Detecting Toxicity Pathways with a Formal Framework based on Equilibrium Changes

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Abstract. Toxicology aims at studying the adverse effects of exogenous chemicals on organisms. As these effects mainly concern metabolic pathways, reasoning about toxicity would involve metabolism modeling approaches. Usually, metabolic network models approaches are rule-based and describe chemical reactions, indirectly depicting equilibria as results of competing rule kinetics. By altering these kinetics, an exogenous compound can shift the system equilibria and induce toxicity. As equilibria are kept implicit, the identification of possible toxicity pathways is hindered as they require a fine understanding of chemical reactions dynamics to infer possible equilibria disruptions. Paradoxically, the toxicity pathways are base on a succession of very abstract (coarse grained) events. To reduce this mismatch, we propose a more abstract framework making equilibria first-class citizens. Our rules describe qualitative equilibrium changes and the chaining of rules is controlled by constraints expressed in extended temporal logic. This higher abstraction level fosters the detection of toxicity pathways, as we will show through an example of endocrine disruption of the thyroid hormone system.

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

1 Introduction

The purpose of toxicology is to study the adverse effects caused by chemical substances on living organisms. In this perspective, the central paradigm of the discipline assumes that the more an organism is exposed to a compound, the greater the effects of this compound will be.

This dose-response relationship underpins toxicity studies, where toxicologists aim at determining the threshold of toxicity of a compound (*i.e.* the lowest exposure from which an induced toxicity is observable). These studies also aim at identifying how a chemical disrupts physiological equilibria, and how these disruptions propagate in an organism, linking the exposure to a chemical to its observable toxicity. This causal chain of equilibrium changes, also known as pathway of toxicity, is widely used by regulating authorities to assess the toxicity of a compound.

Indeed, as our exposure to chemical products is becoming an area of great concern for society, authorities are implementing increasingly strict regulations. As a consequence, chemical manufacturers must now conduct extensive toxicity studies to demonstrate the innocuousness of their products, skyrocketing the development cost of such products.

This context provides ground for modeling toxicity, and so far, most of these modeling approaches are quantitative [8]. They aim at either inferring the toxic threshold of a chemical substance or confirming its specific pathway of toxicity. These objectives require a lot of biological data, which can be restrictive given the current acquisition cost of such data. An alternative approach consists in shifting the focus from toxic thresholds to toxicity pathways. Indeed, describing these pathways in a *qualitative* manner would allow to focus only on equilibrium changes and would therefore require comparatively less biological data. Moreover, such an approach would allow to use automated reasoning tools.

Several generic formalisms have already been developed to qualitatively model biological processes [3, 5, 13, 14, 17]. These formalisms use formal methods to reason about these standard processes. However, expressing toxicology problems in manageable terms for the formalism is frequently troublesome. Several specificities of toxicology make these environments not optimal. For instance, the possibility for a reaction to be modulated, a key notion in toxicology, is difficult to handle with the existing formalisms. In addition, these formalisms describe chemical reactions, only depicting equilibria as indirect results of competing rule kinetics. Yet, toxicity pathways are sequences of equilibrium changes. As such, keeping equilibria implicit while building a toxicological model can thus prove to be confusing for toxicologists, hindering the identification of possible toxicity pathways.

To solve these limitations, we present in this article a domain-oriented formalism directly describing qualitative equilibrium changes. First, a rule-based language allows to express the different equilibrium changes present in a biological system. Then, the chaining of rules can be corseted thanks to constraints expressed in an extended temporal logic. These constraints are usually based on toxicological observations regarding specific conditions of the system. Finally, automated reasoning tools can be used on the resulting system dynamics to detect possible toxicity pathways, providing useful insights to improve the experimental strategies of toxicity studies.

As our formalism is presented alongside examples inspired from the thyroid hormone system, the next section sketches an overview of this system. In Section 3, we explain how to use the new formalism to describe the equilibrium changes of a system. In Section 4, we show how to integrate toxicological knowledge in the system using an extended temporal logic. Finally, this formalism is applied to a model of the thyroid hormone system in Section 5.

2 The Thyroid Hormone System in a Nutshell

The thyroid hormone system plays a crucial role in the organism homeostasis. For example, alteration of thyroid hormones (TH) levels leads to troubles in the energy metabolism and in the adaptive thermogenesis in adults. This crucial role is even further highlighted during the organism development, where a slight disruption of the thyroid hormone homeostasis can lead to severe adverse effects such as neuronal defects, deafness or impaired bone and muscle formation [20, 21].

Consequently, as most endocrine systems, the thyroid hormone homeostasis is maintained by a complex regulation network involving a central control carried out by cerebral regions. However, this regulation is unusually strengthened peripherally by dedicated enzymes, the *deiodinases*. Indeed, contrarily to most endocrine systems, the blood circulating form of TH, tetraiodothyronine (T_4) is inactive and must be 5'-deiodinated into triiodothyronine (T_3) to act on its target receptors.

Another metabolite of T_4, reverse triiodothyronine (rT_3), can be obtained through 5-deiodation. Similarly to T_4, rT_3 is not able to activate thyroid hormone receptors and is thus considered to be inactive. It should be noted that recent experiments suggest that both T_4 and rT_3 have other biological activities [12]. However, these actions need further investigations and will not be developed through this article.

