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Multiscale modelling approaches for assessing cosmetic ingredients safety

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ABSTRACT

The European Union's ban on animal testing for cosmetic ingredients and products has generated a strong momentum for the development of *in silico* and *in vitro* alternative methods. One of the focus of the COSMOS project was *ab initio* prediction of kinetics and toxic effects through multiscale pharmacokinetic modeling and *in vitro* data integration. In our experience, mathematical or computer modeling and *in vitro* experiments are complementary. We present here a summary of the main models and results obtained within the framework of the project on these topics. A first section presents our work at the organelle and cellular level. We then go toward modeling cell levels effects (monitored continuously), multiscale physiologically based pharmacokinetic and effect models, and route to route extrapolation. We follow with a short presentation of the automated KNIME workflows developed for dissemination and easy use of the models. We end with a discussion of two challenges to the field: our limited ability to deal with massive data and complex computations.
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1. Introduction

The European decision to ban animal testing for cosmetic ingredients has generated a strong momentum for the development of *in silico* and *in vitro* alternative methods. One of the aims of the COSMOS project (funded by the European Commission and by Cosmetics Europe under the 7th Framework Programme) was to develop approaches for the *ab initio* prediction of kinetics and toxic effects through multiscale pharmacokinetic modeling and *in vitro* data integration. We present here a summary of the relevant models and results obtained by the COSMOS team. Our major activities were focused on modeling toxicokinetics and toxicodynamics (effects) *in vitro* and *in vivo*, which are required to perform quantitative *in vitro* to *in vivo* extrapolation (QIVIVE) (Adler et al., 2011; Bessems et al., 2014; Coecke et al., 2012; Quignot et al., 2014).

Kinetic modeling is a relatively well developed field, with well established compartmental or physiologically based pharmacokinetic (PBPK) models (Corley, 2010; Gibaldi and Perrier, 1982; Peters, 2011). In PBPK models, the transport and overall fate of the substance administered is governed by anatomic and physiological considerations. The models can have a generic structure, which makes them easier to use (no need to develop new equations for a new substance) (Beaudouin et al., 2010; Corley, 2010; Jamei et al., 2009; Willmann et al., 2007). Most of their parameters are physiological, meaning that they do not depend on the chemical considered, but solely on the subject exposed (at least when exposure to the chemical does not alter appreciably the body functions, such as blood flows). Compilations of average parameter values for several species are available, and even values for specific sub-groups (children, pregnant women, elderly people . . .) (Bois et al., 2010). The few remaining parameters, which depend on chemical structure, are sufficiently mechanistic to be obtained by quantitative structure-property relationships (QSPR), or *in vitro* experiments (Hamon et al., 2015). *In vitro* kinetic models are different from PBPK models, in that they are not particularly physiological, but rather represent the *in vitro* system modeled. Some generic models have been proposed for simple *in vitro* systems – including by us, the virtual cell based assay (VCBA)

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model, see below – but more complex systems (*e.g.*, bi-compartmental systems, micro-chips) require specific developments (Armitage et al., 2014; Crean et al., 2014; Ouattara et al., 2011; Truisi et al., 2015; Wilmes et al., 2013; Zaldívar et al., 2010).

The state of the art is far less advanced for "extrapolatable" toxicodynamic models. Traditional toxicodynamic models are similar to compartmental models (and in fact extend those with *ad hoc* "effect" compartments). They are data fitted and thus specific for a given experiment or clinical trial (Csajka and Verotta, 2006); they are not designed for extrapolations (except very basic time and dose extrapolations). The equivalent of PBPK models in toxicodynamics are biologically-based models. Among the earliest of such models were the biologically based carcinogenesis models (Armitage, 1985; Moolgavkar and Knudson, 1981). However, given the obscurity and complexity of the cancer process (still not elucidated), those models were at the same time too simple (to avoid criticisms) and too complex (to be used in a regulatory framework). They were never really used for risk assessment, except in the extremely simplified form of the multistage cancer dose-response model (Crump and Howe, 1984). A new generation of models is emerging with "systems biology" models (Geenen et al., 2012; Jusko, 2013) and "physiome" (or virtual human) models (Bassingthwaighte, 2000; Hunter and Borg, 2003). Systems biology models are bottom-up models rooted in biochemistry and benefiting from our increasing understanding of cellular signaling and transcriptional control pathways (facilitated by the explosion of omics data). Physiome models are inherently top-down and multiscale and therefore the closest equivalent to PBPK models (PBPK models can in fact be thought of as vascular body-level solute transport models). They started as high-level descriptions of organ physiology and are increasing their resolution to the cell level (arguably the right level to start understanding the origin of most toxic injuries). Originating from two different research communities (biochemists vs. physiologists) these two approaches are slowly merging as they meet each other at the tissue level. Furthermore, the two approaches did not escape the attention of the members of the 21st century toxicology panel of the US National Academy of Sciences, who placed them at the heart of their vision statement (National Research Council (NRC), 2007), gaining much attention, given the authority of its authors. The ensuing consideration of toxicity pathways and modes of action (MOA) met happily the adverse outcome pathway (AOP) thinking in vogue in the ecotoxicology community (Tollefsen et al., 2014), and is still hesitating about changing name . . . Meanwhile, virtual organ programs have been heavily funded (*e.g.*, by the Virtual Liver project of the German Ministry of Research) (Holzhütter et al., 2012) given their potential impact for predictive drug safety assessment. In short, we are witnessing a convergence of systems biology and virtual organs modeling around the concept of quantitative MOAs, fully amenable to QIVIVE and risk assessment.

This paper follows a bottom-up integration logic: A first section presents our work at the organelle/cellular level. We then go toward modeling cell levels effects (monitored continuously), multiscale PBPK and effect models, and route to route extrapolation. We end with a short presentation of the automated KNIME workflows developed for dissemination and easy use of our models.

