Application of physiologically-based toxicokinetic modelling in oral-to-dermal extrapolation of threshold doses of cosmetic ingredients

M. Gajewska, A. Worth, C. Urani, H. Briesen, K.-W. Schramm

A R T I C L E   I N F O

Article history:
Received 3 January 2014
Received in revised form 18 March 2014
Accepted 19 March 2014
Available online 13 April 2014

Keywords:
Physiologically based toxicokinetic (PBTK) modelling
Route-to-route extrapolation
Coumarin
Caffeine
Hydroquinone

A B S T R A C T

The application of physiologically based toxicokinetic (PBTK) modelling in route-to-route (RtR) extrapolation of three cosmetic ingredients: coumarin, hydroquinone and caffeine is shown in this study. In particular, the oral no-observed-adverse-effect-level (NOAEL) doses of these chemicals are extrapolated to their corresponding dermal values by comparing the internal concentrations resulting from oral and dermal exposure scenarios. The PBTK model structure has been constructed to give a good simulation performance of biochemical processes within the human body. The model parameters are calibrated based on oral and dermal experimental data for the Caucasian population available in the literature. Particular attention is given to modelling the absorption stage (skin and gastrointestinal tract) in the form of several sub-compartmental models. This gives better model prediction results when compared to those of a PBTK model with a simpler structure of the absorption barrier. In addition, the role of quantitative structure–property relationships (QSPRs) in predicting skin penetration is evaluated for the three substances with a view to incorporating QSPR-predicted penetration parameters in the PBTK model when experimental values are lacking. Finally, PBTK modelling is used, first to extrapolate oral NOAEL doses derived from rat studies to humans, and then to simulate internal systemic/liver concentrations – Area Under Curve (AUC) and peak concentration – resulting from specified dermal and oral exposure conditions. Based on these simulations, AUC-based dermal thresholds for the three case study compounds are derived and compared with the experimentally obtained oral threshold (NOAEL) values.

© 2014 The Authors. Published by Elsevier Ireland Ltd. This is an open access article under the CC BY-NC-SA license (http://creativecommons.org/licenses/by-nc-sa/3.0/).

1. Introduction

Due to concerns about the potentially adverse effects of human exposure to cosmetic ingredients, combined with animal testing and marketing bans under the EU cosmetics legislation (Parliament and Union, 2009), recent studies are increasingly focusing on the development of predictive models as an alternative to animal testing. In particular, physiologically based toxicokinetic/pharmacokinetic (PBTK/PBPK) models simulate concentration–time profiles of a parent chemical and its metabolites in organs and the blood following a compound-specific kinetic scenario of absorption, distribution, metabolism and excretion (ADME). These models can be used to fill missing toxicity data gaps by means of interspecies and route-to-route (RtR) extrapolations. The use of PBTK modelling for RtR extrapolation is based on comparisons of simulated internal blood or organ concentrations which are typically characterized by a peak value (\(C_{\text{max}}\)) and area under curve (AUC) after inhalation, oral or dermal absorption of the same absolute dose (Chiu and White, 2006; Mielke et al., 2011). In this way, experimentally determined safe limits such as the oral...
Three cosmetic ingredients are the subject of this study: coumarin, hydroquinone and caffeine. Coumarin is used as a fragrance and is a natural ingredient of several herbs, beverages, tobacco and spices. It shows hepatotoxic and carcinogenic properties mainly in rodents. An oral NOAEL of 10 mg/kg BW/day has been concluded in the dog. In rats, no-adverse-effect levels for hepatotoxic effects ranged from 50 to 130 mg/kgBW/day (EFSA, 2004). In this study, 50 mg/kg BW/day is taken as the NOAEL in the rat. Coumarin is extensively metabolized by the liver. There are species-dependent differences in the pathways for its metabolic conversion. In humans, it appears that coumarin is extensively metabolized to 7-hydroxycoumarin (7-HC), and in a smaller amount to 3-hydroxycoumarin (3-HC), while in the rat and mouse the major route of metabolism is by a 3,4-epoxidiation pathway resulting in the formation of coumarin 3,4-epoxide which is rearranged to a toxic metabolite, o-hydroxyphenylacetaldehyde (o-HPA) and further detoxified to o-hydroxyphenylacetic acid (o-HPAA) and o-hydroxyphenylethanol (o-HPE). The construction of simple physiologically based predictive models incorporating the metabolic pathways of coumarin has already been described in the literature (Born et al., 2000; Mielke et al., 2011; Rietjens et al., 2008). In these studies, the RfR extrapolation of a single tolerable daily intake dose of coumarin (0.1 mg/kgBW) was carried out by comparing the AUC and Cmax of coumarin in blood and liver. Only dermal AUC of blood was found to be higher (Mielke et al., 2011).

Hydroquinone is used as a skin whitening agent in creams to reduce the color of the skin, in oxidative hair dyes, and in artificial nail (manicure) preparations. Repeated oral dosing caused tremors and reduced activity (>=64 mg/kg), reduced body weight gain (>=200 mg/kgBW), convulsions (>400 mg/kgBW), and nephropathy in F-344 rats (>100 mg/kgBW). An overall rat NOAEL (for all adverse effects) of 20 mg/kg BW/day was derived (OECD SIDS, 1996; Poet et al., 2010). Metabolism to reactive intermediates in the liver is involved in the renal toxicity and exacerbation of chronic progressive nephropathy associated with hydroquinone ingestion. The formation of benzoquinone is the first critical step towards the formation of toxic metabolites. Following oral administration, the majority of metabolites are conjugates of glucuronide (up to 67%) and sulphuric (up to 33%) acids (Corley et al., 2000; McGregor, 2007; Poet et al., 2010). Skin enzymes responsible for metabolism of hydroquinone are reported to have activities ranging from <1% up to 25% of the activity in the liver (Poet et al., 2010). PBTK model for hydroquinone has been already used in the literature to compare human internal dose metrics following oral and dermal exposures of estimated glutathione conjugates at the applied NOAEL dose (human equivalent concentration) (Corley et al., 2000; McGregor, 2007; Poet et al., 2010).

Caffeine is found in varying quantities in the seeds, leaves, and fruit of some plants. It is used in many creams and lotions since it is believed to slow down the photoaging process of the skin and to absorb ultraviolet radiation thereby preventing the development of tumours after skin exposure to sunlight. It is also used as an active compound in anti-cellulite products because it prevents excessive accumulation of fat in cells. This alkaloid has potent antioxidant properties. It increases the microcirculation of blood in the skin and also stimulates the growth of hair through inhibition of the 5-alpha-reductase activity. Recent animal studies have demonstrated, that depending on the method of administration and species, the developmental NOAEL in rodents is approximately 30 mg/kgBW/day, the teratogenic NOAEL is 8100 mg/kgBW/day, and the reproductive NOAEL approximately 80–120 mg/kgBW/day. In this study, the rat NOAEL of 10 mg/kgBW/day is used (Brent et al., 2011; OECD SIDS, 2002). Caffeine is metabolized in the liver to xanthine and uracil derivatives. Paraxanthine is quantitatively
the main demethylated product, followed by theobromine and theophylline, respectively. The metabolism and toxicokinetics of caffeine, and the use of this information in PBTK modelling have been described in previous studies (Csajka et al., 2005; Ginsberg et al., 2004; Lelo et al., 1986; Thorn et al., 2012; Zandvliet et al., 2005).

The aims of this study were to determine whether: (a) the use of sub-compartments in the absorption stage of the PBTK model, i.e. the gastrointestinal tract (GI tract) and skin, gives a better prediction of blood/plasma levels of selected substances when compared to a model of simpler structure; and (b) the final model, calibrated using individual and population mean literature experimental data, can be successfully applied in the oral-to-dermal extrapolation of toxicity threshold values of three cosmetic ingredients. The oral NOAEL doses determined in rat studies were first converted to human NOAEL values via simulated internal systemic concentrations following oral absorption. Finally, dermal safe limits were determined based on calculated Area Under Curve (AUC) and peak concentration (Cmax) for a parent compound and its potentially toxic metabolite.

