A_1 + \cdots + A_m \;\; \Rightarrow \;\; A_{m+1} + \cdots + A_n \;\; up(\varphi) \;\; down(\psi)$$

Each rule includes two sets of entities, the first one, for all i in $[1, m]$, constitutes the set of "reactants" while the other one, for all i in $[m+1, n]$, represents the set of "products." A rule also includes two modulating conditions $up(\varphi)$ and $down(\psi)$ (φ and ψ being formulas) representing respectively a positive and a negative possible modulation of the rule. The $up(\varphi)$ (resp. $down(\psi)$) 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, it is not displayed in the rule representation.

Definition 5 [Biological action network] *A* biological action network on a signature E *is a set of rules where each rule is an expression of the form :*

$$A_1 + \cdots + A_m \;\Rightarrow\; A_{m+1} + \cdots + A_n \;\; up(\varphi) \;\; down(\psi)$$

where :

- $\forall i \in \{1 \dots n\}, A_i \in E$.
- φ *and* ψ *are formulas on* E.

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 levels of entities present in the rule.

As a basic example of rule, the complexation of TH with TR can be represented by the following rule :

$$\text{TH} + \text{TR} \;\Rightarrow\; \text{TR-TH}$$

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 regulating condition $down()$ applies, namely if the corresponding formula is satisfied.

Definition 6 [Applicable rule] *Let* η *be a state and let us consider a rule* r *of the form* $A_1 + \cdots + A_m \Rightarrow A_{m+1} + \cdots + A_n \;\; up(\varphi) \;\; down(\psi)$. *The rule* r *is* 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 deiodination of T4 into T3 by the type 2 deiodinase (dio2). If we assume that $\tau(\text{dio2}) = \{\varepsilon, \iota, \Delta, \theta\}$, the deiodination can be written as :

$$\text{T4} \;\Rightarrow\; \text{T3} \;\; down(\text{dio2} < \Delta) \quad (a)$$

This rule is applicable if and only if the level of T4 is strictly greater than ε and the level of type 2 deiodinase is at least Δ, namely if there is T4 in the system and a normal concentration of type 2 deiodinase. Note that the catalysis, namely the necessary presence of an enzyme to the proper conduct of a reaction, can be expressed using the $down()$ condition as in the previous example.

When a rule is applied, part of its entities can vary to a potential next level. Since a reactant is consumed during the application of a rule, it is possible for its level to cross a downward threshold and become lower than its initial value. Therefore, the next level of a reactant is the one returned by the decrementation function applied to that reactant, or the current level if the threshold is not crossed.

The next potential level of a product is determined by the levels of reactants participating in the rule. The idea is simple: a product can increase only if each reactant is at a level sufficient to allow the considered product to increase. This is represented here by the condition that the level of every reactant must be strictly greater that the level of the product. Therefore, the next potential level of a product is returned by the incrementation function applied to it only if the level of every reactant in the rule is strictly greater than the initial level of the product.

The notable exception to this qualitative evaluation is the over-activated rules, namely, rules where the $up()$ condition applies. If a rule is over-activated, then the next potential level of a product is always returned by the incrementation function applied to it, independently of the reactant levels.

Definition 7 [Potential next level] *Let η be a state and r be a rule of the form $A_1 + \cdots + A_m \Rightarrow A_{m+1} + \cdots + A_n \;\; up(\varphi) \;\; down(\psi)$, applicable in η,*

- *for each reactant $R \in \{A_1 \dots A_m\}$, the potential next level of R by r is $decr_{_R}(\eta(R))$.*
- *if $\eta \vDash \varphi$, then for each product $P \in \{A_{m+1} \dots A_n\}$, the potential next level of P by r is $incr_{_P}(\eta(P))$.*
- *if $\eta \nvDash \varphi$, then for each product $P \in \{A_{m+1} \dots A_n\}$, the potential next level of P by r is $incr_{_P}(\eta(P))$ only if*
$$\eta(P) < \min_{R \in \{A_1 \dots A_m\}} (\eta(R)).$$
with $\min_{R \in \{\}} (\eta(R)) = \Delta$.

Notice that the constitutive production of an entity, represented by a rule devoid of any reactant, does not inherently lead to an abnormally high level of the entity. Therefore, when the set R of reactant is empty, its minimum is considered to be Δ.