2. From organelles to cells

Before manifesting themselves at the cellular level, most toxicity effects start at the scale of organelles. Mitochondria in particular are often targets of toxicity. They perform two critical functions in the cell: the production of more than 90% of the cell's energy, and the control of cell survival as an integral part of programmed cell death (apoptosis). Three general adverse effects result from mitochondrial toxicity: 1. Disrupted energy metabolism; 2. Increased free radical generation; and 3. Altered apoptosis. We addressed the disruption of mitochondrial energy metabolism by measuring and simulating mitochondrial membrane potential (MMP). The measurement of MMP provides information on the mitochondrion's ability to carry out oxidative phosphorylation (which couples electron transfer to ATP synthesis), and transfer ions and substrates across its inner membranes (Nicholls and Ward, 2000). Thus, one of the most common methods to detect mitochondrial toxicity is the monitoring of the cells' MMP. A variety of fluorescent dyes can be used to that effect in high throughput screening. For example, cationic dyes distribute to the mitochondrial matrix in accordance with Nernst's equation (Mitchell and Moyle, 1969), so that the MMP is given by:

$$\mathrm{MMP} = (\alpha \times V)\frac{R \times T}{F}\log\left(\frac{C_{cyt}}{C_{mit}}\right) \quad (1)$$

where R is the gas constant, T the temperature, F the Faraday constant, C_{cyt} the concentration of the chemical in the cell cytosol, C_{mit} its concentration in mitochondria, α a proportionality constant, and V the cell viability. C_{mit} is computed by integration of the following differential equation:

$$\frac{\partial C_{mit}}{\partial t} = K_{mit}(C_{aq} - C_{mit}) \quad (2)$$

where C_{aq} is the concentration in the aqueous phase of the cell, and K_{mit} is a diffusion rate constant dependent on the chemical and cell line used.

We report here results on the *in vitro* MMP disruption of HepaRG cells by caffeine, carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP), amiodarone and estragole. The MMP was measured and modeled using an extension of the VCBA model (Zaldívar et al., 2011, 2010). That model, like some others (Armitage et al., 2014; Crean et al., 2014; Hamon et al., 2014; Pomponio et al., 2014; Truisi et al., 2015; Wilmes et al., 2013) takes into account the fate of the test compound *in vitro*: partitioning between plastic vial walls, headspace, serum proteins and cells. It includes a model of cell growth and death and has been linked to a threshold model for cell killing. In the course of the COSMOS project, we developed VCBA models for amiodarone, caffeine, FCCP, coumarin, estragole, ethanol, and nicotine. To simulate MMP data, a mitochondrial sub cellular compartment and Nernst's equation were added to the VCBA model. *In vitro* HepaRG MMP data were used to optimize two parameters of Nernst's equation (α and K_{mit}) by least-square minimization. Fig. 1 shows the measured and simulated MMP as a function of the exposure concentration of the four chemicals assayed. The model was able to correctly reproduce the amiodarone and estragole data. The caffeine data show a peak at 0.01 M, which could not be reproduced by the model. The FCCP induced fast decrease of MMP at concentrations lower than 0.1 mM was not well captured by the model either. More experiments with different chemicals will be needed to fully understand the determinants of prediction accuracy.

3. Modeling *in vitro* kinetics and continuously measured cell effects

Two complementary models were used to analyze toxic effects *in vitro*, at the cell level. The first model is the VCBA model introduced above (Zaldívar et al., 2011, 2010). This model is well suited to analyze fixed point cytotoxicity data (as in high-content imaging assays). The second model can be used for continuous cytotoxicity monitoring, using electrical impedance measurements (Xing et al., 2006).

The latter model describes HepaRG cell viability loss following exposure to hepatotoxic molecules. It was applied it to three

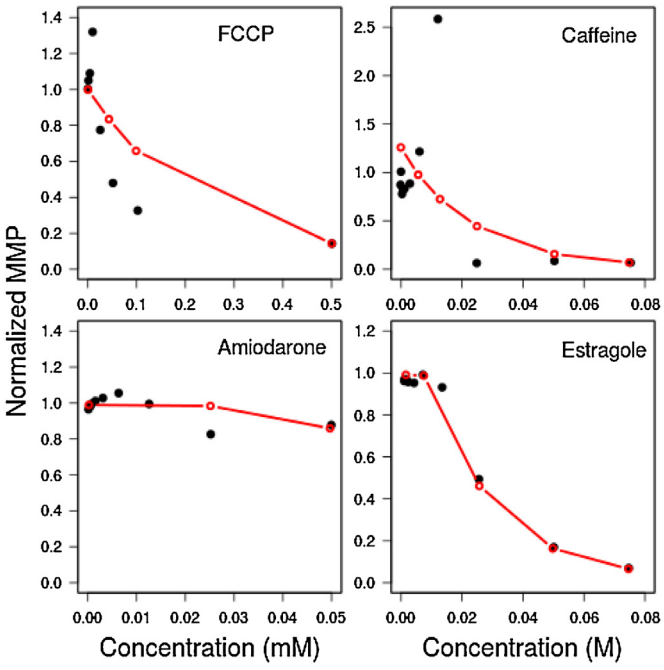


Fig. 1. Observed (black dots) and VCBA model simulated values (red circles and lines) of mitochondrial membrane potential in HepaRG cells exposed in vitro to four test compounds; carbonyl cyanide-*p*-trifluoromethoxyphenylhydrazone (FCCP), caffeine, amiodarone, and estragole. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

cosmetic related substances: coumarin, isoeugenol and benzophenone-2. Our model couples dynamic descriptions of the major *in vitro* kinetic processes involved with a simple model of viability loss (see Teng et al., 2015 for details).

The data were obtained using HepaRG cells (Guillouzo et al., 2007), exposed to the selected chemicals either once (at concentrations ranging from 0.008 to 8 mM with a 48 h follow-up) or during repeated exposures (at concentrations ranging from 0.128 to 8 mM, with a follow-up of 4 weeks). Cellular viability was monitored by impedance measurements with an *in vitro* label-free cell-based monitoring system (xCELLigence™, ACEA Biosciences, Roche Diagnostics). The impedance of HepaRG cells was converted to a normalized cell index (NCI). A decrease in NCI is indicative of cell detachment or death (Xing et al., 2006).

