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► To cite this version:

Frédéric Y. Bois. Physiologically based modeling and prediction of drug interactions. Basic & Clinical Pharmacology & Toxicology, 2010, 106 (3), pp.154-161. <10.1111/j.1742-7843.2009.00488.x>. <ineris-00961746>

HAL Id: ineris-00961746

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Submitted on 20 Mar 2014

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Physiologically-Based Modelling and Prediction of Drug Interactions

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Running title: PBPK Modelling of Drug Interactions

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Abstract

A major challenge for drug development and environmental or occupational health is the prediction of pharmacokinetic and pharmacodynamic interactions between drugs, natural chemicals or environmental contaminants. This article reviews briefly past developments in the area of physiologically-based pharmacokinetic (PBPK) modelling of interactions. It also demonstrates a systems biology approach to the question, and the capabilities of new software tools to facilitate that development. Individual SBML models of metabolic pathways can now be automatically merged and coupled to a template PBPK pharmacokinetic model, using for example the GNU MCSim software. The global model generated is very efficient and able to simulate the interactions between a theoretically unlimited number of substances. Development time and the number of model parameter increase only linearly with the number of substances considered, even though the number of possible interactions increases exponentially.

Introduction

The mathematical modelling of interactions between drugs or toxicants is essentially motivated by a search for efficiency. While assessing or predicting the toxicity of single substances is difficult enough, considering them by pairs, triplets, *etc.* is daunting. Even though much advances have been made on *in vitro* systems [1-3], the experimental route suffers the "curse of dimensionality": Beyond pairs of interacting substances, factorial experimental designs of the order of size 2^n are required to assess simply the sign and order of interactions between n drugs or environmental stressors [4]. Modelling, with the aid of computers, has the potential of being much faster, provided that adequate models are available. In that case, "adequate" does not only mean "correctly fitting the data", but also "correctly predicting future observations", a much stricter requirement which early on oriented research toward mechanistic models. The domain of validity of a mechanistic model is conditioned by its structure and parametric assumptions, while the same domain for empirical (data-fitting) models is restricted to the range of the available data.

Therefore, this short review will leave aside empirical statistical models, such as population models [5], or semi-empirical dose-response models [6], however interesting they are. We will not review either qualitative or semi-qualitative data-mining approaches [7, 8], even though they can be a useful first screen. If we simply examine mechanistic models, two classes emerge: A first group of mechanistic models aims at predicting interactions at the enzyme or receptor level, through molecular modelling or QSAR approaches, or a combination of both [9-11]. A second group of models, which will be the subject of this review,

integrates pharmacokinetic and enzyme or receptor pathways modelling. As this "physiological" or "systems biology" approach is the most comprehensive, it tends to couple with advances and tools or concepts from the above empirical and QSAR models [12]. To further reduce the scope of this paper without losing generality, while still capturing some well-known sources of interactions among chemical substances, we will focus here on metabolic interactions. Those occur because a relatively limited number of enzyme species process a majority of xenobiotics and endogenous chemicals. For any of those reactions, the number of enzyme molecules is itself limited, leading to predictable bottlenecks in the detoxification or, conversely, in the activation of chemicals [3, 8].

We mentioned above that experimental methods suffer from the curse of dimensionality, but to be fair modelling also faces the same problem. However, new solutions are emerging from pathways' modelling, which is proceeding at a fast pace (see <http://systems-biology.org>) [13]. Although quite complex, pathways' modelling can actually reduce the dimensionality of the problem, as we will see in the following.

Physiologically based pharmacokinetic (PBPK) models

PBPK models are increasingly used in drug development and regulatory toxicology to predict the kinetics and metabolism of substances in the body [14-19]. In these models, the body is represented by a set of compartments corresponding to specific organs or tissues, and the transfers or transports of drugs are dictated by various physiological flows (blood, bile, pulmonary ventilation, *etc*) [14]. A system of differential equations can be written, with parameters representing blood flow rates, organ volumes *etc.*, for which information is available in the

published scientific literature or may be obtainable *in vitro*. Numerical integration of that differential system computes the quantity and concentration of the drug considered in each compartment, as a function of time and exposure dose. Indeed, such a description of the body is approximate, if not rough, but a balance has to be found between precision (and therefore complexity) and simplicity (ease of use).

The first report of a PBPK model able to describe simultaneously administration, kinetics and metabolic interactions was for warfarin and bromosulfophthalein [20]. The application to drug-drug interactions did not really take on at that time, in part because PBPK model parameterisation was difficult and did not adhere to the standards of statistical model fitting that was imposed to pharmacokinetic data analysis.