TH Synthesis As deiodinases are also present in the thyroid gland, the gland produces both thyroid hormone forms (T_3 and T_4). However, T_4 still accounts for roughly 90% of the gland production [10]. The synthesis process in itself starts with thyroid follicular cells extracting large quantities of iodide from the blood. This import is carried out thanks to dedicated iodide transporters. Thyroid iodide is then used by an enzyme, thyroid peroxidase (TPO), to assemble TH in the follicle [9]. Finally, TH are released in the blood, where they are associated with transporter proteins, as neither T_3 nor T_4 are soluble in water.

Central regulation The thyroid hormone synthesis, from iodide uptake to TPO activity, can be stimulated by thyroid-stimulating hormone (TSH) [11]. TSH synthesis is performed in the pituitary gland when triggered by thyrotropin-releasing hormone (TRH), itself produced in the hypothalamus [21]. Both TSH and TRH synthesis are down-regulated by high concentrations of T_3, creating a negative feedback loop described as the thyroid hormone *central regulation*, or hypothalamo-pituitary-thyroid axis (HPT axis, see Figure 1).

Peripheral regulation Only a minor part of circulating T_3 is synthesized by the thyroid gland, the remaining part is produced by deiodinases directly in tissues sensitive to thyroid hormone [10, 20]. The activation *in situ* of T_4 places deiodinases as key actors in thyroid hormone level regulation. This regulation is even more fine tuned thanks to three types of deiodinases, performing either 5'- or 5-deiodations, respectively activating or inactivating TH.

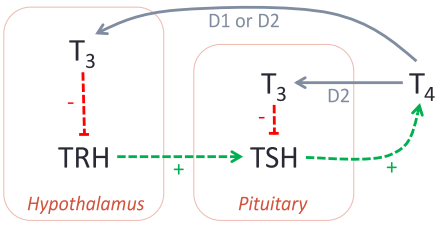

Fig. 1. Representation of the HPT axis integrating deiodinases action. Plain arrows show the deiodination of T_4 in T_3 by D_1 or D_2. Dashed arrows represent positive or negative regulations.

Type 3 deiodinase (D_3) is the main TH inactivator [6]. By catalyzing 5-deiodations, D_3 converts T_4 in rT_3 and T_3 in 3,3'-diiodothyronine, both inactive compounds. D_3 physiological role is to protect tissues from a local hyperthyroidism. As such, high concentrations of T_3 increase D_3 activity and conversely, the activity of the enzyme is reduced in hypothyroidism conditions.

Diametrically opposed to D_3, type 2 deiodinase (D_2) is the main TH activator [20]. D_2 catalyzes the 5'-deiodation of T_4 into T_3 and is down-regulated by T_3. As such, hyperthyroidism inhibits D_2 while low levels of T_3 increases D_2 activity. Interestingly enough, D_2 also plays a crucial role in the HPT axis (see Figure 1). Indeed, D_2 is required to transform T_4 into T_3 in the pituitary gland [20], making D_2 necessary to complete the negative feedback of T_3 on TSH production.

Finally, type 1 deiodinase (D_1) has several roles. This enzyme is able to catalyze both 5- and 5'-deiodations but is extremely inefficient when compared to D_2 or D_3 [20]. Despite this inefficiency, D_1 is able to mitigate the effects of the absence of D_2 by converting enough T_4 into T_3, preventing any global hypothyroidism. On top of this, D_1 primary role actually concerns iodine recycling [10]. Indeed, D_1 is highly affine with sulfated TH (*i.e.* hormones about to be eliminated, see next paragraph). As the thyroid hormone system is extremely dependent on iodine intake, D_1 role is then to recycle as much iodine as possible from sulfated TH before their excretion.

TH Metabolism TH metabolism is mainly carried out by hepatic enzymes. These enzymes are referred to as detoxifying enzymes since they are apt to inactivate a vast range of compounds (either exogenous or endogenous). This inactivation involves the conjugation of the compound with a specific residue, marking the compound for excretion. For instance, the action of the hepatic enzyme sulfotransferase results in sulfated TH [19].

Possible endocrine disruptions The synergy of the different mechanisms evoked previously provides a clockwork regulation of the thyroid hormone system. However, several weak points can hinder this complex machinery:

1. TH synthesis relies heavily on iodide availability in the thyroid gland. An interruption of iodide intake, or the malfunction of the dedicated iodide

transporters can then lead to severe hypothyroidism. Such effects can also result from an impaired TPO activity in the thyroid follicle [21].
2. Disruption in deiodinases activity also leads to troubles, but not necessarily as expected at first sight. Indeed, if considering only thyroid hormone levels, the absence of one of the activating deiodinase (D_1 or D_2) can be counterbalanced by the remaining activating deiodinase. However, D_2 is unable to recycle iodide efficiently. The absence of D_1 can thus lead to a iodide shortage [10]. Conversely, D_2 key role in the pituitary gland cannot be matched by D_1. An absence of D_2 thus leads to local hypothyroidism in the pituitary gland, leading to an unnecessary overproduction of TSH, finally resulting in an global hyperthyroidism [15].
3. The presence of some exogenous compounds in the organism can trigger a dramatic increase of hepatic detoxifying enzymes. This augmentation helps the organism to address the irregular presence of exogenous compounds, but also abnormally increases TH disposal, leading to global hypothyroidism [1].

3 Describing Equilibrium Changes with Transformation Rules

A biological system can be abstracted as a set of biological entities interacting with each other at different concentrations. In parallel, each entity has a concentration regarded as normal in a given organism. This concentration tends to be maintained in normal conditions, and a modification of this concentration can lead to adverse effects. For instance, the normal blood concentration of glucose is about 1 g/L in an adult human, and a concentration greater than 1.3 g/L can lead to several complications.