We modeled the *in vitro* dynamic decrease in concentration for the substance of interest by an apparent linear process (lumping unspecific physico-chemical reactions, plastic binding, evaporation and linear metabolism) or by a Michaelis-Menten equation when saturable cellular metabolism was observed.

For coumarin and isoeugenol, the time-course of cell viability was modeled using Eq. (5), parameterized by a killing rate (b) and a no effect concentration, NEC.

$$\frac{dN}{dt} = \begin{cases} -b(C_i - \text{NEC})N, & \text{if } C_i > \text{NEC}, \\ 0 & \text{otherwise} \end{cases} \quad (5)$$

N being the number of cells in the well. For benzophenone-2, we used a slightly more complex model to describe cell spreading at sub-toxic concentrations (Teng et al., 2015). The model parameters were estimated by least square fitting of the model to the impedance data.

On the basis of goodness of fit, linear loss was used for coumarin. Saturable loss gave better fits for isoeugenol and benzophenone-2. As shown for coumarin in Fig. 2 (for short-exposures) and in Fig. 3 (for repeated exposures), our model described rather well the kinetics of real-time impedance

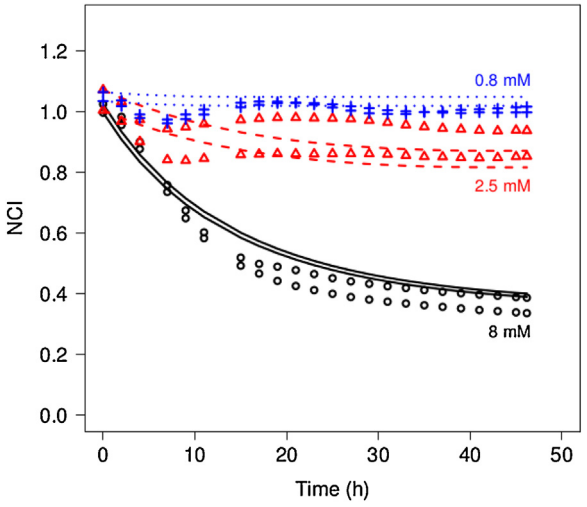


Fig. 2. Normalized Cell Index (NCI) of HepaRG cells exposed once to coumarin at various concentrations. The data and the model predictions are represented by points and lines, respectively (adapted from Teng et al., 2015).

measurements in HepaRG cells at various exposure doses. Similar results were obtained for isoeugenol and benzophenone-2 (Teng et al., 2015).

However for isoeugenol and benzophenone-2, the acute models failed to predict long-term exposures and *vice-versa*. This could be due to various phenomena not accounted for by the model, such at the reversibility of plastic binding, or the complex dynamics of cellular response and adaptation to stress. We have actually observed that *in vitro* repeated exposures experiments tend to lead to complex time-dose-response relationship much more difficult to interpret and analyse than acute toxicity data (Hamon et al., 2014; Wilmes et al., 2013). This should not be taken as a problem to avoid, but as a challenge to resolve, because such complex responses are probably more representative of the *in vivo* cellular responses. In any case, while awaiting better models and deeper understanding, it is recommended to use the proper experimental data to predict the corresponding effect.

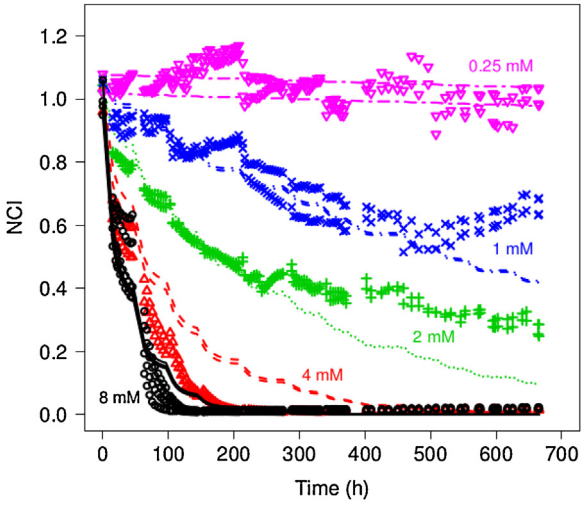


Fig. 3. Normalized Cell Index (NCI) of HepaRG cells exposed every 2–3 days to coumarin at various concentrations for 4 weeks. The data and the model predictions are represented by points and lines, respectively.

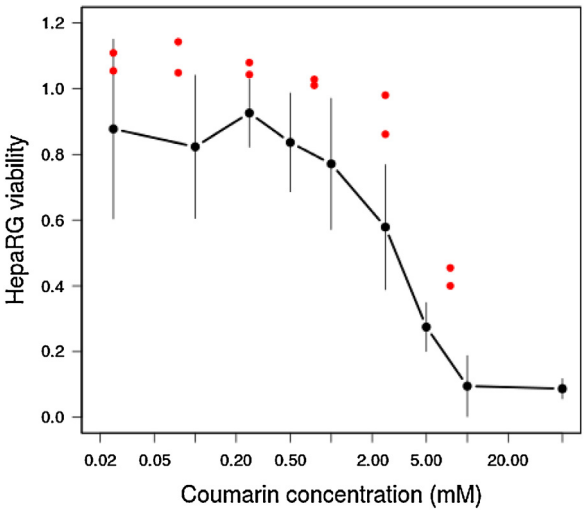


Fig. 4. Comparison of HepaRG cell viability exposed to coumarin at 24 h, as measured by Cellomics and estimated by the VCBA model (black dots) or impedance measurements and Teng's model (red dots). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Fig. 4 shows a comparison between the Teng et al. and VCBA model estimates of HepaRG cells viability after exposure to various concentrations of coumarin. The VCBA model used static Cellomics assay data to assess cell viability. Cell-level toxicodynamics were described by two parameters: a no-effect concentration and a killing rate. Compared to Cellomics, impedance metrics provide slightly higher values of cell viability. However, the two tests give comparable results when experimental variability is taken into account.