Interest in PBPK modelling then shifted to the regulatory toxicology arena, where data were scarce and predictive modelling (for low dose or inter-species extrapolations) much needed [21]. That shift led to sporadic applications of PBPK models to interactions between pairs of substances [22-29]. For reviews of the state of the art at that time, see [30, 31]. The study of more complex mixtures, up to the five-component mixture of dichloromethane, benzene, toluene, ethylbenzene, and xylene was an interesting advance [32, 33]. But, the exercise is quite painstaking and was not extended significantly afterward.

The beginning of this century witnessed a renewed interest in PBPK modelling in the context of drug development, including for drug-drug interactions [34-36], albeit without much awareness of the work being pursued in environmental toxicology [37-40]. However, this is changing, but still, even recent publications focus primarily on binary drug-drug interactions [41-43].

Systems biology pathways modelling

A problem faced by all the above investigators was to efficiently describe the cascades of reactions for the set of substances considered, in addition to the eventual transport and elimination of the metabolites. Assume that we describe each metabolic reaction by a set of four differential equations (for the formation of the enzyme-substrate complex and its dissociation into enzyme plus product), for 50 substances leading to 50 metabolites, all of them distributed primarily in 10 organs or tissues of the body. The corresponding model would consist in about 1200 equations and at least a thousand parameters. The difficulty or even the impossibility to manually develop and check such a model is obvious.

Dealing with a large number of biological reactions is a problem faced daily by systems biologists. To solve it, a set of tools has been developed, and in particular the Systems Biology Markup Language (SBML), a conventional syntax for representing biochemical pathway models [44]. SBML can code for models of metabolism, signalling, transcription *etc.* An international community of software developers and users maintains SBML since the mid-2000 (*cf.* <http://sbml.org>).

SBML defines a uniform symbolic coding system that enumerates reactants, products, parameters, reactions, their formulae *etc.*, needed to define and simulate unambiguously the specified reactions. The model representation is symbolic and does not directly refer to a set of differential equations or any specific mathematical representation. That facilitates the interpretation of the model, and its automatic translation and integration into diverse software. SBML is *de facto* becoming a standard for model specification and exchange in systems biology [45].

Automated coupling of PBPK and systems biology modelling

To be able to deal with more complex mixtures, streamlining the process and taking advantage of the emerging tools of systems biology is a research avenue worth exploring. However, that exercise is still difficult when starting from a Henry-Michaelis-Menten description of metabolic reactions, because the number of parameters needed increases with the square of the number substances considered. In those conditions, modelling is not much more advantageous than experiments.

The challenge is to efficiently describe together cascades of reactions and the eventual transport and elimination of intermediate and final metabolites. The associated model can be very complex, even if limited to pharmacokinetic and metabolic interactions, because the transport of each metabolite requires a set of differential equation of its own. The main steps of a newly proposed "divide and conquer" approach [46] are:

- Develop for each substance and each of its metabolic reaction an SBML-coded model. It is necessary at this step to avoid simplifications of the Henry-Michaelis-Menten type, and to account for the balance of the various quantities of enzymes and enzyme-substrate complex involved, hence using the individual reaction micro-constants, rather than aggregated Henry-Michaelis-Menten constants.
- Define a general PBPK model template, applicable to all the substances considered (for example, the model shown on Figure 1). Such a model is needed to describe the relationship between personal exposure and internal concentrations at the site metabolism, which condition the intensity of interactions between substances;

- Automatically build a global model of transport and metabolism of all the substances in the body, on the basis of the previously defined elements.
 - Use that model to perform predictive simulations of substances' interactions.
- The global model can be used to study the behaviour of complex mixtures, representative of the reality of human population exposures.

The above steps can be performed with the help of version 5.3.1 of the GNU MCSim software. (<http://www.gnu.org/software/mcsim>) [47, 48]. GNU MCSim can use a PBPK model as a template for the description of the pharmacokinetics of each drug (and metabolites) involved in a reaction network. The template equations are copied for each chemical species and aggregated to form a large system of differential equations, able to describe the simultaneous pharmacokinetics and interactions of all the substances considered. Physiological parameters (for example the liver volume) keep the same value for all chemical species (see Table 1), but other parameter values may differ between chemical species. GNU MCSim recognises compartments defined in SBML models, maps them to the PBPK template compartments, and automatically locates the specified biochemical reactions in the correct organs. The substances placed (in SBML) outside of any physiological compartment are automatically distributed in all the compartments of the template PBPK model.