Consequently, our domain-oriented formalism represent the evolution of the concentration of each entity as a change in its equilibrium level. In that line, we introduce four qualitative equilibrium levels depicting increasing concentrations of an entity:

- ε stands for a negligible concentration (*i.e.* a concentration too low to trigger any reaction in the biological system).
- ι stands for an abnormally low concentration (*i.e.* a relative lack of this entity, affecting some mechanisms in the biological system).
- Δ stands for a normal concentration.
- θ stands for an abnormally high concentration (*i.e.* 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 levels are regarded as useful. For example, the modeler may be only interested in the normal (Δ) or excessive (θ) presence of an entity. Therefore, an entity must have at least two levels, but not necessarily more. 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 a map* $\mathcal{E} : E \to \mathcal{P}(\mathbb{L})$ *where* E *is a finite set and for all* $e \in E$, $|\mathcal{E}(e)| \geqslant 2$. *Elements of* E *are called* entities *and for each entity* e, $\mathcal{E}(e)$ *is the set of* admissible levels *of* e.

For instance, $E = \{\mathrm{T}_3, \mathrm{T}_4, \mathrm{TPO}, \mathrm{I}\}$ can be the signature of a thyroid model, with $\mathcal{E}(\mathrm{T}_3) = \{\varepsilon, \iota, \Delta, \theta\}$, $\mathcal{E}(\mathrm{T}_4) = \{\varepsilon, \iota, \Delta, \theta\}$, $\mathcal{E}(\mathrm{TPO}) = \{\varepsilon, \iota, \Delta, \theta\}$ and $\mathcal{E}(\mathrm{I}) = \{\varepsilon, \Delta, \theta\}$.

After defining the system signature, a state of the system is defined as the qualitative level of each entity present in the system. For example, a state η_0 where T_3 is at the level Δ, noted $\eta_0(\mathrm{T}_3) = \Delta$ and where $\eta_0(\mathrm{T}_4) = \varepsilon$, $\eta_0(\mathrm{TPO}) = \iota$ and $\eta_0(\mathrm{I}) = \theta$. This state can also be written:

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

where the entities order is (T_3, T_4, TPO, I).

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 our formalism, the evolution of an entity can follow two functions: the incrementation, *incr*, and the decrementation, *decr*. They return the level of this entity just above (resp. below) its current level. For instance, as $\mathcal{E}(\mathrm{TPO}) = \{\varepsilon, \Delta, \theta\}$, $incr_{\mathrm{TPO}}(\Delta) = \theta$ and $decr_{\mathrm{TPO}}(\Delta) = \varepsilon$. Note that the incrementation (resp. decrementation) function is not defined on the maximal (resp. minimal) admissible levels. As such, $incr_{\mathrm{TPO}}(\eta(\mathrm{TPO}))$ is not defined if $\eta(\mathrm{TPO}) = \theta$.

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

Definition 3 [**Formula**] *The set* $\mathcal{F}$ *of* formulas *on a signature* $\mathcal{E}$ *is inductively defined by:*

- *any atomic formula of the form* $a \leqslant b$ *(where* a *and* b *can be any element of* $E \cup \mathbb{L}$*) belongs to* $\mathcal{F}$.
- *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.*

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

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

Examples of formulas can be $\varphi \equiv (\mathrm{I} = \theta)$, stating an excessive presence of I or $\psi \equiv (\mathrm{T}_4 > \mathrm{TPO})$, stating that the qualitative level of T_4 is strictly greater than the one of TPO. The state η_0, previously described in Equation 1, satisfies φ but not ψ.

To describe possible evolutions of the system, a set of rules of the following form is then used:

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

Beside its identifier r, each rule includes two sets of entities A_i. The first one, for all i in $[1, m]$, constitutes the set of *consumables*, whose level may be reduced by the application of the rule. The other set, for all i in $[m + 1, n]$, represents the set of *produceables* whose level may be increased by the application of the rule. A rule also includes two modulating conditions $when(\varphi)$ and $boost(\psi)$ (φ and ψ being formulas). Intuitively, φ the role of the guard of the rule and ψ will relax some restriction on the increasing of produceable levels.

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:*

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

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 a "state of R" a state on the signature of R.

Let us emphasize that a rule represents *possible equilibrium changes*. Therefore, it makes no sense to have an entity being part of both consumables and produceables of a same rule.

Moreover, a rule can be devoid of any consumable or produceable: In the previous definition, the index m can be equal to zero (the rule does not need any consumable from the signature $\mathcal{E}$) or m can be equal to n (the rule has no produceable from the signature $\mathcal{E}$). A rule without consumable can be considered as a constitutive production of an entity in a given model and a rule without produceable can be interpreted as a constitutive depletion of an entity. In either cases, conventionally, the empty set of entities is denoted Ω, depicting the biological system in the broad sense, outside the signature.

Also, if no modulation is known for a given rule, *when* and *boost* regulations are not displayed in the rule representation, *i.e.* *when*(True) and *boost*(False) are left implicit.

It is worth mentioning that despite the obvious syntactic resemblance between a rule and a chemical reaction, a rule must *not* be interpreted as quanta

of consumables converted into quanta of produceables but as a *possible evolution* of the levels of entities present in the rule, representing possible equilibrium shifts.