The model simulations were performed for single dose applications. This work goes beyond previously published oral-to-dermal extrapolation studies for coumarin and hydroquinone by applying a refined PBTK model in the calculation of oral and dermal safe limits. As far as the authors are aware, this is the first illustrated use of PBTK modelling for the oral-to-dermal extrapolation of caffeine toxicity.

2. Materials and methods

2.1. PBTK model

Three structurally different PBTK models were constructed in order to select one that best simulates ADME processes in human. To distinguish between the models, different names are given (PBTK1–3). The schematic representation of the main organs considered (Fig. 1) applies to all three models and the differences between them are in the GI tract and skin compartments only, as explained in the following paragraphs.

In PBTK1 the GI tract and skin are represented by one compartment with a first order rate of absorption, as described in the literature for coumarin and hydroquinone (Mielke et al., 2011; Poet et al., 2010; Rietjens et al., 2008).

PBTK2 is applied for dermal absorption only. It consists of the surface compartment and two skin compartments with unidimensional diffusion (Fick's second law) across a single skin layer (stratum corneum and viable epidermis are grouped together) into the dermis and there are no hair follicles (Fig. 2).

PBTK3 is the refined PBTK model with various sub-compartments in the skin and GI tract. Depending on the exposure route, multiple sub-compartments are added: GI tract with 6 sub-compartments (for oral exposure only, Fig. 3) or skin with the surface compartment and 4 skin sub-compartments (for dermal exposure only, Fig. 2). In total, the model with all the sub-compartments consists of around 20 state variables (organ concentrations) for the parent compound. The sub-compartments serve to account for the complexity of the absorption process (especially the time-lag in absorption). In the GI tract, three different administration types are considered: gavage, drinking rate, and dissolution from matrix. A first order rate of absorption from stomach, small and large intestine and stomach emptying rate are included according to Loizou and Spendiff (Loizou and Spendiff, 2004). The skin is divided into the stratum corneum, viable epidermis and dermis with blood mix. Unidimensional diffusion describes the transport in fine skin and hair follicles according to Fick's second law with specified initial and boundary conditions. The diffusion coefficient is different for the stratum corneum, viable epidermis and hair follicles. Diffusion via hair follicles is considered for caffeine only.

Skin compartments have continuous spatial and temporal concentration gradients, and are described by partial differential equations (PDEs). The other organs/bio-fluids such as lungs, liver, kidney, adipose tissue, highly perfused tissues (heart, brain, GI tract – for dermal absorption only, rest of body), poorly perfused tissues (muscle, bone, skin or its part unaffected by absorption) and blood are single compartments in the three models. These compartments serve as perfusion and accumulation sites. Metabolism is assumed to occur in the liver and skin (for hydroquinone only) and excretion via urine and bile (to a lesser extent) is described by a first order rate constant. However, since liver metabolism is the major elimination route for coumarin, hydroquinone and caffeine, excretion in urine and bile is mainly considered for their metabolites. The equations for models PBTK1-3 are given in Appendix A.

| Table 1 | Physiological parameters for a reference woman and man. |
|---------|--------------------------------------------------------|
| Quantity | Reference woman | Reference man |
| Average body weight [kg] | 65 | 78 |
| Liver weights fractions (fractions of body weight) | 0.026 | 0.026 |
| Adipose tissue | 0.327 | 0.213 |
| Lungs | 0.0105 | 0.012 |
| Kidney | 0.0044 | 0.0044 |
| GI tract | 0.0265 | 0.025 |
| Stomach | 0.00337 | 0.00318 |
| Small Intestine | 0.0146 | 0.0138 |
| Large Intestine | 0.0085 | 0.0080 |
| Poorly perfused tissues + skin | 0.473 | 0.5855 |
| Highly perfused tissues | 0.0676 | 0.0621 |
| Blood | 0.065 | 0.072 |
| Venous blood | 0.04875 | 0.054 |
| Thickness of: | | |
| skin, viable epidermis, stratum corneum [cm] | | |
| Regional blood flow rates [fraction of cardiac output [L/h]] | | |
| Cardiac output [L/h] | 15 BWM\(^{2}\) | |
| Liver | 0.25 | 0.24 |
| Adipose tissue | 0.055 | 0.04 |
| Lungs | 0.025 | 0.025 |
| Kidney | 0.19 | 0.2 |
| Poorly perfused tissues | 0.135 | 0.16 |
| GI tract | 0.14 | 0.13 |
| Fractions of GI tract flow to stomach, small and large intestine | 0.2, 0.6, 0.2 | 0.2, 0.6, 0.2 |
| Skin | 0.05 | 0.05 |
| Highly perfused tissues | 0.155 | 0.155 |

The three models are based on the following assumptions:

(a) Tissues are homogeneous with respect to the concentration of a chemical. Transport between blood and tissues is assumed to be flow-limited (transport barriers between free molecules of chemical in blood and tissue are negligible) and equilibrium between free and bound fractions in blood and tissue is instantaneous.

(b) Inter-individual differences in metabolism and excretion are not explicitly considered. To partially account for such variations, the metabolic rates are corrected by the subject’s body weight.

(c) The diffusion coefficients in the stratum corneum, viable epidermis and hair follicles are assumed constant throughout the skin absorption process. There is no difference in the mathematical equations describing diffusion in PBTK2 and PBTK3 models.

The mathematical equations were programmed in R by combining functionalities of the following R packages: deSolve, ReacTran, PK, FME, rgenoud and AlgDesign. Ordinary differential equations (ODEs) were solved by the method lsoda available in the deSolve package, which switches automatically between stiff and non-stiff methods. The method of lines was used to solve PDEs.

2.1.1. Physiological parameters

All physiological parameters for a reference man and woman that are independent of the chemical and constitute a constant part of the model equations are given in Table 1. The values were taken from the Brown study (Brown et al., 1997).

2.1.2. Compound-specific model parameters and experimental data used for calibration

2.1.2.1. Coumarin. The physiological and ADME parameters of the rat PBTK model for oral absorption were taken from Rietjens et al. (Rietjens et al., 1975, 1977). A single coumarin dose of 0.857 mg/kg BW dissolved in propylene glycol was administered to four healthy subjects. Immediately after dosing, 200 mL of water was given. Dermal absorption data were taken from Ford et al. (Ford et al., 2001). Three male volunteers received a single dermal application of coumarin 0.5 mL of 0.2% solution of 14C-coumarin in 70% ethanol evenly spread on 100 cm\(^2\).
2.1.2.2. Hydroquinone. The physiological and ADME parameters for the rat model and human in vivo blood concentrations were taken from Corley et al. (Corley et al., 2000). In the latter study, a healthy adult male subject received a single dose of 275 mg of hydroquinone in degassed, distilled water at a concentration of 1.0% (w/w). In the dermal absorption experiment, carried out by Wester et al. (Wester et al., 1998) on four human volunteers, a hydroquinone-containing cream formulation (2.5 mg hydroquinone in 0.125 g cream) was placed on a forehead area of 25 cm² for 8 h.

2.1.2.3. Caffeine. Rat physiological parameters were taken from Corley et al. (Corley et al., 2000), whereas absorption and metabolism parameters were optimized with respect to blood concentrations from English and Deisinger (English and Deisinger, 2005). In the latter study, a single dose of 50-mg/kg of hydroquinone was administered via gavage to Fischer 344 rats and mean results were published. Three different oral studies in humans were selected for the model calibration and

Fig. 1. General structure of PBTK1-3 models.

Fig. 2. Skin divided into sub-compartments in PBTK2 (above) and PBTK3 (below) (2-column).
validation. For calibration, plasma concentrations from Lelo et al. (Lelo et al., 1986) and from Csaikja et al. (Csaikja et al., 2005) were used. In the former study, one non-smoking male volunteer ingested 270 mg of caffeine in a gelatin capsule whereas in the latter study, in addition to a gelatin capsule (200 mg of caffeine sulphate administered to 16 subjects), caffeine was applied in the commercial dietary supplement (a mixture containing 200 mg caffeine and 20 mg ephedrine alkaloids, administered to 8 subjects). For model validation, plasma concentrations from the oral study by Newton et al. (Newton et al., 1981) were selected, in which a gelatin capsule containing 300 mg of caffeine was given to one male subject. To calibrate the model for dermal exposure, the experiment of Otberg et al. (Otberg et al., 2008) was used, in which caffeine in the ethanol/propylene glycol vehicle was administered to 6 male volunteers by applying the liquid onto a chest area of 25 cm² for 24 h. In contrast to the models for coumarin and hydroquinone, in the case of caffeine, the additional impact of hair follicles in the overall absorption process was considered.