Analysis of a PBPK-coupled metabolic network

To illustrate the automated approach just described, and to explore the interaction properties of a sufficiently complex network, a set of primary metabolic reactions were simulated for a list of 50 hypothetical parent chemicals. For each of those, one or two metabolic reactions involving one of 10 possible enzymes were

randomly generated, each with equal probability. The differential equations corresponding to a given substrate (S) and enzyme (E) pair are:

$$\left\{ \begin{array}{l} \frac{dS}{dt} = -K_1 \times S \times E + K_2 \times ES \\ \frac{dE}{dt} = -K_1 \times S \times E + K_2 \times ES - K_3 \times ES \\ \frac{dES}{dt} = K_1 \times S \times E - K_2 \times ES + K_3 \times ES \\ \frac{dP}{dt} = K_3 \times ES \end{array} \right. \quad (1)$$

where ES represents the transient enzyme-substrate complex, P the reaction product, and the K_i the reactions' micro-constants. The product species were randomly drawn among the 50 parents (thereby generating cross-linked reactions) plus another 150 other species (purely terminal products). In addition 5 parent species (S18 to S20) were supposed to induce an increased synthesis of enzymes 7 and 9, according to the following equations:

$$\frac{dE}{dt} = K_4 \times E + K_5 \times S \quad (2)$$

The random draws mentioned above were all from discrete uniform distributions. The enzymes and enzyme-substrate complexes were supposed to stay local to the liver. Parent species and metabolites were defined as circulating, and therefore distributed according to the generic PBPK model of Figure 1. The metabolic network generated by that procedure is presented graphically in Figure 2. The network is relatively complex and highly interconnected, like natural metabolic networks can be. That network was used as a basis for all the following simulations and results.

For simplicity, and without loss of generality, all substances were supposed to be non-volatile and excreted by the kidney, in addition to being eventually metabolised. Substances S1 to S20 (group *A*) were supposed to be natural chemicals, such as those present in food (phenols, *etc.*), absorbed daily at relatively high dose. Substances S21 to S50 (group *B*) were drugs absorbed at lower rate. The group *C* substances, with numbers between 51 and 200, correspond to terminal metabolites. There are only 46 of those metabolites because the random drawing of substances for possible reactions did not select all of the 150 possible choices.

The PBPK model template shown in Figure 1 was used to describe the kinetics of group *A*, *B* and *C* substances in the human body. It corresponds to four differential equations (the lung exchange kinetics are modelled with a simple algebraic equation under an instantaneous equilibrium hypothesis) and ten physiological parameters (see Table 1). The template model does not include the metabolic reactions affecting the substances to which the body is exposed, those were automatically generated, using equations 1 and 2 above, for all reactions of the network. The substance-specific parameters of the PBPK template (partition coefficients, urinary elimination clearance) and the metabolic rate constants were set to the arbitrary values given in Table 2.

GNU MCSim version 5.3.1 (<http://www.gnu.org/software/mcsim>) was used to generate and simulate the global interaction model. That model had 466 state variables and differential equations for the quantities of parent species and metabolites in the various compartments of the PBPK model, and quantities of enzymes and enzyme-substrate complexes in the liver, and 818 parameters.

CellDesigner 4.0.1 was used to generate Figure 2. The other graphs were plotted with Kaleidagraph v4.02 and R v2.8.1.

A first step is to evaluate the "extent" of the possible interactions between substances of the network presented on Figure 2. In that example case, every substance affects the metabolism of any other one either directly or indirectly (there is a path connecting every two nodes of Figure 2). Yet, the previous statement is qualitative and solely based on the structure of the metabolic network. In reality, to paraphrase Paracelsus, the dose makes the interaction. If we turn to quantitative analyses, Figure 3 shows the simulated time-course of venous concentrations of all substances during the co-administration of groups *A* and *B* substances. These results were obtained by numerical integration of the global model for joint pharmacokinetics and metabolism of the substances. Parameters were set the values given in Tables 1 and 2. The initial quantities of substances in the body were set to zero, except for the enzymes, set at an arbitrary initial liver concentration of 1 mM each. Ingestion rates per minute for group *A* and *B* substances were set at a high value (0,1 mM) for 4 hours and to zero afterward. Since the parameter values for all substances were the same in that simulation, the noticeable differences observed on Figure 3 between substances are entirely due to the cascading network structure and to interaction effects. The global model is able to predict the kinetics of parent and daughter species in any compartment of the associated PBPK model for any exposure schedule. Such a simulation only takes a fraction of second on an i686processor.