The restriction on the possible evolution of product levels (third item of definition 7) relies on the assumption that the levels of entities do not spontaneously evolve towards anormal conditions but, indeed, need an initiating factor such as a pre-existing disorder in the reactant levels or the over-activation of the rule to reach abnormal levels. If we keep the T4 deiodination as an example, we can also specify that an excess of type$\tilde{2}$ deiodinase can cause trouble in T3 levels by adding a $up()$ condition to the rule (a) :

$$\text{T4} \;\; \Rightarrow \;\; \text{T3} \;\; down(\text{dio2} < \Delta) \;\; up(\text{dio2} > \Delta)$$

Here, assuming that the rule is applicable at the state η_0 and that $\eta_0(\text{T3}) = \Delta$, the potential next level of T3 by this rule can be θ only if $\eta_0(\text{T4}) = \theta$ or if $\eta_0(\text{dio2}) > \delta$ (so, $\eta_0(\text{dio2}) = \theta$).

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 evolves 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 neither reactant nor product levels are updated simultaneously. Similar ideas have been firstly developed for discrete gene models by Thomas and Snoussi [21, 22]. This behaviour reflects the possibility for the level of a reactant to cross a threshold without all the other reactant levels having to also cross a threshold.

In brief, starting from a given state, it is possible to determine which rules of the system are applicable at that state. Among these rules, the application of one rule changes the level of one entity, modifying the system state. It is then possible to establish a transition graph, mapping all the possible transitions between the states of a system.

Definition 8 [Transition graph] *A biological action network N being given, the associated* transition graph *is the graph $G = (V, T)$ whose set V of vertices is the set of states on the signature E of N, and such that there exists an edge from a state η to a state η', called transition and noted $\eta \to \eta'$, if and only if :*

- *there exists a rule r of the form $A_1 + \cdots + A_m \Rightarrow A_{m+1} + \cdots + A_n$ $up(\varphi)$ $down(\psi)$ applicable at η.*
- *there exists a unique index $i \in [1 \dots n]$ such thaot the potential next level of A_i by r is $\eta'(A_i)$ and $\forall e \in E \setminus \{A_i\}$, $\eta'(e) = \eta(e)$. In other words,o the only changed level is the level of A_i becoming $\eta'(A_i)$.*

In fact, it is possible to loop on an state even if there is an outgoing transition. This means that self loops are present on every state but they are not included here to avoid an overburden of the transition graph.

Once the transition graph of the biological system is established, it can be used as a basis for testing properties about the system dynamics.

4 Application of the Formalism to the Thyroid Hormone System

According to Figure 2, the signature of the system is the set of entities {TSH, TH, TR, TR-TH, ED}. The set of admissible levels of each entity is determined according to the rules where this entity intervenes, so we first detail the rules of the system representing the different interactions presented in Figure 2 :

1. _ $\Rightarrow$ TR
2. TR + TH $\Rightarrow$ TR-TH
3. TR-TH $\Rightarrow$ TR + TH
4. _ $\Rightarrow$ TSH $down(\text{TR-TH} \geqslant \Delta)$ $up(\text{TR-TH} = \varepsilon)$
5. TSH $\Rightarrow$ TH $down(\text{ED} > \varepsilon)$ $up(\text{TSH} > \Delta)$

Since TR receptors are formed constitutively in tissues sensitive to TH, the first rule abstracts the production of TR. Rules 2 and 3 represent respectively the complexation of TR and TH into TR-TH and their decomplexation. As a reminder, TR-TH is the entity that will determine the negative feedback strength applied on TSH production.

Rule 4 abstracts the molecular machinery allowing the production of TSH. This machinery is inhibited by the presence of TR-TH (in accordance with the negative feedbacks paragraph in Section 2), via the $down()$ condition. Conversely, when the TR-TH concentration is insignificant, the production of TSH is over-activated in a compensating effort by the organism, as formalised by the $up()$ condition. So, TSH is considered to be produced normally only when the concentration of TR-TH is at an abnormally low level but still significant in the organism, namely ι.

Finally, Rule 5 represents the TH production preconditioned by the level of TSH. TSH is considered as a reactant to take into account the inherent degradation of TSH during the TH production. The $down()$ condition introduces the endocrine disruptor action that blocks TH production and the $up()$ condition allows for the possibility of TH reaching an abnormally high concentration when over-stimulated by TSH.