The experiments for all the three substances were performed on individuals in the fasting state. Tissue-to-blood partition coefficients ($K_{PBTK}$) were either taken from the literature (hydroquinone) or calculated (coumarin, caffeine) according to the Schmitt method (Schmitt, 2008). In this method, steady state tissue-to-plasma partition coefficients are calculated based on: a) the composition of the tissues in terms of water, neutral lipids, neutral and acidic phospholipids, and proteins as physiological parameters; and b) lipophilicity, phospholipid membrane binding, pKa and the unbound fraction in blood plasma as compound specific parameters.

2.2. Evaluation of the goodness-of-fit of the PBTK models

To compare the performance of the models PBTK1-3, the coefficient of determination (R2), mean squared error (MSE) and Akaike’s Information Criterion (AIC) (Kletting and Glattling, 2009) were used. For model discrimination, AIC penalizes models with a large number of parameters when having the same agreement in terms of mean squared error. The model with the lowest algebraic AIC therefore indicates the best agreement with experimental data while minimizing the risk of over fitting. In addition, a corrected AIC (AICc) was applied to adjust for a small number of data points (Kletting and Glattling, 2009).

2.3. Sensitivity analysis

Sensitivity analysis and parameter identifiability were performed to identify the most important and sensitive parameters with respect to the blood/plasma AUC for the case study chemicals according to Soetaert and Petzoldt (Soetaert and Petzoldt, 2010; Soetaert, 2010) prior to their optimization.

In local sensitivity analysis (Eq. (1)), all parameters were evaluated individually in a very small region close to their nominal value. A parameter value divided by the average of simulated outputs was used as a scaling factor (SF).

$$x = f(x, u, \theta) \quad y = g(x, \theta) \quad S_\theta = \left( \frac{\partial y}{\partial \theta} \right)_{x=x_0} \quad S_\theta$$

$$L1 = \frac{\sum |S_\theta|}{N} \quad L2 = \sqrt{\frac{\sum (S_\theta^2)}{N}}$$

$$y' = \frac{1}{\text{min}([1 - S_\theta^2])}$$

where $y'$ is vector of function outputs for a specific variable; $x$ is vector of state variables; $\theta$ is vector of parameters ($\hat{\theta}$ parameter estimate); $u$ is vector of inputs; $N$ is number of time points; $S$ is columns of the sensitivity matrix that correspond to the parameters included in the set; $EV$ is estimation of the eigenvalues.

The following kinetic and compound-specific parameters were analyzed: GI tract absorption rates ($Diss$ or $DF$, $k_{a,m}$, $k_{a,b}$, $k_{b,m}$, $k_{b,a}$), liver metabolic rates ($\beta_m$, $k_{m}$, $k_{max}$), skin absorption parameters ($D_s$, $D_{ve}$, $D_{kg}$, $k_{liq}$, $k_{fat}$, $P_{ve}$, $P_{vev}$, $P_{cart}$), blood-to-plasma ratio and tissue-to-blood partition coefficients: $P_{cart}$, $P_{ve}$, $P_{vev}$, $P_{cart}$, $P_{cart}$, $P_{cart}$, $P_{cart}$, $P_{cart}$, $P_{cart}$, $P_{cart}$.

The higher the absolute sensitivity value, the more important is the parameter. These sensitivity functions are collapsed into summary values ($L1$ and $L2$ are used as selection criteria) (Eq. (2)). Based on the sensitivity functions of blood AUC to selected parameters, the identifiability of a set of parameters to be fine-tuned by calibration is then calculated. As a rule of thumb, a collinearity value ($y'$) less than about 0.8 is regarded “identifiable” (in general, when the collinearity index exceeds 20, the linear dependence is assumed to be critical (Eq. (3))) (Brun et al., 2001; Omlin et al., 2001). The collinearity is a measure of approximate linear dependence between sets of parameters. The higher its value, the more the parameters are related. In this context, “related” means that several parameter combinations may produce similar values of the output variables.

Monte Carlo simulations were used to quantify the impact of variability and uncertainty in parameter distributions separately by drawing parameter values according to a predefined distribution (normally distributed random samples), running the model with each of these parameter combinations, and calculating the values of the selected output variables at each output interval.

Optimization was carried out by applying the Levenberg–Marquardt algorithm to nonlinear data fitting (Moré, 1978).

2.4. Quantitative structure–property relationships for dermal absorption

Quantitative structure–property relationships (QSPRs) for dermal penetration were compiled from the literature. These QSPRs, listed in the supplementary material along with supporting references, comprise: 20 equations for overall skin permeability coefficient ($K_p$), 5 for stratum corneum permeability coefficient ($K_{pcm}$), 5 for viable epidermis permeability coefficient ($K_{pve}$), 10 for stratum corneum/water partition coefficient ($P_{pcm}$), 7 for stratum corneum/viable epidermis partition coefficient ($P_{pcmve}$) and 7 for maximal flux ($j_{max}$). All these quantities can be used as input parameters in PBTK models. The predictions closest to in vitro or in vivo experimental values (if available) were selected. In case no experimental data were available, the median of all predicted results was calculated. As can be seen in the supplementary material, the following physicochemical properties are considered in the QSPR equations: octanol–water partition coefficient, molecular weight and radius, molar volume, hydrogen bond acceptors, hydrogen bond donors, melting point, the number of carbons not involved in a $\equiv\text{O}$ bond, molecular refractivity and Abraham descriptors (linear free energy descriptors).

3. Results

3.1. Parameterization of the PBTK model

3.1.1. Liver metabolism and skin metabolism

Liver metabolism rates were taken from the literature and were optimized with respect to in vivo blood concentrations for the main metabolites only. Table 2 shows the values used together with their reference sources. Metabolism was either assumed to follow Michaelis–Menten kinetics (4) or was described by the first order reaction (5).

$$v_{max} = \frac{C_{in}}{K_m + C_{in}}$$

$$k_m + \frac{C_{in}}{K_m}$$

(4)
Table 2
Metabolites formed in liver and skin.

| Metabolite                  | Organ          | Metabolite of | Metabolic rates        | Reference                   |
|-----------------------------|----------------|---------------|------------------------|-----------------------------|
| o-HPA                       | Liver          | Coumarin      | vmax = BW 12.70; km = 397 | Rietjens et al. (2008)      |
| 7-HC                        | Liver          | Coumarin      | vmax = BW 2.5; km = 0.277 |                             |
| 3-HC                        | Liver          | Coumarin      | Kmet = BW 0.001         |                             |
| o-HPAA                      | Liver          | oHPA          | vmax = BW 144.84; km = 0.136 |                             |
| Benzoxquinone               | Liver          | Hydroquinone  | vmax = BW4.4; km = 5    | Corley et al. (2000)        |
| Hydroquinone                | Liver          | Hydroquinone  | vmax = BW60; km = 5     |                             |
| Gluthationine               | Liver          | Benzoquinone  | vmax = BW20; km = 5     |                             |
| Benzoquinone                | Skin           | Hydroquinone  | vmax = 0.05; km = 4     |                             |
| Paraxanthine                | Liver          | Caffeine      | vmax = 0.351 BW; km = 1 | Lefo et al. (1986)          |
| Theobromine                 | Liver          | Caffeine      | vmax = 0.0432 BW; km = 1| and Zandvliet et al. (2005)|
| Theophylline                | Liver          | Caffeine      | vmax = 0.0072 BW; km = 1|                             |
| 1,3,7-Trimethyluric acid    | Liver          | Caffeine      | Kmet = 0.001 BW         |                             |

* Optimized value.

\[ K_{\text{met}} = \frac{C_{\text{L}}}{P_{\text{C}_{\text{L}}}} \]

where \(C_{\text{L}}\) is the liver concentration and \(P_{\text{C}_{\text{L}}}\) is the liver-to-blood partition coefficient of a substance undergoing metabolism.