To have an overview of the magnitude of the possible interactions, it is possible to compute an "interaction index" for each chemical species. For a given species, we define that index as the ratio of the concentration resulting from a

joint exposure to groups *A* and *B* substances over the sum of the concentrations resulting from *separate* exposures to the same substances, at the same dose. In the absence of interactions the index value is 1, it is superior to 1 if the joint exposure leads to a disproportionate increase in the concentration of the substance considered, and lower than 1 otherwise. Figure 4 gives that index for all species, as a function of the ingestion rate of substances of groups *B* (the same rate was used for all substances of that group), 24 hours after the start of 4-hour exposures. Substances of group *A* were administered at the rate of 0.1 mM/min. During exposure to the mixture, the internal concentrations of groups *A* and *B* substances were in general increased (we will call that "synergy"). On the contrary, metabolites (group *C*) had reduced concentrations on average (an effect we will term "antagonism"). Synergy (which would correspond to an increased toxicity if the substances were toxic) was observed in 60% of the cases. The concentration increase at 24 h, due to interactions, reached a factor 7 (substance S6). Antagonism (by inhibition of metabolite formation) lowered concentrations by a factor 2 at most (for S39, metabolite of S20). At lower doses of group *B* substances, interaction effects dampened. Maximal synergies went down to a factor 2 at most (still for S6). Antagonisms reached at most a factor 1,5 (for S59 and S38), but interactions with group *A* substances (which are supposed to represent natural substrates, ingested at relatively high doses) were still observed, so that interactions indices did not return to 1. Note that for a few substances, the sign of the interaction changed with dose.

The above results were obtained with a model whose metabolic parameter values were all set to the arbitrary values given in Table 2. They are simply results of a stylised case study, aimed at exploring the possible extent of interactions and

their behaviour in a realistically large metabolic network. In the real world, the parameters characterising each drug or substance might be very different and exposure levels and routes will be highly variable. To simulate such a variability, it is possible to perform Monte-Carlo simulations [49], by randomly sampling parameter values. Besides, the metabolic network studied here is only a plausible example and our results depend on it. A specific real world network would probably respond differently, and this should be the subject of specific investigations.

Advantages of a micro-constants approach

The potential complexity of interaction effects between drugs, food constituents, and environmental chemicals, is an open problem since many decades. From a sheer mathematical point of view it is clear that combinatorial approaches (*e.g.*, exploring all possible pairs, triplets *etc.*), be they experimental, statistical or mechanism-based, are impractical as soon as the complexity of the mixtures considered exceeds a small handful of substances. Modelling approaches do also suffer that "curse of dimension" unless they decompose the problem and take advantage of their power for synthesis. Our approach tends toward that goal and takes advantage of recent advances in bioinformatics and biomathematics at three levels: Automatic reconstruction of a metabolic network on the basis of individual reactions; Automatic coupling to an ADME prediction model; Fewer parameters through the use of reaction micro-constants (instead of the Henry-Michaelis-Menten approximations).

To illustrate the latest point, crucial to the feasibility of the proposed approach, Figure 5 shows the number of model parameters required by various approaches as a function of the number of interacting substances considered. When using

reaction micro-constants, the number of parameters required increases only linearly. The use Michaelis-Menten inhibition constants imposes at least a quadratic increase (when dealing only with pairs of chemicals, that is with order two interactions) and quickly becomes prohibitive.

The above advantages translate in feasibility for large networks of substances, ease of development and rapidity. The development of the template PBPK model of Figure 1 took about an hour and that of SBML models about three hours (using JDesigner version 2.1c). The compilation of all SBML models with the PBPK template took about a second. Using the global model and the GNU MCSim software, 10000 stochastic simulations can be run in 6 minutes on a i686 processor under GNU/Linux. The analysis and interpretation of the results take much longer.

However, to be fully operational, the systems biology approach presented here still requires gathering qualitative and quantitative data on the individual metabolism of the target substances. In that context, going back to experimentation becomes useful, with the advantage of being better targeted and therefore more efficient. In particular, the reaction micro-constant required (K_1 , K_2 and K_3 , in Table 2) are seldom available in the published literature, as everyone got used to Michaelian V_{max} , K_m , and K_i constants (which suffice when considering simple binary interactions). However, at least for some enzymes, simple modifications of standard activity measurements allow an easy determination of the micro-constants *in vitro* (work in progress in our laboratory.) We are also exploring quantum chemistry predictions of those micro-constants.