4. From *in vitro* to *in vivo* with multiscale PBPK models

4.1. In silico *predictions of PBPK model parameters*

Quantitative structure activity/property relationships (QSARs/QSPRs) are theoretical (*in silico*) models that relate the structure of chemicals to their biologic activities or properties (*e.g.*, physico-chemical, partitioning or fate property). QSAR models can be used to parameterize PBPK models by providing predictions for basic physico-chemical properties (*e.g.*, ionization constant, octanol over water partition coefficient, distribution coefficient or aqueous solubility) as well as key ADME properties (*e.g.*, extent of gastrointestinal absorption, oral bioavailability, plasma protein binding, metabolism rate by cytochromes or clearance). A number of QSAR models and software tools are available for the prediction of ADME related properties (Mostrag-Szlichtyng and Worth, 2010; Worth et al., 2011). In the context of the COSMOS project we used the ACD/Labs Percepta software (ACD/Labs Percepta, release 2014. Advanced Chemistry Development Inc., Toronto, Canada. www.acdlabs.com) to predict several physico-chemical parameters (*e.g.*, Log K_{ow}, pK_a, F_{up}) and ADME properties (*e.g.*, passive oral absorption, oral bioavailability, total body clearance) that were used for the calibration of PBPK models.

4.2. From cells to tissues to whole body

As shown in the previous sections, not only the assessment of concentrations in organelles, but also the assessment of cell concentrations in a tissue is relevant to perform *in vitro* to *in vivo* extrapolations (Martin et al., 2015). Since the liver as a whole is a rather homogeneous organ, it is possible to represent it as an average single cell containing relatively complex micro-organelles enveloping transport and molecular pathways (Krauss et al., 2012). This approach balances the complexity of PBPK models and that of molecular interactions, while reducing to a minimum the complexity of the organ structure.

However, when the liver structure is analyzed in detail it becomes clear that liver substructures are heterogeneous (Pang and Rowland, 1977; Pang et al., 2007). In the liver tissue, blood is distributed by portal veins into functional subunits, called lobules, which carry out diverse functions including the detoxification of xenobiotics at the cellular level. The lobules capillaries distribute blood and dissolved substances to various cells, and in particular hepatocytes. In a lobule, blood transport produces a natural oxygen gradient, introducing a zonation of the lobule, which in turn induces a heterogeneous distribution of CYP expression factors in the hepatocytes radially aligned in the lobule. The metabolism of substances and the toxic response of the organ depend on this heterogeneity.

That issue has attracted the attention of several groups and projects (Holzhütter et al., 2012), and has motivated several experimental initiatives, such as novel forms of cell cultures (Soldatow et al., 2013). Mathematical models for the multi-scale description of the liver have also been developed (Diaz Ochoa et al., 2013; Hunt and Ropella, 2008; Pang et al., 2007; Wambaugh and Shah, 2010).

Several publications have shown how such models can be implemented to improve predictions of the *in vivo* toxic response to small molecules. In our case (Diaz Ochoa et al., 2013), we described the metabolism and detoxification of acetaminophen in the lobule using six sinusoids, which are capillary structures, ordered to form a hexagonal shaped lobule. In each hepatocyte, a metabolic network sub-model allows the estimation of the rate of production of relevant metabolites and the production of oxidative species. On this basis, we can estimate the clearance by the simulated liver and apply it to the liver compartment in a PBPK model. The explicit calculation of clearance using a parallel liver model requires a concurrent parallelization. That opens a novel computational way to integrate liver cells with an ever growing molecular description complexity, depending on the information available for the reconstruction of molecular pathways. This kind of approach is essentially a "divide and conquer" strategy where different sub-models are defined in separate programs run in parallel. Current developments are not restricted to the generation of models or algorithms implemented in conventional machines. For instance the use of cloud computing has also opened the possibility to simulate very complex liver models with a high degree of biological detail (Ropella and Hunt, 2010).

That approach allowed us to estimate local concentrations of acetaminophen that potentially trigger cell necrosis, helping to visualize liver damage after human exposure to a toxic dose of acetaminophen (Fig. 5). Due to the coupling of the toxic response and detoxification it was possible to qualitatively estimate the effect of the toxic response on the concentration of the substance in the whole organism. Implementing cell dynamics and mechanical cell interactions made it also feasible to visualize in detail liver necrosis, closing the gap between *in silico* predictions and *in vivo* outcomes (Drasdo et al., 2014).

However, for quantitative estimation, it is sometimes more appropriate to directly couple the sub-cellular units to a PBPK model. Simpler approaches combining PBPK modeling and systems biology have investigated the coupling of a single complex liver cell with internal metabolism (Bois, 2009; Péry et al., 2013). Slightly more complex models describe the liver as a 1D tube representing a liver sinusoid where the transport of substances is coupled to the metabolism in the hepatocytes (Andersen et al., 1997; Jones et al., 2012). Fig. 6 illustrates our implementation of such a model.

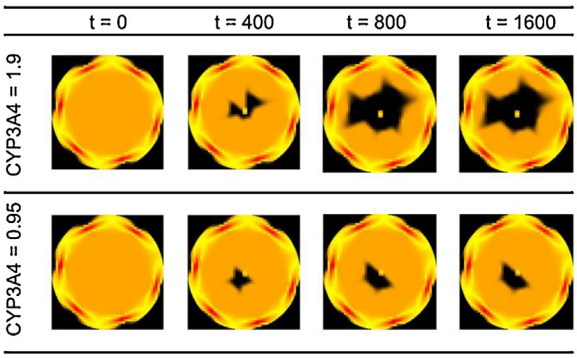


Fig. 5. Visualization of liver damage at different times (in minutes) after the oral application of acetaminophen for two virtual patients with different CYP expression factors. The orange and black center area represents healthy and damaged liver tissue, respectively. The six red ellipsoids at the periphery are the periportal veins (reprinted from Diaz Ochoa et al., 2013; with permission).