These rules induce a precise definition of the different sets of admissible levels :

- Since TH is the main concern of the model, its level should be as accurate as possible, therefore $\tau(\text{TH}) = \{\varepsilon, \iota, \Delta, \theta\}$.
- In order to allow an over-activation of the TH production (rule 5), the TSH level must be able to reach θ. On the contrary, ι is not required here, thus $\tau(\text{TSH}) = \{\varepsilon, \Delta, \theta\}$.
- The same applies to the TR-TH set of admissible level: ι is necessary to allow a normal production of TSH (rule 4) but not θ. Therefore, $\tau(\text{TR-TH}) = \{\varepsilon, \iota, \Delta\}$.
- In this model, we are only interested in the presence or absence of TR and ED. We can then assimilate their levels to Boolean values: $\tau(\text{TR}) = \{\varepsilon, \Delta\}$ and $\tau(\text{ED}) = \{\varepsilon, \Delta\}$.

The complete graph has five dimensions and includes 144 different states ($144 = 4 \times 3 \times 3 \times 2 \times 2$). Here we focus only on the specific region of this graph where the TR level is Δ (*i.e.* we consider it constitutively expressed) and the ED level is ε (because we first want to see the behaviour of the system when not disrupted). These restrictions limit the graph to 36 states (represented in three dimensions) and make it representable as in Figure 3.

It is easily observable that the only way to reach the plane where $\eta(\text{TH}) = \theta$ is to go through the dashed green arrows present on the plane $\eta(\text{TSH}) = \theta$. These arrows represent the transitions allowed by an over-activation of the production of TH (rule 5) when TSH is in excess. Furthermore, the plane $\eta(\text{TSH}) = \theta$ is only reachable by the dashed red arrows on the the plane $\eta(\text{TR-TH}) = \varepsilon$ corresponding to the over-activation of the production of TSH (rule 4). Finally, we also see that in order to reach the plane $\eta(\text{TR-TH}) = \Delta$, the predecessor state must have a TH level of at least Δ, illustrating Definition 7.

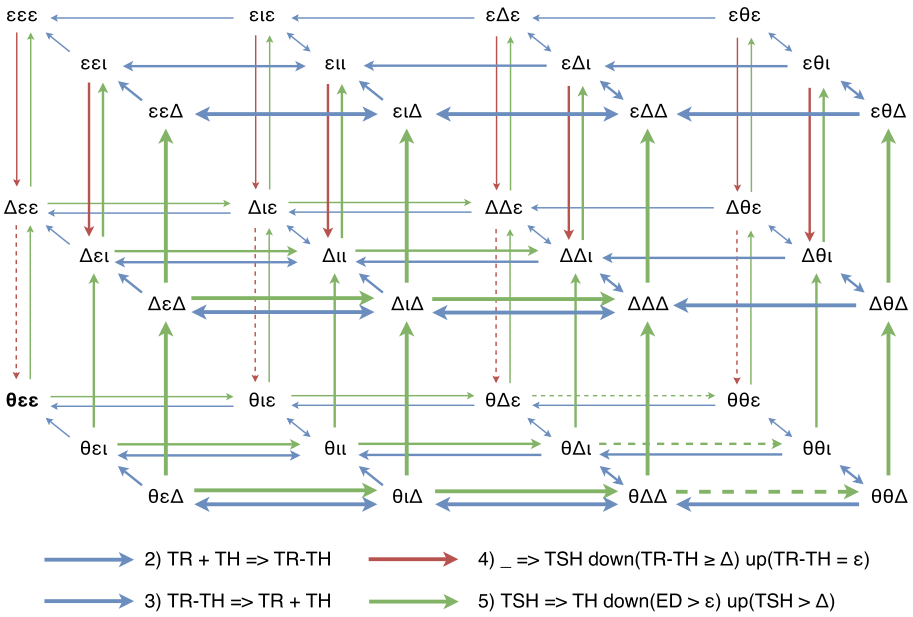

Figure 3: Partial transition graph of the over-simplified thyroid hormone model. The states are represented by a 3-letters string where the first one (resp. second and third) is the level of TSH (resp. TH and TR-TH). The levels of TR and ED are set to Δ and ε respectively. A dashed arrow indicates the application of an over-activated rule, where the $up()$ condition applies.

If we introduce an endocrine disruptor to the system and thus put the level of ED to Δ, we can see that all the green arrows would disappear because of the $down()$ condition on the production of TH (rule 5). At this point, all the transitions converge towards the state $\theta\varepsilon\varepsilon$ at the bottom left in the background. This state corresponds to an excess of TSH combined to a lack of TH and TR-TH, namely the same condition as observed biologically when an endocrine disruptor is introduced into an organism.