In case of skin metabolism (for hydroquinone only), Michaelis–Menten kinetics was also assumed due to lack of experimental data. The equation rates were optimized in such a way that metabolism was less than 10% of the applied dose (Corley et al., 2000; McGregor, 2007; Poet et al., 2010).

3.1.2. Absorption and distribution parameters

The absorption and distribution parameters for parent compounds are given in Table 3. These parameters include literature and optimized values. The literature values comprise: stomach emptying rate, absorption rates (diffusion coefficient in stratum corneum for caffeine, small intestine absorption rate for hydroquinone, etc.) and tissue-to-blood partition coefficients for hydroquinone. QSARs (listed in the supplementary material) were used to calculate median predicted values of diffusion coefficients in viable epidermis and partition coefficients between stratum corneum and viable epidermis. The following parameters were optimized with respect to blood/plasma concentrations:

(a) coumarin: oral data: stomach absorption rate (\(k_{\text{STM}}\)), small intestine absorption rate (\(k_{\text{STI}}\)), metabolic rate to 7-HC (\(v_{\text{MAX}}\)); dermal data: diffusion coefficient in stratum corneum (\(D_{\text{SC}}\)), partition coefficients stratum corneum/vehicle (\(P_{\text{SC}}\)), formulation rate intake (\(k_{\text{FORM}}\)).

(b) hydroquinone: oral data: stomach absorption rate (\(k_{\text{STM}}\)), kinetic constant of stomach emptying rate to small intestine (\(k_{\text{MAX}}\)), metabolic rate to hydroquinone-glucuronide (\(v_{\text{MAX}}\)); dermal data: diffusion coefficient in stratum corneum (\(D_{\text{SC}}\)), partition coefficients stratum corneum/vehicle (\(P_{\text{SC}}\)), formulation rate intake (\(k_{\text{FORM}}\)).

(c) caffeine: oral data: dissolution rate from a tablet (\(D_{\text{ISS}}\)), stomach absorption rate (\(k_{\text{STM}}\)), small intestine absorption rate (\(k_{\text{STI}}\)), metabolic rate to paraxanthine (\(v_{\text{MAX}}\)); dermal data: diffusion coefficient in hair follicles (\(D_{\text{HF}}\)), partition coefficient follicles/vehicle (\(P_{\text{HF}}\)), formulation rate intake in skin (\(k_{\text{FORM}}\)) and hair follicles (\(k_{\text{FOL}}\)).

3.2. PBTK model simulations and goodness-of-fit statistics

Simulated concentration–time profiles in blood or plasma generated by the PBTK3 model following oral and dermal absorption are presented in Fig. 4 for coumarin, Fig. 5 for hydroquinone, and Fig. 6 for caffeine. Experimental data (Exp. Data) are added to the plots together with reference sources. Simulations were carried out either for published individual or group mean data. In the latter case, a so-called mean subject was built based on the mean physiological information provided and used in the model optimization.

Three statistical criteria, the coefficient of determination (R2), mean squared error (MSE) and Akaike’s Information Criterion (AIC) (Kletting and Glattling, 2009), which were used to compare the simulation performance of the models, are given in Tables 4 and 5. This comparison was made for the oral and dermal simulation separately.

3.3. Sensitivity analysis

For coumarin, the AUC of simulated blood/plasma is more sensitive to kinetic parameters compared to tissue-to-blood partition
coefficients. Based on the sensitivity function statistics and Monte Carlo simulations, the most sensitive parameters are found to be intestine absorption rates (ka11, ka12), skin absorption rates (Dxc, Dev, kform) and coumarin metabolic rate to 7-HC. The most sensitive partition coefficients are PCpin, PCppt and PCing (oral model) and PCppt, PClpt and PCing (dermal model).

For hydroquinone, both oral and dermal absorptions tissue-to-blood partition coefficients show lower sensitivity to blood/plasma AUC than kinetic parameters. As expected, the most sensitive parameters are GI tract absorption rates (kmin, kastm, Dt), skin absorption rates (Dxc, Dev, kform) and hydroquinone metabolic rate to hydroquinone glucuronide. Among the most

![Fig. 4. Simulations of coumarin blood concentrations following oral administration for 4 volunteers (left) and plasma concentrations following dermal absorption for 3 volunteers (right) (2-column).](image1)

![Fig. 5. Simulations of hydroquinone blood concentrations after oral administration to 1 volunteer (left) and plasma concentrations following dermal absorption for the “mean” volunteer (right) (2-column).](image2)

**Table 4**

| Compound          | BW [kg] | PBTK3  | PBTK1  |
|-------------------|---------|--------|--------|
|                   | MSEa    | R2b    | AICc   | MSEa    | R2b    | AICc   |
| Coumarin          |         |        |        |         |        |        |
| 70                | 1.245e-05 | 86.736 | -95.325 | 1.234e-05 | 86.855 | -96.477 |
| 46                | 2.356e-05 | 80.369 | -90.861 | 3.514e-05 | 70.727 | -89.154 |
| 68                | 3.149e-05 | 83.245 | -115.407 | 8.435e-05 | 55.129 | -108.688 |
| 87.7              | 4.091e-06 | 96.367 | -133.774 | 2.727e-05 | 79.822 | -120.491 |
| Hydroquinone      |         |        |        |         |        |        |
| 69                | 6.568e-05 | 93.225 | -83.569 | 5.430e-05 | 94.398 | -85.978 |
| Caffeine          |         |        |        |         |        |        |
| 83                | 0.450   | 90.867 | -41.3517 | 4.158   | 15.560 | -18.376 |
| 65                | 1.828   | 20.923 | -18.964 | 7.462   | -22.691 | -7.101 |
| 65                | 1.422   | 38.316 | -24.4098 | 4.078   | -76.849 | -15.7049 |
| 69                | 0.8117  | 85.280 | -34.268 | 5.151   | -72.967 | -8.443 |

a Mean squared error.
b Coefficient of determination, R-squared.
c Akaike’s Information Criterion with the correction for a small number of data points (Kletting and Glatting, 2009).
sensitive partition coefficients are: $PC_{\text{ppt}}, PC_{\text{adp}}, PC_{\text{ing}}$ (oral model) and $PC_{\text{ing}}, PC_{\text{ppt}}, PC_{\text{adp}}$ (dermal model).

For caffeine and the case of oral administration, tissue-to-blood partition coefficients show comparable sensitivity to kinetic parameters in terms of blood/plasma AUC; however, lower sensitivity is observed in the case of dermal absorption. The most sensitive kinetic parameters are caffeine metabolic rate to paraxanthine ($v_{\text{max}}$), skin absorption rates ($D_{\text{sc}}, D_{\text{ve}}, D_{\text{h}}$) and blood-to-plasma ratio. The most sensitive partition coefficients are: $PC_{\text{adp}}, PC_{\text{ppt}}, PC_{\text{kid}}$ (oral model) and $PC_{\text{ing}}, PC_{\text{adp}}, PC_{\text{ppt}}$ (dermal model).

3.4. QSPR predictions of dermal absorption

A compilation of published in vitro, in vivo and QSPR predictions of properties related to skin penetration is given in Table 6.