Such 1D-liver models have disadvantages as well as advantages: they are less realistic because they assume that the dispersion rate is homogeneous, implying that the substance concentration is at equilibrium at any time in each compartment. In general the lack of hydrodynamics, as well as the incorrect representation of boundary conditions may hide some effects (like differences in blood velocities within a sinusoid) related to the transport of substances from the sinusoidal space to the hepatocyte, as well as hinder the representation of the inherent fractal structure of the organ (Dokoumetzidis and Macheras, 2003). However, the elimination of the explicit spatial dependence allows a simple mathematical description using ordinary differential equations. This kind of less granular 1D-liver still captures the essentials of the liver zonation and simultaneously increases the calculation performance when cellular networks (metabolism or cell regulation) are coupled to the hepatocyte. In summary, the selection of the dimensionality of the liver model depends on the level of complexity required for the quality of the *in silico* predictions.

In Fig. 7 we present an example of the use of a zonated 1-D liver for acetaminophen. In this example, an oral dose of 5 mg/kg was assumed to be given to a man weighing 73 kg. The values of the PBPK model parameters are based on those reported by Péry et al. (2013). We assumed that primary metabolism in each hepatocyte was adequately described by a Michaelis-Menten term, multiplied by a scaling factor representing the differences in metabolic rate in each zone of the liver. Without zonation this factor is equal 1 for all hepatocytes. We first compared the predictions of our model without zonation to those obtained with a previously published unstructured model (Péry et al., 2013).

We assumed a heterogeneous metabolism along the liver sinusoid. Different signals like gradients of oxygen, nutrients, metabolites, hormones and cytokines have an influence on zonation since they modulate the activity of various enzymes involved in the metabolic pathways (Gebhardt and Matz-Soja, 2014). However, the estimation of zonation factors is not trivial, since they vary continuously in response to fluctuating sinusoidal patterns (Oinonen and Lindros, 1998). Glucuronidation is the dominant pathway of conjugation at high APAP concentrations, and is faster in the pericentral than in the periportal region, as has been shown in experimental studies (Anundi et al., 1993). Correspondingly, we selected a factor of 1.0 for the pericentral zone, 0.5 for the midlobular zone, and 0.5 for the periportal zone (Oinonen and Lindros, 1998). As a consequence, an overall increase in acetaminophen concentrations and risk of toxic response was observed (Fig. 7).

That model has been implemented as a KNIME work-flow (Berthold et al., 2007), increasing the portability of the model. Based on this methodology, it is possible to write general PBPK models that can easily be applied to different substances.

A significant limitation is the difficulty to observe zonation *in vivo*, limiting the possibility to validate the corresponding models. Several advances in on-chip bioreactor technology mimicking a 1D liver could help validate *in silico* models (Allen and Bhatia, 2003). For example, advances in microfluidics have facilitated the development of organs on-chip containing cells coupled to micro vessels mimicking the capillaries of real organs (Dash et al., 2009; Kimura et al., 2015), opening the possibility to directly observe liver zonation and changes in metabolic rates. These advances represent a potential method to deliver complementary results or even couple *in vitro* and *in silico* methods to increase the confidence in both methods.

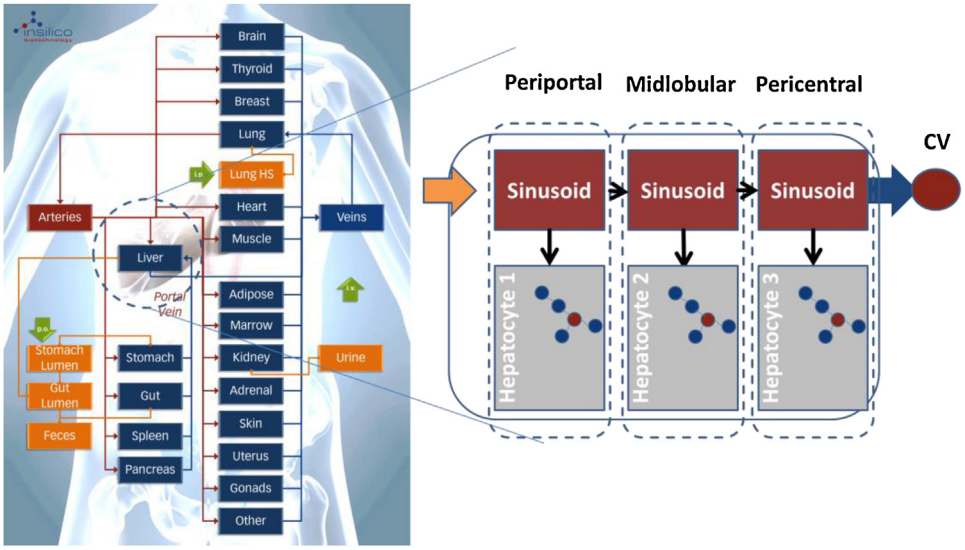


Fig. 6. 1D-liver model of the transport of a substance from portal veins to central veins in the sinusoid as well as its transport into the hepatocytes (parameters have been defined according to Table 1). In each hepatocyte the metabolism is described by a molecular network. This enables the description of a heterogeneous metabolism and phenotype along the sinusoid (zonation).

Table 1
Parameters required for the modeling of sinusoids in our 1D liver model (Fig. 6). All the parameters are scaled proportionally to the total liver volume.