Perspectives

The *ab initio* modelling of metabolic interactions in cocktails of drugs or substances is feasible and even promising. Computing can be fast and the

development of the model is limited to that of individual drug sub-models, reusable each time that that substance is to be included in a mixture. The latest developments of the systems biology approach presented here requires slightly different parameters than the Henry-Michaelis-Menten constants usually available, and its application to concrete cases will require a specific experimental effort, probably worth the investment on a medium to long term.

This system biology approach can obviously be extended to the description of pharmacodynamic interactions arising from signalling and regulation cascades. However, while that approach gives access to the study of the behaviour of complex networks, the number of such networks and of realistic mixtures of drugs, food-borne chemicals and environmental contaminants is extremely large. The future in this area, with the new tools available, may well lie in formulating general and robust statements about complex mixtures, with specific investigations of a few very relevant ones.

Acknowledgements

I thank Karen Watanabe and Stefanie Stöckl for helpful discussions. This work was supported by the European Commission, 6th FP Priority 6, project 2-FUN [contract #036976] and the French MEEDDAD (PRG189_08_DRC03).

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Tables

Table 1: Physiological parameter values for the PBPK model shown in Figure 1.

Parameter ^(a)	Symbol	Value
Total blood flow	F_t	6.4
Fractional blood flows		
Fat	f_f	0.09
Liver	f_l	0.24
Muscle and skin	f_m	0.18
Viscera	f_v	— ^(b)
Volumes ^(c)		
Total body volume	V_t	75
Fat	V_f	18
Liver	V_l	1.7
Muscle and skin	V_m	— ^(d)
Viscera	V_v	6

^(a) Units: volumes are in litres, total blood flow in litres/min.

^(b) The fraction of blood flow to the viscera is computed by difference between 1 and the sum of the fractional flows to the other compartments.

^(c) Those volumes correspond to those of a 30 years old woman [50].

^(d) The volume of the muscle and skin compartment is computed by difference between the total body volume, 10% of it (corresponding to bones) and the other volumes.

Table 2: Values of the substance-specific non-physiological parameters.

Parameter ^(a)	Symbol	Value
Tissue over blood partition coefficients		
Fat	P_f	1
Liver	Pl_f	1
Muscle and skin	P_m	1
Viscera	P_v	1
Metabolic rate constants		
$E + S \rightarrow ES$ reaction	K_1	50
$ES \rightarrow E + S$ reaction	K_2	50
$ES \rightarrow E + P$ reaction	K_3	0.1
Enzyme turn-over parameters		
Base rate	K_4	0.1
Induction rate constant	K_5	1
Renal clearance	K_e	0.05

^(a) Units: Partition coefficients are unitless, metabolic rate constants are in min^{-1} and clearance in litres/min.

Figure legends

Figure 1: Graphical representation of a physiologically based pharmacokinetic model of the human body. This model was used here as a template to describe the kinetics and interactions of substances in mixture. Urinary excretion takes place in the kidney, part of viscera. Metabolism is not figured, and is specified in separate Systems Biology Markup Language models. For symbols, see Table 1.

Figure 2: Computer-generated hepatic metabolic network for 50 hypothetical substances. Parent substances (groups A and B, see text) and metabolites (group C) distribute in the body according to the physiologically based pharmacokinetic model of Figure 1 (distribution compartments are not figured, for clarity of the graph.)

Figure 3: Simulated blood kinetics of the group A, B and C substances. The metabolic network is shown on Figure 2. Each parent and metabolites are distributed in the body according to the physiologically based pharmacokinetic model of Figure 1. Panel I: concentration of the groups A and B substances; panel II: group C of pure metabolites. All parent substances were administered together. Differences between profiles are entirely due to network structure and metabolic interactions.

Figure 4: Interaction indices for the substances of groups A, B and C, as a function of the ingestion rate of group B substances. For a given chemical species, the interaction index is defined as the ratio of the concentration resulting from a *joint* exposure to groups A and B substances over the sum of the

concentrations resulting from *separate* exposures to the same substances, at the same dose. Exposure to all substances was supposed to last for 4 hours, simulated concentrations were recorded after 24 hours.

Figure 5: Number of model parameters required by various approaches as a function of the number of interacting substances considered. Solid line: using reaction micro-constants; short dashes: using Michaelis-Menten inhibition constants for pair of chemicals; long dashes: using Michaelis-Menten higher order inhibition constants for triplets of chemicals.