This example, although simple, shows that the abstraction made by this formalism is adequate to reproduce known biological behaviours.

5 ToxBioNet, a Software Platform Dedicated to Toxicology

The aim of ToxBioNet is to help toxicologists in their search for potential toxic pathways explaining the mechanism of a toxicity. One of the main goals is to be able to extract all the possible pathways between an initiating event and an adverse outcome, compatible with a particular biological action network. The resulting pathways can be further filtered if some key events are experimentally known to be involved or not involved in the studied toxicity. Similarly, some successions of key events are known

to be highly unlikely in biology (for example an event A known to be never followed by the event B). Pathways including such successions can thus be also filtered out. Finally, we would like to develop a heuristic suggesting the most informative and relevant experiences when trying to determine which remaining pathways are actually involved in the studied toxicity. To achieve this purpose, our formalism has to be combined with a second language able to express properties such as successions of key events.

Formal methods will be helpful to assist toxicologists to construct a mechanistic model. These can reveal previously unsuspected relations between pathways or key events. When trying to enumerate the pathways leading from an event A to an event B, the filtering step is facilitated by the search for inconsistencies between existing knowledge and hypotheses. As a trivial example, if an event of a pathway P from A to B is involved in another pathway which is certain to lead to B', and if we know that B never happens alongside B', the pathway P can be easily filtered out.

ToxBioNet is currently under development: it is already possible to run simulations on biological action networks. This simulator, written in Java, is able to parse an input file containing all the rules describing a biological system and to create the corresponding toxicological model. As shown in Section 4, the number of states in the transition graph grows exponentially with the number of entities (a system including n entities can have up to 4^n states). This makes the generation of the state graph technically difficult if not impossible for systems including more than fifteen entities (which implies approximately a billion possible states). In order to avoid the pitfall of the construction of a huge state graph, the simulator can exhibit as many traces as wanted in the state graph without constructing it. It was tested using a complex thyroid hormone system model: this model includes more than fifty rules based on a hundred of scientific references.

6 Conclusion

In this chapter is presented a new formal framework able to handle several specificities of toxicology not taken into account so far, such as the possible presence of a compound in abnormal concentrations or the possibility, for a reaction, to be modulated. This modelling framework is applied to the simple model of the thyroid hormone system and its expressive power allows us to describe the biological system with enough precision to reproduce existing behaviours such as the disruption of TH levels resulting in abnormally high TSH levels.

In the future, the current formalism will be combined with a formal language able to express properties on successions of key events: a simple idea would be to adapt classical temporal logics to our toxicological framework. Formal methods such as model checking, will be useful to find new potential pathways of toxicity satisfying some given properties. Besides, checking the successions of key events could also highlight gaps in the current toxicological knowledge. The platform ToxBioNet will therefore be useful as an experiment-aid tool to select the most informative experiments to conduct.

References

[1] Stig Andersen, Klaus Michael Pedersen, Niels Henrik Bruun, and Peter Laurberg. Narrow individual variations in serum t4 and t3 in normal subjects: a clue to the understanding of subclinical thyroid disease. *The Journal of Clinical Endocrinology & Metabolism*, 87(3):1068–1072, 2002.

[2] Gerald T. Ankley, Richard S. Bennett, Russell J. Erickson, Dale J. Hoff, Michael W. Hornung, Rodney D. Johnson, David R. Mount, John W. Nichols, Christine L. Russom, Patricia K. Schmieder, et al. Adverse outcome pathways: a conceptual framework to support ecotoxicology research and risk assessment. *Environmental Toxicology and Chemistry*, 29(3):730–741, 2010.

[3] Thomas Backhaus, Hans Blanck, and Michael Faust. *Hazard and risk assessment of chemical mixtures under REACH: state of the art, gaps and options for improvement*. Swedish chemicals agency, 2010.

[4] Robert A. Barter and Curtis D. Klaassen. Reduction of thyroid hormone levels and alteration of thyroid function by four representative udp-glucuronosyltransferase inducers in rats. *Toxicology and applied pharmacology*, 128(1):9–17, 1994.

[5] Laurence Calzone, François Fages, and Sylvain Soliman. Biocham: an environment for modeling biological systems and formalizing experimental knowledge. *Bioinformatics*, 22(14):1805–1807, 2006.