Parameter	Value or formula	Source
Permeability (neutral substances)	$P = (\log P - 6.7)$ cm/s. Valid for neutral substances	Trapp et al. (2008)
Transport through sinusoid	0.05 mm/s	Vollmar and Menger (2009)
Mean sinusoid length	~368 μm	Grisham (2009)
Volume sinusoidal space	2.12×10^{-13} L	estimated by ourselves using hepatocyte volume
Volume Hepatocyte	5.09×10^{-14} L	Lodish (2000)
Sinusoid diameter	10 μm	Grisham (2009)
Thickness of barrier between sinusoid and hepatocyte	2 μm	Ostrovidov et al. (2004)
Approximated number of hepatocytes in the liver	$\sim 10^9$	Grisham (2009)

The introduction of additional structures in a PBPK model has a double effect: they can improve the predictions made with such a model, but they can also limit its use if new and unknown parameters cannot be easily estimated (Poggesi et al., 2014). Despite the difficulty of experimentally validating structured liver models, they can be useful to study qualitatively the effect of population variability in cytochromes expression on liver zonation, to estimate risks involved in changes in zonation (induced for example by other substances or liver damage), or to study the impact of zonation on liver toxic response.

4.3. Route-to-route extrapolation

To predict safe levels of human exposure to general chemicals we have so far usually relied on animal studies carried out mainly by the oral route. However, for cosmetic ingredients the main route of consumer exposure is expected to be dermal. For existing chemicals, rather than carrying out additional animal studies using a new exposure route (which goes against 3R principles and the European Union ban on animal testing for cosmetics), route-to-route extrapolation can be performed by modeling if reliable data

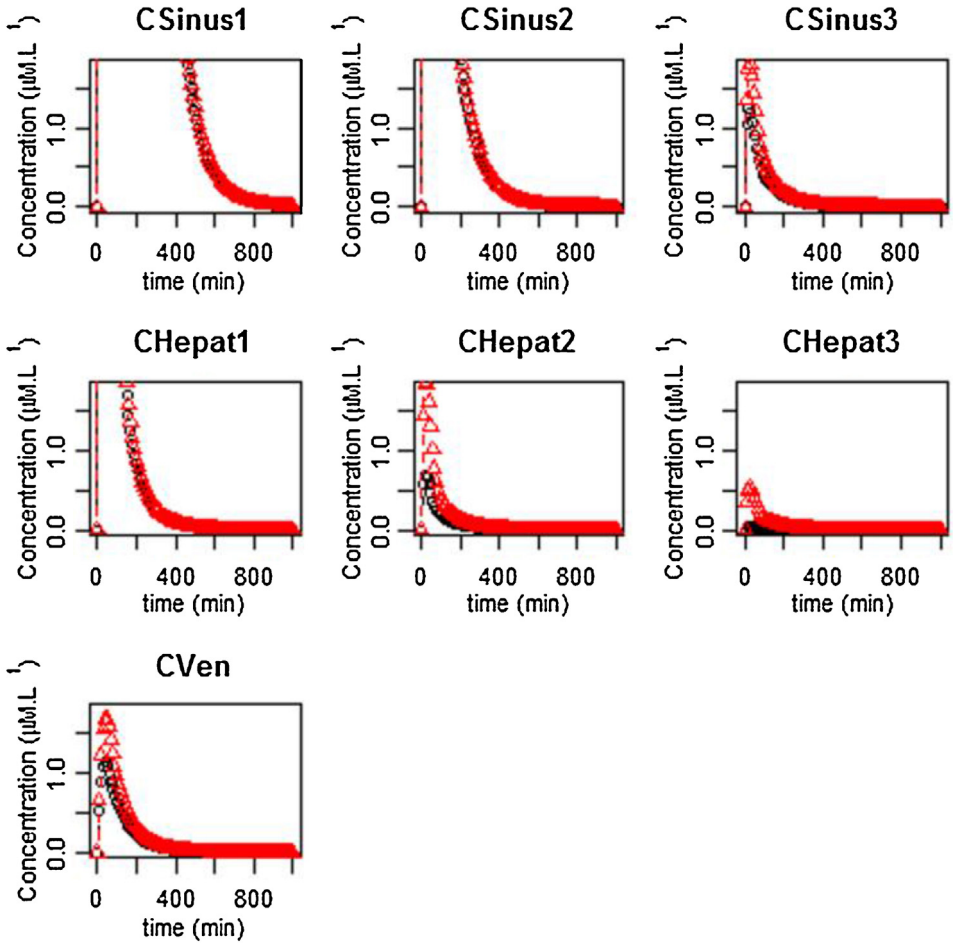


Fig. 7. Model prediction of acetaminophen concentration-time profiles in three zones (1: periportal; 2: midlobular; 3: pericentral) of the liver sinusoid vessels or hepatocytes, and in venous blood, with zonation of metabolism (red curves) or without zonation (black curves), after a 5 mg/kg oral administration of acetaminophen of an adult man. The transport rate to hepatocytes was 1.40×10^{-1} L/min, and 1.08×10^{-2} L/min along the sinusoids. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

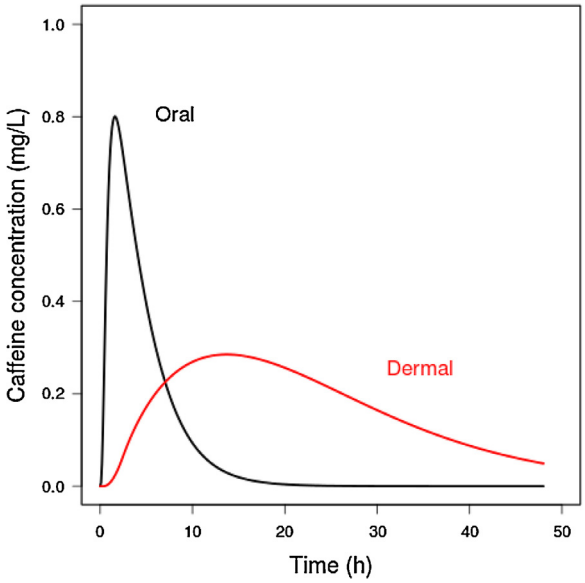


Fig. 8. Concentration-time profiles of caffeine in blood after either bolus oral or single application dermal exposure to an oral NOAEL dose (2.1 mg per kg of body mass) (adapted from Gajewska et al., 2014).