As a basic example of rule, the production of T_3 and T_4 from I can be represented by the following rule:

$$r_A : \mathrm{I} \Rightarrow \mathrm{T}_3 + \mathrm{T}_4 \quad when(\mathrm{TPO} > \varepsilon)$$

In order to be applicable at a given state, a rule must meet basic criteria. First, since the level ε is interpreted as a negligible concentration, a rule is applicable only if all its consumables are present at least at the level ι. In addition, a rule cannot be applied if the formula φ of the modulating condition *when* is not 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:*

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

is said applicable *at the state η if and only if:*

- $\forall i = 1 \ldots m,\ \eta(A_i) \neq \varepsilon$.
- $\eta \vDash \psi$.

For instance, the rule r_A is applicable if and only if the levels of I and TPO are strictly greater than ε. By the way, note that the catalysis, namely the necessary presence of an enzyme to the proper conduct of a reaction, can be expressed using the *when* condition as in the previous example, but the catalyst cannot be present both on the left and right parts of the rule.

Definition 7 [Potential next level] *Let R be an $\mathcal{E}$-action network, let η be a state of R and r be a rule of R of the form:*

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

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 one of the following conditions is satisfied:

- $e \in \{A_1 \ldots A_m\}$ *and* $\eta_r^{\triangleright}(e) = decr_e(\eta(e))$.
- $e \in \{A_{m+1} \ldots A_n\}$, $\eta(e) < max(\mathcal{E}(e))$, *and:*
  - *if* $\eta \nvDash \varphi$ *and* $\eta(e) < \min_{i \in \{1 \ldots m\}}(\eta(A_i))$ *then* $\eta_r^{\triangleright}(e) = incr_e(\eta(e))$
  - *if* $\eta \vDash \varphi$ *then* $\eta_r^{\triangleright}(e) = incr_e(\eta(e))$.

  Where conventionally, $\min_{i \in \varnothing}(\eta(A_i)) = \eta(\Omega) = \Delta$.

If the entity A_i acts as a consumable, its potential next level is the one returned by the decrementation function.

If it acts as a produceable, its potential next level depends on the *boost* statement:

- if the *boost* statement ψ is not satisfied, a produceable level can increase *only if all the consumables levels are strictly greater*. In this case, the potential next level of a produceable is thus the one returned by the incrementation function applied to the produceable.

– if the *boost* statement ψ is satisfied, the previous restriction no longer applies. In such cases, the potential next level of a produceable is returned by the incrementation function applied to it, independently of the consumable levels. These levels must still be greater than ε, as the rule is applicable.

So, in the case of a rule deprived of consumables, produceables levels cannot exceed Δ unless the *boost* statement is satisfied.

Moreover, 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 synthesis of T_3 and T_4 as an example, we can also specify that an excess of TPO can cause trouble in T_3 and T_4 levels by adding a *boost* condition to the rule r_A:

$$r_B : \mathrm{I} \Rightarrow \mathrm{T}_3 + \mathrm{T}_4 \quad when(\mathrm{TPO} > \varepsilon) \quad boost(\mathrm{TPO} > \Delta)$$

Here, assuming that the rule is applicable at the state η_0 and that $\eta_0(\mathrm{T}_3) = \Delta$, the potential next level of T_3 by this rule can be θ only if $\eta_0(\mathrm{I}) = \theta$ or if $\eta_0(\mathrm{TPO}) = \theta$.

The dynamics is fully asynchronous. Among all the applicable rules at a given state, at most 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. Similar ideas have been firstly developed for discrete gene models by Thomas and Snoussi [16, 18]. This behavior 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 is not required but if so, it changes the level of one entity. It is possible to stay indefinitely at a same system state thanks to a special transition called 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 labeled 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:*
  • *$\eta'(e) = \eta_r^{\triangleright}(e)$*
  • *$\forall\ e' \in E \setminus \{e\},\ \eta'(e') = \eta(e')$.*

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

– *$\mathcal{L}(\eta) = \{\alpha \in \mathcal{A} \mid \eta \vDash \alpha\}$ where $\mathcal{A} \subset \mathcal{F}$ is the set of atomic formulas.*

– $\Sigma = R \cup \{Id\}$.
– 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 $(\pi \equiv \eta_0 \xrightarrow{r_0} \eta_1 \xrightarrow{r_1} \dots \xrightarrow{r_{i-1}} \eta_i \xrightarrow{r_i} \dots)$ *is then an infinite sequence of labeled 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.

4 Integrating Toxicological Knowledge into Constraints

As the transition graph of a biological system includes many toxicologically improbable paths, it is necessary to filter out the irrelevant ones and to characterize the interesting paths for toxicologists. Temporal logic and model checking tools have already been successfully applied to biological systems [2, 7]. Here, since we seek to filter paths in details, we need a logic able to express both state and transition properties, such as the state/event linear temporal logic (SE-LTL) developed by Chaki [4].

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

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* $(\pi \equiv \eta_0 \xrightarrow{r_0} \eta_1 \xrightarrow{r_1} \dots) \in \Pi_R$, *the* satisfaction relation $\models \subset \Pi_R \times \mathcal{T}_R$ *is inductively defined by:*

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

Furthermore, for all $r \in R$ of the form $r : A_1 + \cdots + A_m \Rightarrow A_{m+1} + \cdots + A_n$ *when*(φ) *boost*(ψ), 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:

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

We proceed similarly for $\uparrow e$.