[6] Theo Colborn, Dianne Dumanoski, and John Peterson Myers. *Our stolen future: are we threatening our fertility, intelligence, and survival?: a scientific detective story*. Dutton, 1996.

[7] Ricardo H. Costa-e Sousa and Anthony N. Hollenberg. Minireview: the neural regulation of the hypothalamic-pituitary-thyroid axis. *Endocrinology*, 153(9):4128–4135, 2012.

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

[9] Josef Köhrle. Local activation and inactivation of thyroid hormones: the deiodinase family. *Molecular and cellular endocrinology*, 151(1):103–119, 1999.

[10] Frank Lezoualc'h, A. H. Hassan, Pierre Giraud, Jean-Philippe Loeffler, Stephanie L. Lee, and Barbara A. Demeneix. Assignment of the beta-thyroid hormone receptor to 3, 5, 3'-triiodothyronine-dependent inhibition of transcription from the thyrotropin-releasing hormone promoter in chick hypothalamic neurons. *Molecular Endocrinology*, 6(11):1797–1804, 1992.

[11] R. Malik and H. Hodgson. The relationship between the thyroid gland and the liver. *Qjm*, 95(9):559–569, 2002.

[12] PT Männistö, T Ranta, and J Leppäluoto. Effects of methylmercaptoimidazole (mmi), propylthiouracil (ptu), potassium perchlorate (kclo4) and potassium io-

dide (ki) on the serum concentrations of thyrotrophin (tsh) and thyroid hormones in the rat. *Acta endocrinologica*, 91(2):271–281, 1979.

[13] M. E. Meek, Alan Boobis, Ila Cote, Vicki Dellarco, George Fotakis, S. H. A. R. O. N. Munn, Jennifer Seed, and Carolyn Vickers. New developments in the evolution and application of the who/ipcs framework on mode of action/species concordance analysis. *Journal of Applied Toxicology*, 34(1):1–18, 2014.

[14] Lars C. Moeller, Xia Cao, Alexandra M. Dumitrescu, Hisao Seo, and Samuel Refetoff. Thyroid hormone mediated changes in gene expression can be initiated by cytosolic action of the thyroid hormone receptor β through the phosphatidylinositol 3-kinase pathway. *Nuclear receptor signaling*, 4:e020, 2006.

[15] OECD. In vitro & ex vivo assays for identification of modulators of thyroid hormone signalling. Technical report, Organisation for Economic Cooperation and Development, 2013.

[16] Paracelsus. *Das Buch Paragranum / Septem Defensiones*. Hofenberg, 2014.

[17] Koichi Saito, Hideo Kaneko, Keiko Sato, Akira Yoshitake, and Hirohiko Yamada. Hepatic udp-glucuronyltransferase (s) activity toward thyroid hormones in rats: induction and effects on serum thyroid hormone levels following treatment with various enzyme inducers. *Toxicology and applied pharmacology*, 111(1):99–106, 1991.

[18] Edith Sánchez, Miguel Angel Vargas, Praful S. Singru, Isel Pascual, Fidelia Romero, Csaba Fekete, Jean-Louis Charli, and Ronald M. Lechan. Tanycyte pyroglutamyl peptidase ii contributes to regulation of the hypothalamic-pituitary-thyroid axis through glial-axonal associations in the median eminence. *Endocrinology*, 150(5):2283–2291, 2009.

[19] Ulrich Schweizer, Joachim M. Weitzel, and Lutz Schomburg. Think globally: act locally: new insights into the local regulation of thyroid hormone availability challenge long accepted dogmas. *Molecular and cellular endocrinology*, 289(1):1–9, 2008.

[20] A. I. Shih, Shenli Zhang, H. James Cao, Heng-Yuan Tang, Faith B. Davis, Paul J. Davis, and Hung-Yun Lin. Disparate effects of thyroid hormone on actions of epidermal growth factor and transforming growth factor-α are mediated by 3*, 5*-cyclic adenosine 5*-monophosphate-dependent protein kinase ii. *Endocrinology*, 145(4):1708–1717, 2004.

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

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

[23] Paul M. Yen, Shinichiro Ando, Xu Feng, Ying Liu, Padma Maruvada, and Xianmin Xia. Thyroid hormone action at the cellular, genomic and target gene levels. *Molecular and cellular endocrinology*, 246(1):121–127, 2006.