on the ADME properties of the substance are available (Gerrity and Henry, 1990). Obviously here, technical considerations as to the type of *in vitro* assays to perform are essential. The mode of route-to-route extrapolation should be decided on a case-by-case basis (Nielsen et al., 2008). For example, the threshold of toxicological concern (TTC) approach can eventually be applied (Worth et al., 2012), and Kroes et al. (2007) give examples of how the TTC approach can be applied to cosmetic ingredients and impurities. They proposed that default factors should be used to adjust from an external topical dose to an internal dose, followed by application of the oral TTC values derived by Munro et al. (1996). PBPK modeling provides a more mechanistic approach to route-to-route extrapolation (Dourson and Felter, 1997; Reitz et al., 1988). In our recent work on cosmetic ingredients, a series of PBPK models coupled to VCBA models was developed to extrapolate between routes of exposure for caffeine, coumarin, ethanol, hydroquinone, isopropanol and nicotine (Gajewska et al., 2014).

We report here, as an example, the results for caffeine, based on the use of a full PBPK model. Concentration-time profiles of caffeine in blood, following either oral or dermal absorption were simulated by our PBPK model at the oral NOAEL dose of 2.1 mg per kg of body mass and per day (Fig. 8). These simulations were performed for a male subject, weighing 75 kg. The concentration versus time profiles for the two administration routes are clearly different, which indicates that the NOAEL dose should probably not be the same after oral or dermal exposures, as discussed in detail in (Gajewska et al., 2014).

PBPK models can predict concentration-time profiles of a given compound at the organ level or cell level (see above). In turn, the predicted intracellular concentrations can be linked to cellular or tissue level effect through the use of a concentration-effect relationship (*i.e.*, a model), such as the VCBA one or similar (Gajewska et al., 2015; Paini et al., 2012), or more complex pathway model (Hamon et al., 2014, 2015). Such a modeling approach has the potential to predict effective target organ dose. Dose response curves obtained based on *in silico* simulation and *in vitro* toxicity data can be used to derive benchmark dose levels, usable as points of departure for risk assessment. Acceptable human daily intake, tolerable intakes, health based recommendation and exposure limits can be set (Rietjens et al., 2011). In the specific case of a cosmetic, the amount of an ingredient used in the final formulation can be restricted in order to obtain a final product that does not pose a health concern to the consumer.

5. From *in silico* models to automated workflows

Future human safety assessments are likely to rely strongly on the use of multi-scale models, implemented through a combination of computational tools, in order to perform extrapolations such as QIVIVE. The development of automated software tools is now seen as an important step for harmonizing and expediting chemical safety assessments. The KNIME workbench (Berthold et al., 2007) provides a user-friendly graphical workflow interface for data processing and analysis. Most of the mathematical models developed within the framework of COSMOS – mostly written in R (R Development Core Team, 2013) – have been implemented in the KNIME platform. They already are or will soon be publicly available through the COSMOS KNIME WebPortal (http://knimewebportal.cosmostox.eu), with documentation and user guidance available at

Table 2
List of the biokinetics workflows developed in COSMOS and which are or will be available in the COSMOS KNIME WebPortal at http://knimewebportal.cosmostox.eu.

Type	Chemical	Exposure	Species	Output
PBPK	Caffeine	Oral, dermal	Human, rat	Time kinetics
PBPK	Coumarin	Oral, dermal	Human, rat	Time kinetics
PBPK	Ethanol	Oral, inhalation	Human, rat	Time kinetics
PBPK	Estragole	Oral, dermal	Human	Time kinetics
PBPK	Hydroquinone	Oral, dermal	Human, rat	Time kinetics
PBPK	Isopropanol	Oral, dermal	Human, rat	Time kinetics
PBPK	Methyliodide	Oral, dermal, inhalation	Human, rat	Time kinetics
PBPK	Nicotine	Intra-venous (IV)	Human	Time kinetics
PBPK	Styrene	Inhalation	Human	Time kinetics
PBPK	Methotrexate	Oral, dermal	Human	Time kinetics
PBPK	Valproic acid	Oral, dermal	Human	Time kinetics
PBPK	Piperonyl butoxide	Dermal	Human	Time kinetics
PBPK	General	Oral, dermal, inhalation	Human	Time-dose kinetics
PBPK and bioaccumulation	90 chemicals	Oral	Human	Bio-accumulation factor
	General	Oral	Human	Bio-accumulation factor
QIVIVE	Caffeine	Oral, dermal	Human	Time-dose kinetics
QIVIVE	Coumarin	Oral, dermal	Human	Time-dose kinetics
QIVIVE	Estragole	Oral, dermal	Human	Time-dose kinetics
QIVIVE	General	Oral, dermal	Human	Time-dose kinetics
VCBA	30 chemicals	*In vitro*	HepaRG, HepG2, 3t3balb/c, A549, cardiomyocytes	Time-dose kinetics and effects
1D liver	Coumarin	Oral, inhalation, IV	Human	Time kinetics
1D liver	Caffeine	Oral, inhalation, IV	Human	Time kinetics