Table 5
Goodness of fit: dermal model.

| Compound     | BW [kg] | PBTK3  |          | PBTK1  |          | PBTK2  |
|--------------|---------|--------|----------|--------|----------|--------|
|              |         | MSE$^a$ | R$^b$    | AICc   | MSE$^a$ | R$^b$  | AICc   |
|              |         |         |          |        |         |        |        |
| Coumarin     | 78      | 1.274e-05 | 88.973   | −123.225 | 1.009e-04 | 12.658 | −106.809 | 4.379e-05 | 62.108 | −112.971 |
|              | 76      | 1.084e-04 | 59.587   | −103.959 | 1.029e-04 | 61.618 | −106.633 | 3.096e-05 | 88.455 | −116.090 |
|              | 91      | 1.381e-05 | 81.902   | −122.499 | 9.633e-05 | −26.223 | −107.229 | 4.857e-05 | 36.359 | −112.037 |
| Hydroquinone | 60      | 1.292e-05 | 92.862   | −67.192  | 4.938e-05 | 72.722 | −61.008  | 3.221e-05 | 82.209 | −62.797  |
| Caffeine     | 75      | 9.565e-07 | 91.052   | −163.465 | 2.305e-05 | −115.627 | −134.960 | 1.671e-06 | 84.367 | −159.227 |

$^a$ Mean squared error.  
$^b$ Coefficient of determination, $R^2$-squared.  
$^c$ Akaike’s Information Criterion with the correction for a small number of data points (Kletting and Glatting, 2009)
Table 6
Comparison with QSPR-predicted and in vitro/in vivo experimental parameters.

| Substance | Kp | Kp<sub>sc</sub> | Kp<sub>sc</sub> | PC<sub>sc</sub> | PC<sub>sc</sub> | J<sub>max</sub> | %Abs |
|-----------|----|--------------|--------------|--------------|--------------|--------------|------|
| Coumarin  | 0.011<sup>a</sup> | 0.009<sup>a</sup> | –            | –            | –            | 0.172<sup>4e</sup> | 64.4<sup>4e</sup> |
| in vitro  | 0.0025 | 0.003 | 0.020 | 4.530 | 1.829 | 0.0018 | – |
| QSPRs     | 2e-05<sup>a</sup> | 9.33e-06<sup>a</sup> | – | – | – | 0.807<sup>b</sup> | – |
| Hydroquinone | – | – | – | – | – | – | – |
| in vitro  | 2e-05<sup>a</sup> | 9.33e-06<sup>a</sup> | – | – | – | 0.807<sup>b</sup> | – |
| in vivo   | 0.001 | 0.0025 | 0.0017 | 2.113 | 0.800 | 0.0012 | – |
| QSPRs     | 0.003<sup>a</sup> | – | – | – | – | 7.006<sup>a</sup> | 69.4<sup>a</sup> |
| Caffeine  | 1.80e-04<sup>a</sup> | – | – | – | – | 7.006<sup>a</sup> | 69.4<sup>a</sup> |
| in vitro  | 1.05e-04<sup>a</sup> | 3.16 e-04 | 0.0032 | 0.864 | 0.600 | 0.0011 | – |

<sup>a</sup> EDETOX database http://edetox.ncl.ac.uk/.
<sup>b</sup> Ford et al. (2001).
<sup>c</sup> Wester et al. (1998).

Table 7
Rat simulated blood and liver AUC and C<sub>max</sub> for the parent compound and toxic metabolite following NOAEL doses.

| Substance | Rat oral NOAEL dose [mg/kg BW] | AUC liver [mg/L/h] | AUC blood [mg/L/h] | C<sub>max</sub> liver [mg/L] | C<sub>max</sub> blood [mg/L] |
|-----------|-------------------------------|--------------------|--------------------|-----------------------------|-----------------------------|
| Coumarin (O-HPA) | 10, 50 | 2.214, 26.773 (0.0171, 0.130) | 0.326, 3.760 (0.0009, 0.0065) | 8.241, 138.442 (0.062, 0.511) | 0.450, 6.389 (0.0008, 0.0077) |
| Hydroquinone (Benzoquinone) | 20 | 0.819 (0.029) | 0.081 (0.0035) | 1.037 (0.0342) | 0.056 (0.0028) |
| Caffeine | 10.1 | 245.285 | 4.728 | 123.105 | 1.867 |

The table shows predictions closest to experimental values for overall skin permeability coefficient (Kp) and stratum corneum permeability coefficient (Kp<sub>sc</sub>). For the remaining properties, median values were calculated, excluding outliers. In addition, the last column shows literature findings on total absorption percentage in skin (%Abs). For all three substances, the predictions of skin permeation coefficients are close to available experimental values, and therefore so are the median values. Slight errors in estimating the permeability/partition coefficients and maximal flux may arise from the use of water as an application vehicle in tested substances for which QSPR models have been derived. Therefore, calibration of the values is necessary if in vivo dermal data are available. Based on the results for coumarin, hydroquinone and caffeine, the QSPRs prove to be a useful alternative to in vivo skin penetration experiments as they give easily generated predictions close to the observed values and can be taken as suitable starting values for the absorption parameters in the PBTK model. The diffusion and partition coefficients in stratum corneum, were selected for optimization with respect to experimental blood data to provide a better simulation of the in vivo absorption as these parameters are more dependent on the type of vehicle.

3.5. Interspecies extrapolation

The rat PBTK model for oral absorption for the three substances has a simple structure (Rietjens et al., 2008) with a first order rate of absorption in the GI tract, whereas, the human PBTK 3 model includes sub-compartments as described above. Table 7 shows the rat PBTK model results in terms of the simulated AUC and C<sub>max</sub> values at experimentally determined NOAEL doses (application via gavage). Liver and blood concentrations of the parent compound and its main toxic metabolite were examined. The absolute doses applied in the human PBTK model that give AUC and C<sub>max</sub> values in blood and liver comparable to the rat results were estimated (Table 8). Out of these results, the lowest value was chosen and assumed to be the extrapolated human NOAEL value, that is, 8.5 mg/kgBW for coumarin, 1.9 mg/kgBW for hydroquinone, and 2.1 mg/kgBW for caffeine based on the parent compound C<sub>max</sub> or AUC in blood. AUC/C<sub>max</sub> values of toxic metabolites were found to show internal concentrations comparable to those in rat at much higher external doses. The dose conversion of coumarin via o-HPA in blood and liver by the PBTK model at 50 mg/kgBW was found to be very high (the external dose would need to be more than 100 mg/kgBW, therefore it is indicated as higher than 100 in the table). Due to this fact and resulting high NOAEL dose extrapolated to human level, 10 mg/kg BW (dog NOAEL value) was additionally considered and extrapolated to the value of 3.1 mg/kgBW. High nonlinearity in results (AUC and C<sub>max</sub> values) was observed when simulating external doses of 10 and 50 mg/kgBW in rats. This non-linearity could arise for several reasons including: a) wrongly assumed unchanged and constant first rate of absorption from the rat GI tract and interspecies differences in absorption; b) higher delivery of a parent compound to liver than its metabolic clearance.

3.6. Oral to dermal extrapolation

The application conditions, concentrations and vehicles (coumarin: 2 mg/mL in ethanol; hydroquinone: 16 mg/mL in cream; caffeine: 4.562 mg/mL in ethanol/proplylene glycol) for RRR extrapolation were chosen in accordance with the literature.
Table 9

Internal concentrations predicted by oral-to-dermal extrapolation at oral NOAEL doses.

| Substance            | AUC liver | AUC blood | C<sub>max</sub> liver | C<sub>max</sub> blood |
|----------------------|----------|-----------|------------------------|-----------------------|
|                      | Oral     | Dermal    | Oral                   | Dermal                |
| Coumarin (O-HPA)     | 1.222 (3e-05) | 5e-02 (1e-06) | 0.322 (1e-05) | 1.74 (4e-07) | 3.592 (1e-04) | 8e-03 (2e-07) | 0.449 (1e-05) | 0.030 (6e-08) |
| Hydroquinone (benzoquinone) | 0.082 (0.002) | 0.026 (6e-04) | 0.062 (0.002) | 0.532 (6e-04) | 0.091 (0.002) | 6e-04 (1e-05) | 0.045 (0.002) | 0.013 (1.6e-05) |
| Caffeine             | 29.395   | 26.735    | 4.201                  | 8.162                 | 7.434                  | 0.946                  | 0.800                  | 0.285                  |

**Fig. 7.** Oral and dermal human exposure to coumarin (top, left), hydroquinone (top, right) and caffeine (bottom) at single oral NOAEL doses (2-column).

source used in the calibration step with a slight modification of exposure time (2–6 h) and exposed skin area (896 cm²), in order to be consistent for all three substances. As the absolute amount of dose applied refers to estimated NOAEL values, the volume of vehicle had to be increased to ensure unchanged concentrations and consequently the exposed skin area is larger. In the case of coumarin study, only the dose of 3.1 mg/kg BW could be extrapolated (from the dog NOAEL value of 10 mg/kg BW/day) under realistic conditions. Use of the rat NOAEL value (50 mg/kg BW/day) extrapolated to the human (8.5 mg/kg BW) would require very high vehicle volume to maintain the formulation concentration of 2 mg/kg BW applied on the skin. Table 9 provides C<sub>max</sub> and AUC results for blood and liver after oral and dermal absorption. Model simulations for the two exposure routes and parent compounds (only) are presented in Fig. 7.