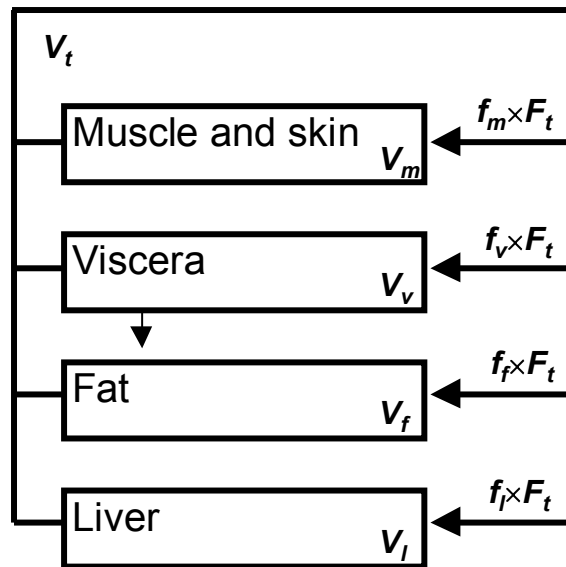


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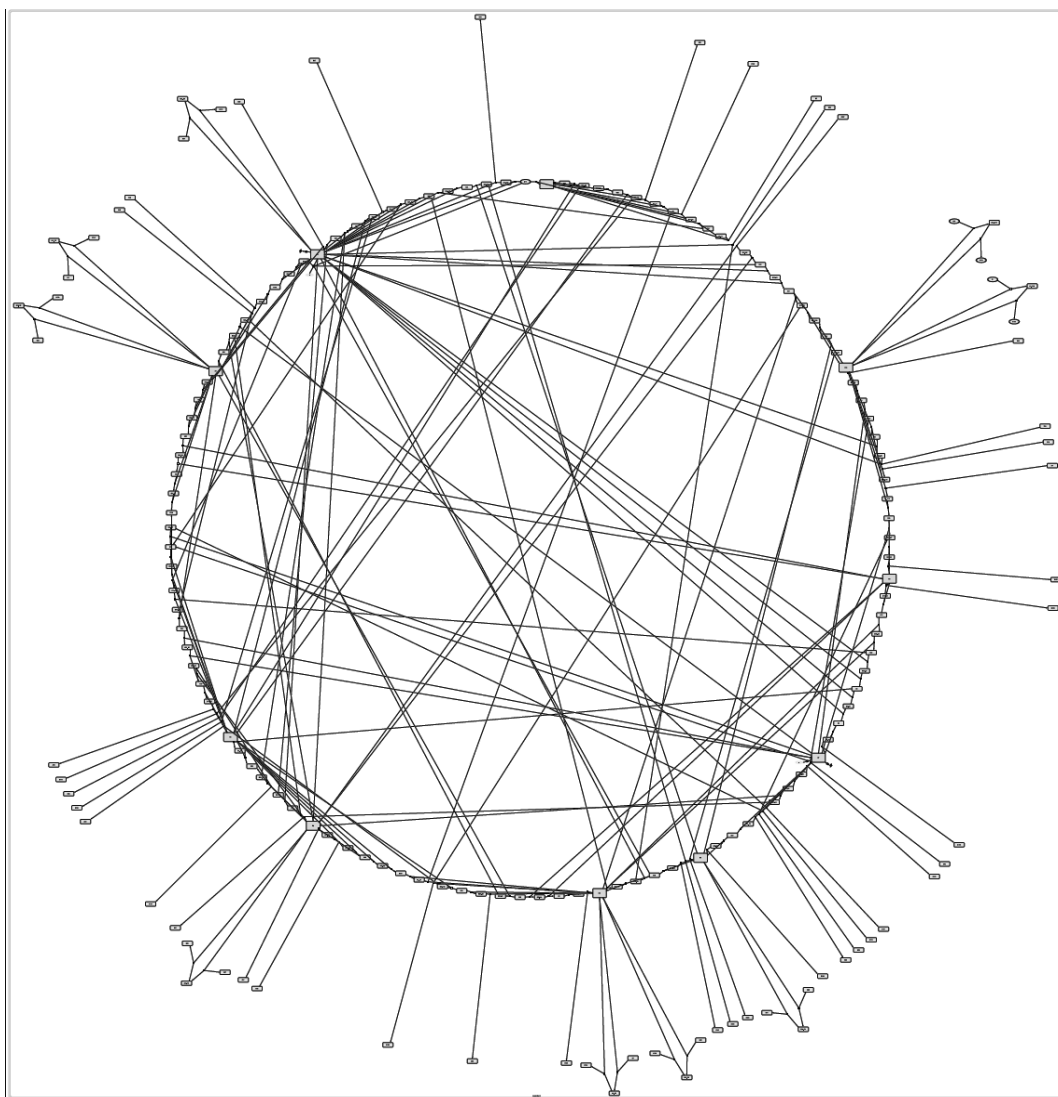