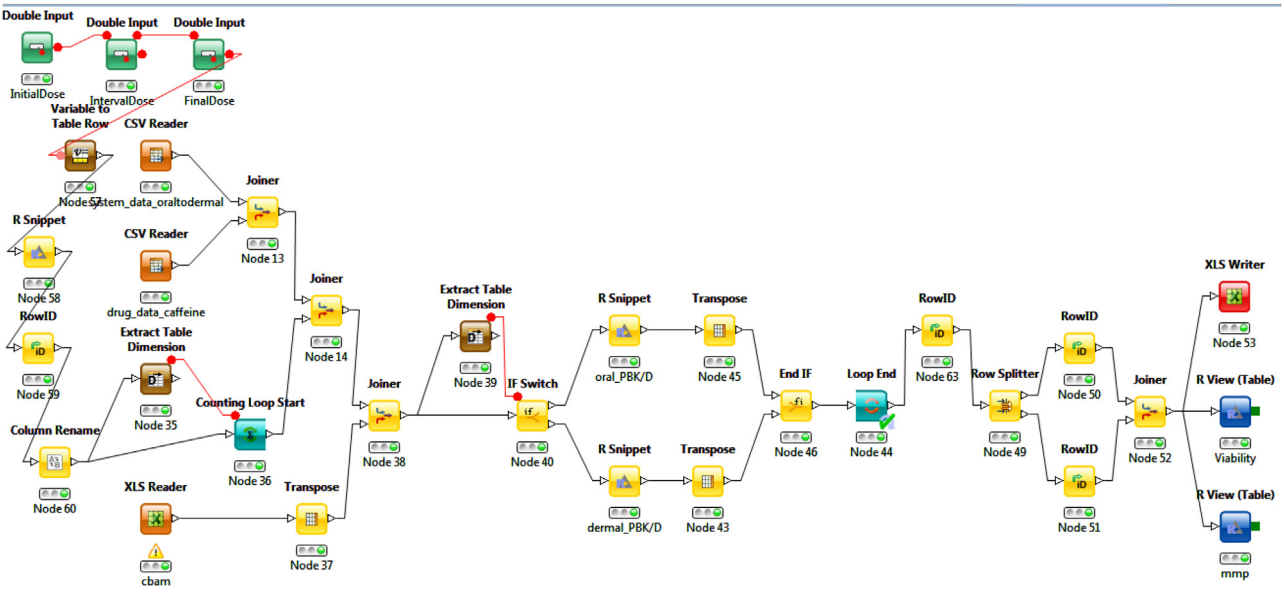


Fig. 9. Schematic representation of a KNIME work-flow developed for PBPK modeling for caffeine effects after oral and dermal exposure. The work flow automatizes data input, model parameters' setting, simulation calls and output to different data streams. The core calculation are done in the "R snippet" nodes in the middle.

http://cosmosspace.cosmostox.eu (Table 2). Fig. 9 shows a screen capture of the KNIME caffeine work-flow graphical interface. Workflows offer a graphical interface for automatizing data input, model parameters' setting, simulation calls and output to different data streams.

6. Conclusion

The work presented here was performed to develop a safety assessment approach for cosmetic ingredients that does not rely on the use of animal experiments and which is therefore compliant with the European Union ban on animal testing. Two approaches are available to maintain an acceptable safety level for those products: The first would be to use human based *in vivo* biomarker assay. The second, and more realistic, is the combination of predictive chemistry (*in silico*) assessments, based on legacy knowledge from past animal experiments, *in vitro* testing, and QIVIVE. We focused here on the latter. Mathematical or computer modeling and *in vitro* experiments are complementary. A purely *ab initio* chemistry-based approach is not currently feasible for toxicity predictions, since the knowledge gap is too wide and has to be filled with *in vitro* data. Such data by themselves are also insufficient. *In vitro* systems are still far from able to mimic a human body and the data collected, essentially finite, need to be interpolated and extrapolated in various ways. Modeling can help there. We have made a modest contribution to the large international endeavor in this field, addressing various scales of complexity: from the sub-cellular level (mitochondrial stress), through the cellular one (cytotoxicity models), the tissue and organ scale (liver), up to the whole body (with PBPK models).

The models we present are all relevant to the safety assessment of cosmetic ingredients, but at various degrees, depending of the depth of the assessment sought. Dermal exposure simulations are available in all the PBPK models we developed. The KNIME workflows for *in vitro* and *in vivo* kinetics' simulations (Table 2) are clearly the most readily applicable, in particular for QIVIVE in the context of *ab initio* assessments (*e.g.*, to predict *in vivo* cellular levels which can be used in input to dose-response models calibrated with *in vitro* data) (Hamon et al., 2015; Quignot et al., 2014). We are actually using them in case studies demonstrating the applications of the various tools developed in COSMOS and other projects of the SEURAT cluster (the results will be reported later in separate publications). The other models presented here are more specific and have narrower scope. The liver model, for example, should for now be reserved for in depth mechanistic studies of particular chemicals and is too complex for high-throughput screening. On the other hand, the models we developed can be transposed to drugs or general chemicals without problems.

One apparent issue in the overview we presented is the definition of the boundaries between models. For example, where should a PBPK model stop and where does systems biology modeling start? The question may seem purely definitional, as we know that a continuum exists from transport and metabolism to metabolism control and signaling pathways, and that continuum extends to cell–cell communications, to the tissue level *etc*. However, it is also an operational question: What should toxicologists, risk assessment practitioners and decision makers expect from PBPK modeling? Certainly not an all-encompassing tool. Our opinion is that PBPK models should focus on transport and leave the details of metabolism and transport to systems biology, so that the norm should be coupled PBPK – systems biology – physiome models (Geenen et al., 2013; Hamon et al., 2015). Or maybe PBPK models should rather be viewed as particular (vascular system) physiome models? Not everyone may agree with this view, as we tend to endorse the labels imposed by customs and norms. Yet we should be open to changes, particularly when they broaden our views and capabilities.

One limitation in this field is our ability to deal with complex computations based on massive data sets. This includes, for example, the integration of omics data. Beyond multivariate statistics, clustering and *ad hoc* approximate pathway analysis, the integration of such data in a mechanistic framework is a scientific and computational challenge (US EPA, 2014). Virtual tissues and virtual body models are obviously extremely complex and few research organizations have the capabilities to run them to a full extent not to mention risk assessment practitioners, so that simplified versions are still the norm. Computational and mathematical methods still need improvement to meet this challenge.

The modeling of adverse effects in humans is based on the fact that toxicity pathways and mechanisms are in fact high-jacked

biological pathways, and toxic effects are a particular class of physiological effects. While the use of modeling approaches and *in vitro* methods to replace animal experiments remains a considerable challenge, advances in the field are being made due to the efforts of a very large community of scientists.

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