For instance in our running example, the property χ characterizing paths where an excess of I leads to a future excess of T_3 can be written as: $G((\mathrm{I} > \Delta) \Rightarrow F(\mathrm{T}_3 > \Delta))$ and the formula ξ stating that the rule r_B is the first applied when T_4 is absent from the system can be written as: $G((\mathrm{T}_4 = \varepsilon) \Rightarrow r_B)$. In this situation, the path beginning with $(\eta_0 \xrightarrow{r_B} \eta_1)$, where $\eta_0 = (\Delta, \varepsilon, \iota, \theta)$ and $\eta_1 = (\theta, \varepsilon, \iota, \theta)$ satisfies both χ and 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 SE-LTL 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 satisfies basic biological and toxicological properties as well as specific properties related to the studied issue.

5 Application to the Thyroid Hormone System

The formalism described in the previous section can be illustrated with the thyroid hormone system developed in Section 2. This system contains the following entities: blood iodide ($\mathrm{I_B}$), thyroid iodide ($\mathrm{I_T}$), thyroid peroxydase (TPO), blood triiodothyronine ($\mathrm{T_{3B}}$), blood tetraiodothyronine ($\mathrm{T_{4B}}$), pituitary triiodothyronine ($\mathrm{T_{3Pit}}$), thyroid-stimulating hormone (TSH), type 1 to 3 deiodinases ($\mathrm{D_1}$, $\mathrm{D_2}$, $\mathrm{D_3}$) and hepatic detoxifying enzymes (Detox).

On top of these endogenous entities, we can also introduce exogenous compounds able to disrupt the thyroid hormone system. Each compound is an endocrine disruptor triggering one of the disruptions listed in Section 2: $\mathrm{X_I}$ impacts the dedicated iodide transporters in thyroid, $\mathrm{X_{D1}}$ and $\mathrm{X_{D2}}$ respectively inactivates $\mathrm{D_1}$ and $\mathrm{D_2}$, and $\mathrm{X_{Hep}}$ increases hepatic enzymes levels.

Finally, the signature of our example corresponds to the set:
$\mathcal{E}_{\text{thy}} = \{\text{I}_\text{B}, \text{I}_\text{T}, \text{TPO}, \text{T}_\text{3B}, \text{T}_\text{4B}, \text{T}_\text{3Pit}, \text{TSH}, \text{D}_1, \text{D}_2, \text{D}_3, \text{Detox}, \text{X}_\text{I}, \text{X}_\text{D1}, \text{X}_\text{D2}, \text{X}_\text{Hep}\}$

The $\mathcal{E}_{\text{thy}}$-action network R_{thy} is made of 21 rules. However, for the sake of clarity, only a part of these rules is presented in this section. The complete model, including the list of rules, is available in appendix.

Central regulation The HPT axis is modeled thanks to the following rules:
$I_{transfer} : \text{I}_\text{B} \Rightarrow \text{I}_\text{T} \;\; when(\text{TSH} > \varepsilon \wedge \text{X}_\text{I} = \varepsilon)$
$TPO_{synth} : \Omega \Rightarrow \text{TPO} \;\; when(\text{TSH} > \varepsilon) \;\; boost(\text{TSH} = \theta)$
$TPO_{destr} : \text{TPO} \Rightarrow \Omega \;\; when(\text{TSH} = \varepsilon \vee (\text{TPO} = \theta \wedge \text{TSH} < \theta))$
$TH_{synth} : \text{I}_\text{T} \Rightarrow \text{T}_\text{3B} + \text{T}_\text{4B} \;\; when(\text{TPO} > \varepsilon) \;\; boost(\text{TPO} = \theta)$
$Pit_{synth} : \text{T}_\text{4B} \Rightarrow \text{T}_\text{3Pit} \;\; when(\text{D}_2 > \varepsilon) \;\; boost(\text{D}_2 = \theta)$
$Pit_{destr} : \text{T}_\text{3Pit} \Rightarrow \Omega \;\; when(\text{D}_2 = \varepsilon \vee (\text{T}_\text{3Pit} = \theta \wedge \text{D}_2 < \theta))$
$TSH_{synth} : \Omega \Rightarrow \text{TSH} \;\; when(\text{T}_\text{3Pit} < \theta) \;\; boost(\text{T}_\text{3Pit} = \varepsilon)$
$TSH_{destr} : \text{TSH} \Rightarrow \Omega \;\; when(\text{T}_\text{3Pit} = \theta \vee (\text{TSH} = \theta \wedge \text{T}_\text{3Pit} > \varepsilon))$

Rule TPO_{synth} expresses the ability of the organism to restore normal levels of TPO only when TSH is present in the system. Conversely, TPO_{destr} conveys that levels of TPO tend to decrease when TSH is absent. Moreover, TSH is also required for the production of the dedicated iodide transporters. Note that these transporters are abstracted in this model. Consequently, actions of TSH and X_I directly apply to $I_{transfer}$.

The synthesis of TH requires the presence of both I_T and TPO. However, TPO levels are not affected by TH_{synth} since TPO is a catalyst of the reaction.

The negative feedback of T_4B on TSH production mediated exclusively by D_2 (as illustrated in Figure 1) is highlighted in TSH_{synth} and TSH_{destr}. Indeed, T_3Pit can only be obtained through deiodination of T_4B by D_2 (rules Pit_{synth} and Pit_{destr}).