4. Discussion

In this study, all steps involved in the application of PBTK modelling for RtR extrapolation (to derive dermal threshold levels from oral values) were made. First, the PBTK model parameters were calibrated with respect to available in vivo literature data, the role of QSAR predictions was evaluated for skin penetration, and then the best model structure was selected to simulate the ADME profiles of coumarin, hydroquinone and caffeine.

Finally, the selected PBTK model was used for interspecies (rat to human) extrapolation and to calculate oral and dermal internal concentrations at the external (oral NOAEL) doses under strictly specified conditions.

Calibration was performed using in vivo blood/plasma concentrations of either a single healthy volunteer or mean group results published in the literature. Except for caffeine, the PBTK model could not be validated against different experimental data, which means the applicability domains of the coumarin and hydroquinone models are less certain. In addition, absorption parameters were optimized for a given experimental design (vehicle and administration type, skin site, etc.) and metabolic/elimination rates were subject-specific.

Based on the calibrated parameters in this work, within the investigated dose ranges, coumarin shows the highest absorption rate from stomach and diffusion rates within the skin layers. Hydroquinone is quickly absorbed from the small intestine. Caffeine shows the highest partitioning in the stratum corneum (the most limiting absorption step) and the viable epidermis.

Refined PBTK3 model was compared with the two simpler models (PBTK 1 and 2) in simulating blood/plasma concentrations with respect to available literature in vivo data. The coefficient of
determination \((R^2)\), mean squared error (MSE) and Akaike’s Information Criterion (AIC) \((\text{Kletting and Glattling, 2009})\) were used as decision criteria. As the statistics in Tables 4 and 5 show, the PBTK3 model structure (with sub-compartments in the absorption stage therefore with more parameters to determine) is better in most of the instances \((7/9\) in oral and \(4/5\) in dermal absorption), as indicated by the lower AICc value whenever individual or mean data are available for the same substance. PBTK1 has the advantage of fewer parameters to determine and can easily be set for a given subject but when applied to a different one, the model does not show equally good predictions \((\text{e.g. coumarin oral data})\). In the dermal coumarin experiment, PBTK1 does not give good results, PBTK2 is close to PBTK3 in predictions, but still PBTK3 performs the best. In the hydroquinone oral and dermal experiments, all model structures seem to be good candidates; however in this case there is no comparison between different subjects. The availability of many oral experimental data for caffeine makes it a good test substance for choosing the best model. In addition, different types of dosing were considered, either pure caffeine or its mixture with herbs/substances. PBTK1 does not perform well either in the case of pure caffeine or in case of the mixture. PBTK3 gives good predictions for pure caffeine in a gelatin coating \((\text{Lelo et al., 1986; Newton et al., 1981})\) but performs poorly when applied to the data obtained in the second study \((\text{Csajka et al., 2005})\). It seems likely that either PBTK3 does not work well when applied to mixtures or the experimental results were more variable due to population effects \((\text{e.g. use of contraceptives})\) which cannot be easily captured by the present PBTK model. In the dermal model the inclusion of the hair follicles compartment, in accordance with the experimental design \((\text{Otberg et al., 2008})\), helps obtaining better prediction performance. However, PBTK2, without this compartment, gives also sufficiently good results \((R^2, \text{AICc, MSE})\). Based on these observations, it is concluded that PBTK3 is the best model for the chosen substances and can be used in further applications with higher reliability.

Due to lack of realistic acute toxicity data, rat NOAEL doses \((\text{which are based on repeated dose experiments})\) were used in this study as a surrogate. The rat values were extrapolated to human level prior to \(\text{Rat} \text{Rat} \text{or} \text{Rat} \text{Extrapolation}. \) For the sake of conservative selection, the lowest human exposure doses giving similar internal concentrations, either in terms of \(C_{\text{max}}\) or AUC, to those calculated by the rat PBTK model were chosen. These internal concentrations refer to the parent compound, not a toxic metabolite \((\text{because in humans the metabolites show internal concentrations that are comparable to those of the rat at much higher external doses})\). Assuming that the toxic effects of a parent compound are relatively low \((\text{if any, including caffeine})\), no further correction of extrapolated doses with respect to toxicodynamics was made, even though this has been suggested by some authors \((\text{Bessems and Geraets, 2013})\). More simplistic than the use of PBTK modelling, human equivalent doses can also be calculated according to Eq. (6) \((\text{Reagan-Shaw et al., 2008})\):

\[
\text{HEQ} = \frac{\text{mg}}{\text{kgBW}} = \text{NOEL}_{\text{Rat}} \cdot \frac{K_{\text{mRAT}}}{K_{\text{mHUMAN}}} \tag{6}
\]

where \(K_{\text{mRAT}} \approx 6\) and \(K_{\text{mHUMAN}} \approx \frac{\text{BW}}{1000}\)

This approach gives the following results: \(1.51\) mg/kgBW for coumarin \(2\) times lower than the PBTK model result; for \(3.24\) mg/kgBW for hydroquinone \(1.5\) times higher; and \(1.57\) mg/kgBW for caffeine \(1.3\) times lower. There is a clear difference in both scaling approaches; however it is only the PBTK model that considers differences in biochemical processes between species \((\text{especially metabolism})\) and is thus the more reliable approach.

In order to calculate dermal safe limits for the three substances, the effect of varying absolute dermal dose on AUC and \(C_{\text{max}}\) in blood was investigated. The surface area of exposed skin was increased proportionately with the applied dose. The diffusion coefficients were not changed from the values determined in the calibration step for specific skin sites although the exposed area had to be increased. This is a limitation in the extrapolation, as is the lack of skin absorption data for doses close to the investigated levels. First, dermal exposure levels at NOAEL doses were translated to internal concentrations via the PBTK model and compared with those derived from oral exposure. Then, a cycle of dermal exposure simulations was performed within a range of external absolute doses to establish the dermal threshold value. In the case of hydroquinone, the dermal doses used for simulations were from \(1/10\) to \(5\) times the oral NOAEL value indicating a dermal threshold value based on AUC in blood that is \(9\) times lower than the oral NOAEL dose applied to a skin surface area of \(896/9\) cm\(^2\) \((2\) h of exposure). However, oral \(C_{\text{max}}\) values were always higher. For caffeine, doses ranging from \(1/5\) to \(5\) times the oral NOAEL value were simulated. At the highest dose, the dermal \(C_{\text{max}}\) in blood was close to the oral one but still slightly lower. The dermal threshold value based on the AUC in blood was \(2\) times lower than the oral NOAEL dose applied to a skin surface area equal to \((448.2)/2\) cm\(^2\) \((2\) h of exposure). Coumarin simulations were carried out for dermal doses from \(0.5\) to \(5\) times the oral NOAEL. The dermal safe limit based on coumarin AUC in blood \((6\) h of exposure) was \(1.8\) times higher than the oral NOAEL dose applied to a skin area of \((1.8896)\) cm\(^2\). The same investigations were carried out for liver \(C_{\text{max}}\) and AUC- calculated values for the three substances, but matching of rat internal concentrations would require higher external doses than in case of blood.