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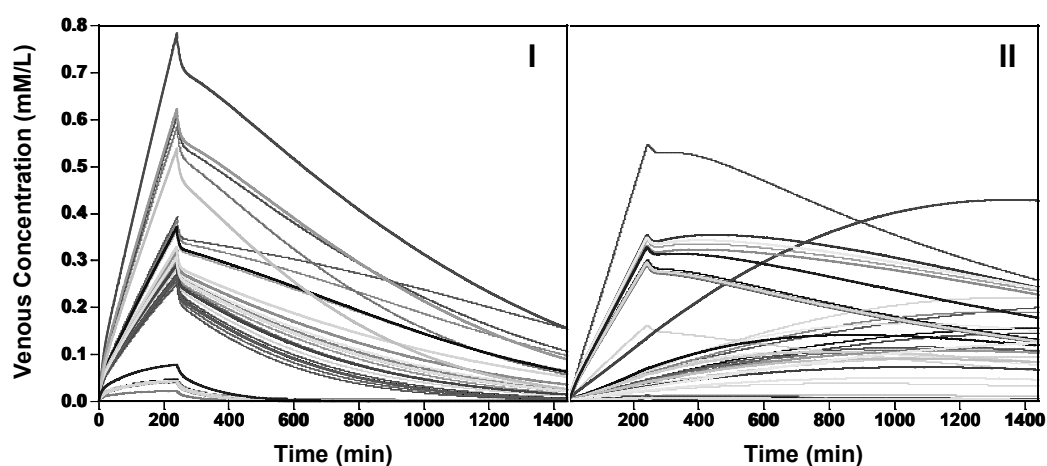


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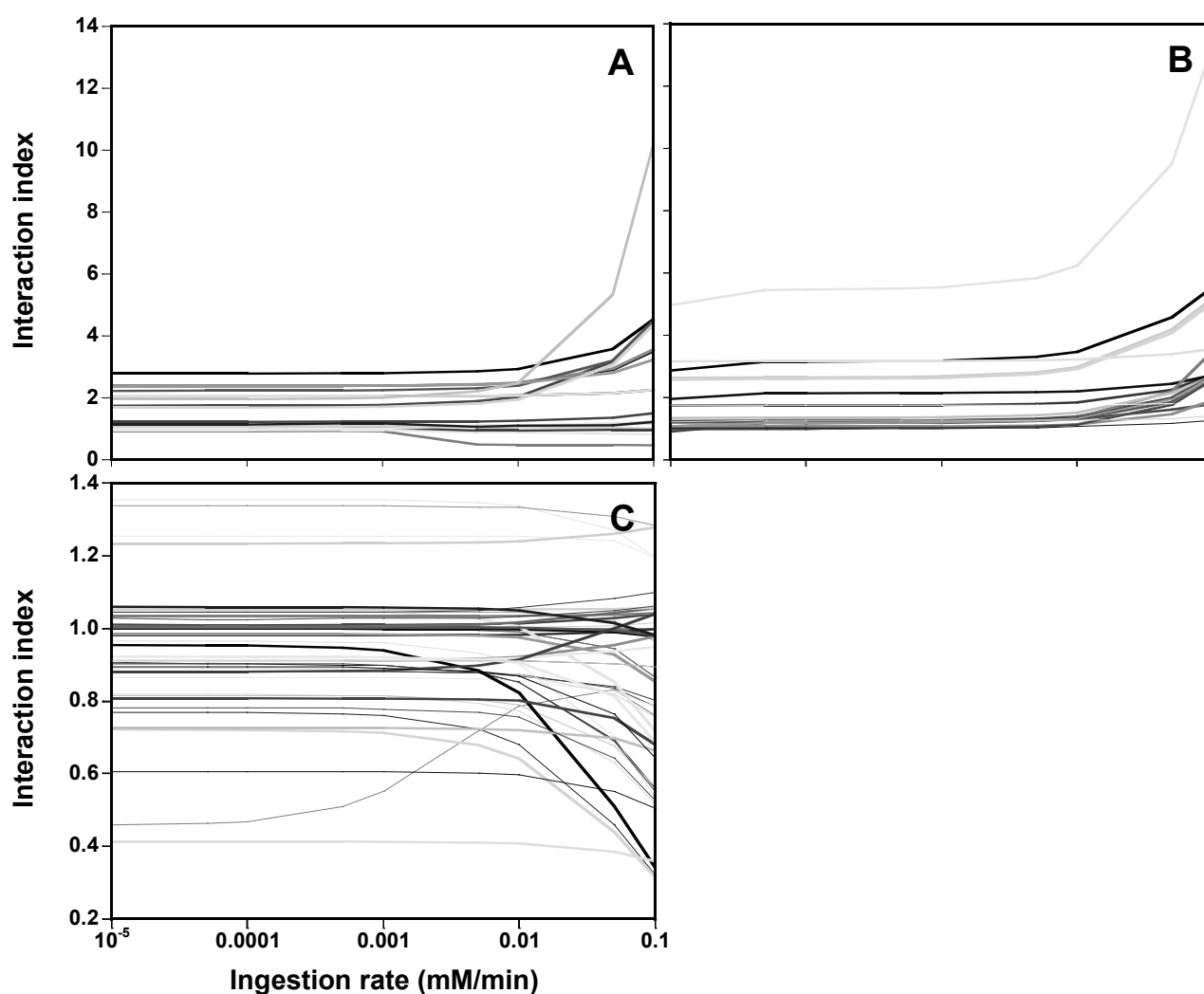