Activation and metabolism of TH The activation of blood TH is handled by D_1 and D_2. Their equilibria and their impact on the system are handled thanks to the following rules:
$D1_{synth} : \Omega \Rightarrow \text{D}_1 \;\; when(\text{X}_\text{D1} = \varepsilon) \;\; boost(\text{T}_\text{3B} = \varepsilon)$
$D1_{destr} : \text{D}_1 \Rightarrow \Omega \;\; when(\text{X}_\text{D1} > \varepsilon \vee (\text{D}_1 = \theta \wedge \text{T}_\text{3B} > \varepsilon))$
$D2_{synth} : \Omega \Rightarrow \text{D}_2 \;\; when(\text{T}_\text{3B} < \theta \wedge \text{X}_\text{D2} = \varepsilon) \;\; boost(\text{T}_\text{3B} = \varepsilon)$
$D2_{destr} : \text{D}_2 \Rightarrow \Omega \;\; when(\text{T}_\text{3B} = \theta \vee \text{X}_\text{D2} > \varepsilon \vee (\text{D}_2 = \theta \wedge \text{T}_\text{3B} > \varepsilon))$
$I_{recycling} : \text{T}_\text{4B} \Rightarrow \text{I}_\text{B} \;\; when(\text{D}_1 > \varepsilon)$
$TH_{activation} : \text{T}_\text{4B} \Rightarrow \text{T}_\text{3B} \;\; when(\text{D}_1 = \theta \vee \text{D}_2 > \varepsilon) \;\; boost(\text{D}_2 = \theta)$

Both D_1 and D_2 levels are induced by a lack of T_3B in the system. On the contrary, D_2 levels are reduced by an excess of T_3B. On top of that, the presence of exogenous disruptors such as X_D1 or X_D2 alters D_1 and D_2 levels.

As the vast majority of sulfated TH is composed of T_4B, $I_{recycling}$ models accurately the preponderant recycling role of D_1. D_1 also intervenes marginally in T_4B deiodation, as shown in $TH_{activation}$, where D_1 needs to be at level θ

to satisfy the *when* statement. As for D_2, the enzyme acts essentially on T_{4B} deiodation in T_{3B}, as shown by both *when* and *boost* statements of $TH_{activation}$.

The metabolism of TH is mainly provided by D_3 and Detox. The rules involving these entities are:
$D3_{synth} : \Omega \Rightarrow \mathrm{D_3}$ $\;when(\mathrm{T_{3B}} > \varepsilon)$ $\;boost(\mathrm{T_{3B}} = \theta)$
$D3_{destr} : \mathrm{D_3} \Rightarrow \Omega$ $\;when(\mathrm{T_{3B}} = \varepsilon \vee (\mathrm{D_3} = \theta \wedge \mathrm{T_{3B}} < \theta))$
$Detox_{synth} : \Omega \Rightarrow \mathrm{Detox}$ $\;boost(\mathrm{X_{Hep}} > \varepsilon)$
$Detox_{destr} : \mathrm{Detox} \Rightarrow \Omega$ $\;when(\mathrm{Detox} = \theta \wedge \mathrm{X_{Hep}} = \varepsilon)$
$T3_{destr} : \mathrm{T_{3B}} \Rightarrow \Omega$ $\;when(\mathrm{D_3} = \theta \vee \mathrm{Detox} = \theta \vee (\mathrm{T_{3B}} = \theta \wedge \mathrm{D_3} > \varepsilon))$
$T4_{destr} : \mathrm{T_{4B}} \Rightarrow \Omega$ $\;when(\mathrm{D_3} = \theta \vee \mathrm{Detox} = \theta \vee (\mathrm{T_{4B}} = \theta \wedge \mathrm{D_3} > \varepsilon))$

The regulation of D_3 levels is symmetrical to D_2 regulation, as T_{3B} is an inducer of D_3 levels. The case of Detoxis interesting: since we are only interested in an excessive activity of the hepatic detoxifying enzymes, the set of admissible levels of this entity is $\{\Delta, \theta\}$. An excess of Detox is only triggered by the presence of X_{Hep}, as seen in $Detox_{synth}$ and $Detox_{destr}$.

Furthermore, the *when* statements of rules $T3_{destr}$ and $T4_{destr}$ reflects two important notions on the metabolism of T_{3B} and T_{4B}:

1. An excess of D_3 or Detox is enough to decrease the levels of both TH.
2. If TH are in excess, the presence of D_3 is enough to restore normal TH levels. It is capital to note that when both levels of D_3 and TH are normal, D_3 does not trigger the decrease of TH levels.

The set of all these rules allows to generate the system dynamics. It is then possible to constrain these dynamics thanks to biological observations expressed through SE-LTL properties. For instance, we express the fact that $TH_{activation}$ always primes on $I_{recycling}$ as long as there is no shortage of blood iodide:

$$\varphi_0 \equiv G((\mathrm{I_B} > \varepsilon \wedge app(TH_{activation}) \wedge app(I_{recycling})) \rightarrow \neg I_{recycling})$$

Literally, φ_0 means that if I_B is present in the system, and both $TH_{activation}$ and $I_{recycling}$ are applicable, then $I_{recycling}$ does not apply. The G operator indicates that the property must be satisfied at every step of the path.