5. Conclusions

The use of GI and dermal sub-compartments in the PBTK model gives a better simulation of absorption for the three cosmetic ingredients than a simple model structure. However, the parameters estimated for pure substances may not apply to mixtures. For the three substances studied, QSAR predictions provide a reliable alternative to in vivo dermal penetration experiments, which can be used as reliable input parameters to the dermal PBTK model. Inter-species extrapolation by PBTK modelling gives different results compared with a simple scaling to the human equivalent dose, which takes into account body weight and surface area only. When the same absolute dose is applied orally and dermally, different concentration–time profiles in blood and liver are obtained. Under defined exposure conditions, oral-to-dermal extrapolation at oral NOAEL values indicates that the dermal AUC in blood is higher than the corresponding oral values for hydroquinone and caffeine, and is only slightly lower in the case of coumarin. This indicates that, for a fixed external dose, oral exposure does not always give a higher internal concentration than dermal exposure, especially in the case of low absolute doses.

This study illustrates a strategy for the oral-to-dermal extrapolation of toxicity data based on PBTK modelling using three cosmetic ingredients as case study compounds. This modelling framework could be used to guide the generation of additional experimental data to refine the PBTK models and extend their applicability, with the ultimate aim of applying the models in chemical risk assessment.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

Transparency document

The Transparency document associated with this article can be found in the online version.
Acknowledgements

This research was supported by the Seventh Framework Programme (FP7/2007–2013) COSMOS (Integrated In Silico Models for the Prediction of Human Repeated Dose Toxicity of Cosmetics to Optimize Safety) Project and by Cosmetics Europe.

Appendix A.

PBTK 1: GI tract: 1 compartment with first order rate of absorption

\[
\frac{dA_{abs,GI}}{dt} = -k_{abs,GI} \cdot A_{abs,GI} \quad \text{and} \quad \frac{dA_{abs,GI}}{dt} = 0 \quad (A1.1)
\]

where \( t_{lag} \) is a time lag of absorption in the GI tract.

PBTK 3: GI tract: sub-compartments: stomach content and tissue, small and large intestine content and absorbed quantity

Stomach content:

\[
\frac{dA_{Stm,cont}}{dt} = D_{rt} - k_{Stm} \cdot C_{Stm,cont} - k_{GIT} \cdot A_{Stm,cont} \quad (A1.2)
\]

with \( C_{Stm,cont} = \frac{\text{flow}_{Stm}}{V_{Stm}} \), \( k_{GIT} = \frac{k_{max}}{1 + \text{flow}_{Stm}/V_{Stm}} \)

- admin. via drinking: \( D_{rt} = D \cdot C_{dose} \) and \( C_{dose} = \frac{Dose}{V_{Stm}} \), \( A_{Stm,cont}(t=0) = 0 \)
- admin. via gavage: \( D_{rt} = D \cdot C_{dose} \) and \( A_{Stm,cont}(t=0) = \text{Dose} \)
- admin. via coated tablet: \( D_{rt} = \text{Diss} \cdot \frac{dA_{Stm,cont}}{dt} \) and \( A_{Stm,cont, diss}(t=0) = \text{dose} \), \( A_{Stm,cont}(t=0) = 0 \)

Stomach tissue

\[
\frac{dA_{Stm}}{dt} = k_{abs} \cdot C_{Stm,cont} + f_{rat} \cdot f_{git} \cdot \left( \frac{C_{art} - C_{Stm}}{Pc_{Stm}} \right),
\]

\[
A_{Stm}(t=0) = 0, \quad C_{art} = \frac{A_{art}}{V_{art}}, \quad C_{Stm} = \frac{A_{Stm}}{V_{Stm}} \quad (A1.3)
\]

Small Intestine lumen and absorbed quantity

\[
\frac{dA_{SI, lumen}}{dt} = k_{GIT} \cdot A_{Stm,cont} - (k_{SI} + \text{flow}_{SI}) \cdot C_{SI, lumen},
\]

\[
A_{SI, lumen}(t=0) = 0, \quad C_{SI, lumen} = \frac{A_{SI, lumen}}{\frac{3}{4} \cdot V_{int}} \quad (A1.4)
\]

\[
\frac{dA_{SI}}{dt} = k_{SI} \cdot C_{SI, lumen} + f_{rat} \cdot f_{git} \cdot \left( \frac{C_{art} - C_{SI}}{Pc_{SI}} \right),
\]

\[
A_{SI}(t=0) = 0, \quad C_{SI} = \frac{A_{SI}}{\frac{3}{4} \cdot V_{int}} \quad (A1.5)
\]

Large Intestine lumen and absorbed quantity:

\[
\frac{dA_{LI, lumen}}{dt} = \text{flow}_{LI} \cdot C_{SI, lumen} - (k_{el} + k_{LI}) \cdot C_{LI, lumen},
\]

\[
A_{LI, lumen}(t=0) = 0, \quad C_{LI, lumen} = \frac{A_{LI, lumen}}{\frac{1}{4} \cdot V_{int}} \quad (A1.6)
\]

\[
\frac{dA_{LI}}{dt} = k_{LI} \cdot C_{LI, lumen} + f_{rat} \cdot f_{git} \cdot \left( \frac{C_{art} - C_{LI}}{Pc_{LI}} \right),
\]

\[
A_{LI}(t=0) = 0, \quad C_{LI} = \frac{A_{LI}}{\frac{1}{4} \cdot V_{int}} \quad (A1.7)
\]

PBTK 1: Skin: 1 compartment with first order rate of absorption

\[
\text{if}(t > t_{lag}) \quad \frac{dA_{abs,skin}}{dt} = -k_{abs,skin} \cdot C_{abs,skin} \quad \text{else} \quad \frac{dA_{abs,skin}}{dt} = 0,
\]

\[
C_{abs,skin} = \frac{A_{abs,skin}}{V_{abs,skin}}, \quad V_{abs,skin} = \text{Area} \cdot L \quad (A1.8)
\]

where \( t_{lag} \) is a time lag of absorption in the skin.

PBTK 2: Skin: 2 compartments: one with Fickian diffusion (\( E = \text{stratum corneum + viable epidermis} \)) and one with blood mix.

\[
\frac{dC_{Abs,E,j}}{dt} \approx -q_{Abs,E,j-1} + 2 \cdot q_{Abs,E,j} + q_{Abs,E,j+1} - \frac{\text{BioC}_{Abs,E,j}}{V_{Abs,E,j}},
\]

\[
j = 1 : N, \quad C_{Abs,E,j} = \frac{A_{Abs,E,j}}{V_{Abs,E,j}} \quad (A1.9)
\]

If skin metabolism occurs, it is assumed to follow Michaelis–Menten kinetics:

\[
\text{BioC}_{Abs,E,j} = \text{max}_{j=N}, \quad \text{otherwise BioC}_{Abs,E,j} = 0
\]

Initial and boundary conditions:

\[
C_{Abs,E}(t=0)_{E,0} = 0
\]

\[
\frac{dC_{Abs,E}}{dt} \quad (t > 0)_{\text{vap},0} = k_{abs,form} \cdot C_{form}
\]

\[
C_{Abs,E}(t > 0)_{\text{vap},0} = P_{C_{skin}} \cdot C_{skin}
\]

\[
C_{Abs,E}(t > 0)_{\text{vap},0} = P_{C_{skin}} \cdot C_{form}
\]

where \( P_{C_{skin}} \) is skin-to-blood partition coefficient and PCE is a partition between E and vehicle.

Dermis and Mix with the blood:

\[
\frac{dA_{Skin}}{dt} = f_{skin} \cdot \left( C_{art} - C_{Skin} \right) + q_{Abs,skin,j=N} \cdot \text{Area}, \quad A_{skin}(t=0) = 0 \quad (A1.10)
\]

PBTK 3: Skin: sub-compartments: skin surface, stratum corneum, viable epidermis, hair follicles

Skin surface:

\[
\frac{dC_{form}}{dt} = \text{DesRate} - \text{AbsRate} - \text{EvRate} \quad C_{form} \quad \frac{A_{form}}{V_{0, appliedForm}} \quad (A1.11)
\]

or \( (t < t_{appl}) \) \text{AbsRate} = \text{DesRate} = \text{EvRate} \quad \text{AbsRate} = \text{DesRate} = \text{EvRate}

and \( (t > t_{appl}) \) \text{AbsRate} = 0 \quad (A1.12)

where \( t_{appl} \) is the application time of a formulation. We assume that the deposition rate is instantaneous (DesRate = 0).