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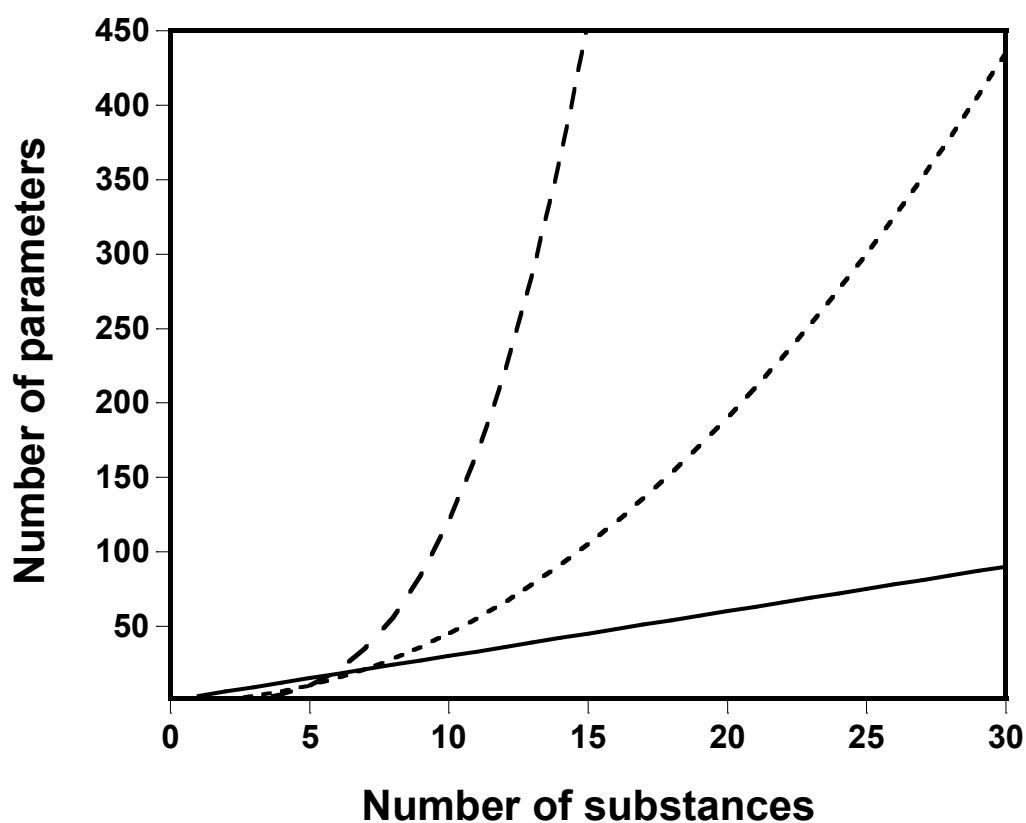


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