It has also been observed that an excess of detoxifying enzymes quickly leads to the depletion of T_{4B}:

$$\varphi_1 \equiv G((\mathrm{Detox} = \theta \wedge app(T4_{destr})) \rightarrow T4_{destr})$$

We can also check that the model verifies global biological properties such as the fact that without any disrupter, an hypothyroidic state (*i.e.* where T_{4B} is lacking) leads to a state where TSH is in excessive concentration:

$$\varphi_2 \equiv G((\mathrm{T_{4B}} = \varepsilon \wedge \mathrm{X_{D2}} = \varepsilon) \rightarrow F(\mathrm{TSH} = \theta))$$

Of course, several paths belonging to R_{thy} do not satisfy the previous properties. For instance, let π_1 and π_2 the portions of path represented in Figure 2. In state η_{12} of π_1, the conditions of φ_0 are satisfied and $I_{recycling}$ should not be applied. Therefore, π_1 does not satisfy φ_0, contrary to π_2.

As such, if we consider $Ax_{thy} = \{\varphi_0, \varphi_1, \varphi_2\}$ and the constrained network $N_{\mathrm{thy}} = (R_{\mathrm{thy}}, Ax_{thy})$, paths including π_1 do not belong to N_{thy}.

Finally, we can use the constrained network N_{thy} to search for existing pathways of toxicity. Indeed, possible disruptions described in Section 2 correspond

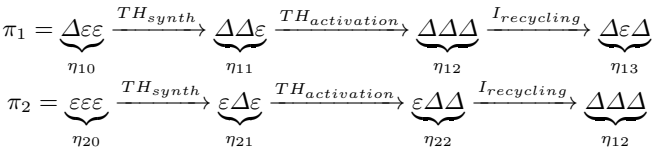

Fig. 2. Possible portions of paths belonging to R_{thy}. For the sake of simplicity, states depicted here only contain the levels of respectively I_B, $\text{T}_{4\text{B}}$ and $\text{T}_{3\text{B}}$.

to sets of paths belonging to N_{thy}. These sets of paths can be identified thanks to temporal formulas. For example, the inactivation of D_2 by X_{D2} leading to hyperthyroidism corresponds to paths satisfying φ_{D2}:
$\varphi_{\text{D2}} \equiv G((\text{X}_{\text{D2}} > \varepsilon) \rightarrow F(\text{D}_2 = \varepsilon) \wedge F(\text{T}_{3\text{Pit}} = \varepsilon) \wedge F(\text{TSH} = \theta) \wedge F(\text{T}_{4\text{B}} = \theta))$

The effect of X_{Hep}, namely the trigger of hepatic detoxifying enzymes leading to decreased levels of $\text{T}_{4\text{B}}$ and then high levels of TSH can also be expressed thanks to φ_{Hep}:

$$\varphi_{\text{Hep}} \equiv G((\text{X}_{\text{Hep}} > \varepsilon) \rightarrow F(\text{Detox} = \theta) \wedge F(\text{T}_{4\text{B}} = \varepsilon) \wedge F(\text{TSH} = \theta))$$

6 Conclusion

We presented a new formal framework able to handle several specificities of the toxicology domain not taken into account so far. This rule-based modeling framework allows for a direct description of equilibrium changes happening in a biological system. This description does not model the speed differences between equilibrium changes, which can affect the global behavior of the system. For this reason, we integrated biological and toxicological knowledge about equilibria kinetics through formulas expressed in SE-LTL.

As demonstrated on a simple model of the thyroid hormone system, its expressive power allows us to describe equilibrium changes in the biological system as well as knowledge about equilibrium kinetics. This knowledge is then used to filter out irrelevant paths from the initial rule-based model.

In the future, our formalism will be coupled with a SE-LTL model checker in order to generate a comprehensive list of the most probable toxicity pathways present in a model. Indeed, it is possible to define pathological states and enumerate the paths leading to these states. These paths shall then be sorted thanks to additional toxicological knowledge regarding their plausibility. Furthermore, filtering the resulting paths could also highlight gaps in the current toxicological knowledge and help toxicologists in their design of new experimental strategies.

Finally, as this formalism is now well-defined, it 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 near future, the platform will also be able to integrate the temporal formulas and to use these biological constraints to filter out irrelevant paths. This will be achieved by generating all the paths allowed by a biological

action network while checking these paths for their biological relevance. Finally, by defining states regarded as pathologic, the platform will then be able to compute all the paths leading to pathologic states and propose putative pathways of toxicity.

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Appendix

Entity	Biological name	Admissible levels
$\mathrm{I_B}$	blood iodide	$\{\varepsilon, \Delta\}$
$\mathrm{I_T}$	thyroid iodide	$\{\varepsilon, \Delta\}$
TPO	thyroid peroxydase	$\{\varepsilon, \Delta, \theta\}$
$\mathrm{T_{3B}}$	blood triiodothyronine	$\{\varepsilon, \Delta, \theta\}$
$\mathrm{T_{4B}}$	blood tetraiodothyronine	$\{\varepsilon, \Delta, \theta\}$
$\mathrm{T_{3Pit}}$	pituitary triiodothyronine	$\{\varepsilon, \Delta, \theta\}$
TSH	thyroid-stimulating hormone	$\{\varepsilon, \Delta, \theta\}$
$\mathrm{D_1}$	type 1 deiodinase	$\{\varepsilon, \Delta, \theta\}$
$\mathrm{D_2}$	type 2 deiodinase	$\{\varepsilon, \Delta, \theta\}$
$\mathrm{D_3}$	type 3 deiodinase	$\{\varepsilon, \Delta, \theta\}$
Detox	hepatic detoxifying enzymes	$\{\Delta, \theta\}$
$\mathrm{X_I}$	iodide transporter inactivator	$\{\varepsilon, \Delta\}$
$\mathrm{X_{D1}}$	$\mathrm{D_1}$ inactivator	$\{\varepsilon, \Delta\}$
$\mathrm{X_{D2}}$	$\mathrm{D_2}$ inactivator	$\{\varepsilon, \Delta\}$
$\mathrm{X_{Hep}}$	detoxifying enzymes inducer	$\{\varepsilon, \Delta\}$

Table 1. The signature of $\mathcal{E}_{\mathrm{thy}}$, including the different set of admissible levels.

I_{intake}	: Ω	$\Rightarrow$	$\mathrm{I_B}$	
$I_{transfer}$	: $\mathrm{I_B}$	$\Rightarrow$	$\mathrm{I_T}$	$when(\mathrm{TSH} > \varepsilon \wedge \mathrm{X_I} = \varepsilon)$
TPO_{synth}	: Ω	$\Rightarrow$	TPO	$when(\mathrm{TSH} > \varepsilon) \quad boost(\mathrm{TSH} = \theta)$
TPO_{destr}	: TPO	$\Rightarrow$	Ω	$when(\mathrm{TSH} = \varepsilon \vee (\mathrm{TPO} = \theta \wedge \mathrm{TSH} < \theta))$
TH_{synth}	: $\mathrm{I_T}$	$\Rightarrow$	$\mathrm{T_{3B}} + \mathrm{T_{4B}}$	$when(\mathrm{TPO} > \varepsilon) \quad boost(\mathrm{TPO} = \theta)$
Pit_{synth}	: $\mathrm{T_{4B}}$	$\Rightarrow$	$\mathrm{T_{3Pit}}$	$when(\mathrm{D_2} > \varepsilon) \quad boost(\mathrm{D_2} = \theta)$
Pit_{destr}	: $\mathrm{T_{3Pit}}$	$\Rightarrow$	Ω	$when(\mathrm{D_2} = \varepsilon \vee (\mathrm{T_{3Pit}} = \theta \wedge \mathrm{D_2} < \theta))$
TSH_{synth}	: Ω	$\Rightarrow$	TSH	$when(\mathrm{T_{3Pit}} < \theta) \quad boost(\mathrm{T_{3Pit}} = \varepsilon)$
TSH_{destr}	: TSH	$\Rightarrow$	Ω	$when(\mathrm{T_{3Pit}} = \theta \vee (\mathrm{TSH} = \theta \wedge \mathrm{T_{3Pit}} > \varepsilon))$
$D1_{synth}$	: Ω	$\Rightarrow$	$\mathrm{D_1}$	$when(\mathrm{X_{D1}} = \varepsilon) \quad boost(\mathrm{T_{3B}} = \varepsilon)$
$D1_{destr}$	: $\mathrm{D_1}$	$\Rightarrow$	Ω	$when(\mathrm{X_{D1}} > \varepsilon \vee (\mathrm{D_1} = \theta \wedge \mathrm{T_{3B}} > \varepsilon))$
$D2_{synth}$	: Ω	$\Rightarrow$	$\mathrm{D_2}$	$when(\mathrm{T_{3B}} < \theta \wedge \mathrm{X_{D2}} = \varepsilon) \quad boost(\mathrm{T_{3B}} = \varepsilon)$
$D2_{destr}$	: $\mathrm{D_2}$	$\Rightarrow$	Ω	$when(\mathrm{T_{3B}} = \theta \vee \mathrm{X_{D2}} > \varepsilon \vee (\mathrm{D_2} = \theta \wedge \mathrm{T_{3B}} > \varepsilon))$
$D3_{synth}$	: Ω	$\Rightarrow$	$\mathrm{D_3}$	$when(\mathrm{T_{3B}} > \varepsilon) \quad boost(\mathrm{T_{3B}} = \theta)$
$D3_{destr}$	: $\mathrm{D_3}$	$\Rightarrow$	Ω	$when(\mathrm{T_{3B}} = \varepsilon \vee (\mathrm{D_3} = \theta \wedge \mathrm{T_{3B}} < \theta))$
$Detox_{synth}$	: Ω	$\Rightarrow$	Detox	$boost(\mathrm{X_{Hep}} > \varepsilon)$
$Detox_{destr}$	: Detox	$\Rightarrow$	Ω	$when(\mathrm{Detox} = \theta \wedge \mathrm{X_{Hep}} = \varepsilon)$
$I_{recycling}$	: $\mathrm{T_{4B}}$	$\Rightarrow$	$\mathrm{I_B}$	$when(\mathrm{D_1} > \varepsilon)$
$TH_{activation}$	: $\mathrm{T_{4B}}$	$\Rightarrow$	$\mathrm{T_{3B}}$	$when(\mathrm{D_1} = \theta \vee \mathrm{D_2} > \varepsilon) \quad boost(\mathrm{D_2} = \theta)$
$T3_{destr}$	: $\mathrm{T_{3B}}$	$\Rightarrow$	Ω	$when(\mathrm{D_3} = \theta \vee \mathrm{Detox} = \theta \vee (\mathrm{T_{3B}} = \theta \wedge \mathrm{D_3} > \varepsilon))$
$T4_{destr}$	: $\mathrm{T_{4B}}$	$\Rightarrow$	Ω	$when(\mathrm{D_3} = \theta \vee \mathrm{Detox} = \theta \vee (\mathrm{T_{4B}} = \theta \wedge \mathrm{D_3} > \varepsilon))$

Table 2. The R_{thy} action network.