Evaporation of a solute (EvRate) is calculated for volatile substances (here vehicles) according to [Tibaldi et al., 2011]:

\[
\text{EvRate} = -\frac{kev}{T}\quad (A1.13)
\]

For a vehicle (solvent) this evaporation is quantified in terms of decrease of an applied solution volume in time rather than mass of the solvent:

\[
\frac{dV_{appliedForm}}{dt} = -\frac{kev \cdot \text{Area}}{1000 \cdot \sigma_{form}} \quad V_{appliedForm}(t=0) = V_{0, appliedForm} \quad (A1.14)
\]
where
\[ k_{	ext{exp}} = \beta \cdot \frac{M \cdot V_p}{K \cdot T \cdot 10} \left[ \frac{\text{mg}}{h \cdot \text{cm}^2} \right] \]
\[ \beta = 0.0111 \cdot 10^{-0.06} \cdot \frac{D_p^{0.19}}{\gamma^{0.15}} \cdot \chi^{0.04} \]
where \( \beta \) is the mass transfer coefficient in vapour phase (m h\(^{-1} \)), \( M \) the molecular weight, \( V_p \) the vapour pressure of the liquid at skin temperature (Pa), \( T \) the gas constant in J mol\(^{-1} \) K\(^{-1} \), \( T \) the skin temperature (Assumed to be 303 K), \( V_{air} \), the velocity of air (at workplaces it ranges from 0.3 – 0.6 m s\(^{-1} \)), \( D_t \) the diffusivity of the liquid in gas phase (range: 0.03 to 0.06 m\(^2\) h\(^{-1} \)), the kinematic viscosity of air (Literature value of 0.054 m\(^2\) s\(^{-1} \)), \( \gamma \) the length of evaporation area in the direction of air stream, \( TL \) a thickness of applied substance layer (cm), \( Area \) the application area (cm\(^2\)) and \( \delta_{\text{form}} \) the density of solvent (g cm\(^{-3}\)).

Stratum Corneum (SC):
\[ \frac{dC_{SC,i}}{dt} \approx -q_{SC,i+1} - \frac{q_{SC,i+1} - 2 \cdot q_{SC,i} + q_{SC,i-1}}{L_{SC}}; i = 1 : N, \quad C_{SC,i} = \frac{A_{SC,i}}{V_{SC,i}} \]

\[ (A1.15) \]

where \( q_{SC,i} = -D_{SC} \cdot \frac{C_{SC,i+1} - C_{SC,i} + C_{SC,i+1}}{L_{SC}}; i = 1 : N \)

Initial and boundary conditions:
\[ C_{SC}(t = 0)_{0 < x < L_{SC}} = 0 \]
\[ \frac{dC_{SC}(t > 0)_{x=0}}{dx} = k_{a \text{form}} \cdot C_{\text{form}} \]
\[ \frac{dC_{SC}(t > t_{\text{app}})_{x=0}}{dx} = 0 \]
\[ C_{SC}(t > 0)_{x=L_{SC}} = PC_{SC} \cdot C_{\text{form}} \]
\[ C_{SC}(t > 0)_{x=L_{SC}} = PC_{SCVE} \cdot C_{VE} \]

Viable Epidermis (VE):
\[ \frac{dC_{VE,j}}{dx} \approx -q_{VE,j+1} - 2 \cdot q_{VE,j} + q_{VE,j-1}}{V_{VE,j}}; j = 1 : M, \]
\[ C_{VE,j} = \frac{A_{VE,j}}{V_{VE,j}} \]

\[ (A1.16) \]

where
\[ q_{VE,j} = -D_{VE} \cdot \frac{C_{VE,j+1} - 2 \cdot C_{VE,j} + C_{VE,j-1}}{L_{VE}}; j = 1 : M \]

\[ M \text{ and } BioC_{VE,j} = \frac{\text{max}_{n} C_{Ve,i} \cdot v_{En}}{\text{max}_{m} C_{Ve,i} \cdot v_{En}} \text{ (for hydroquinine only)} \]

Initial and boundary conditions:
\[ C_{VE}(t = 0)_{0 < y < L_{VE}} = 0 \]
\[ C_{VE}(t > 0)_{y=L_{VE}} = PC_{SCVE} \cdot C_{SC} \]
\[ D_{SC} \frac{dC_{SC}(t > 0)_{y=L_{SC}}}{dy} = D_{VE} \cdot \frac{dC_{VE}(t > 0)_{y=0}}{dy} \]
\[ C_{VE}(t > 0)_{y=L_{VE}} = PC_{skn} \cdot C_{skn} \]

Dermis and mix with the blood:
\[ \frac{dA_{skn}}{dt} = -\frac{3}{4} \cdot f_{skn} \cdot \left( C_{art} - \frac{C_{skn}}{PC_{skn}} \right) + \frac{q_{VE,j=M}}{V_{skn}} \cdot \frac{100 - nf}{100} \cdot Area, \]
\[ A_{skn}(t = 0) = 0, \quad C_{skn} = \frac{A_{skn}}{10^{-3} \cdot \frac{V_{skn}}{V_{ve}}} \]

\[ (A1.17) \]

We assume that the fine skin covers 2/3 of the dermis, whereas hair follicles 1/3 and 3/4 of the overall blood flow goes to the fine skin.

Hair follicles compartment (Bookout et al., 1997):
\[ \frac{dC_{hf,i}}{dt} \approx -q_{hf,i+1} - 2 \cdot q_{hf,i} + q_{hf,i-1}}{V_{hf}}; i = 1 : K, \]
\[ L_{hf} = \frac{388}{560} L \]

\[ (A1.18) \]

The initial and boundary conditions:
\[ C_{hf}(t = 0)_{0 < x < L_{hf}} = 0 \]
\[ \frac{dC_{hf}(t > 0)_{x=0}}{dt} = k_{a \text{hf}} \cdot C_{\text{form}} \]
\[ D_{hf} \cdot \frac{dC_{hf}(t > t_{\text{app}})_{x=0}}{dt} = 0 \]
\[ C_{hf}(t > 0)_{x=L_{hf}} = PC_{hf} \cdot C_{\text{form}} \]
\[ C_{hf}(t > 0)_{x=L_{hf}} = PC_{skn} \cdot C_{skn} \]

Mix with a blood:
\[ \frac{dA_{skn}}{dt} = \frac{1}{4} \cdot f_{skn} \cdot \left( C_{art} - \frac{C_{skn}}{PC_{skn}} \right) + \frac{q_{hf,i=K}}{V_{skn}} \cdot \frac{nf}{100} \cdot Area, \]
\[ A_{skn}(t = 0) = 0, \quad C_{skn} = \frac{A_{skn}}{10^{-3} \cdot \frac{V_{skn}}{V_{ve}}} \]

\[ (A1.19) \]

The rest of compartments/tissues are common for PBTK1, PBTK2 and PBTK 3:

Adipose tissue (adp), highly perfused tissues (hpt) and poorly perfused tissues (ppt):
\[ \frac{dA_{org}}{dt} = f_{org} \cdot \left( C_{art} - \frac{C_{skn}}{PC_{skn}} \right), \quad A_{org}(t = 0) = 0, \quad C_{art} = \frac{C_{art}}{V_{art}} \]
\[ C_{org} = \frac{A_{org}}{V_{org}} \]

where org = organ name (adp, hpt, ppt)
Kidney
\[ \frac{dA_{kid}}{dt} = f_{kid} \cdot \left( C_{art} - \frac{C_{kid}}{PC_{kid}} \right) - CLR \cdot \frac{C_{kid}}{PC_{kid}} \]

\[ (A1.20) \]

Liver
\[ \frac{dA_{liv}}{dt} = Fl_{\text{GIF}} + FORM_{liv} + f_{liv} \cdot \left( C_{art} - \frac{C_{liv}}{PC_{liv}} \right) - MET_{liv} \]
\[ A_{liv}(t = 0) = 0, \quad C_{liv} = \frac{A_{liv}}{V_{liv}} \]

\[ (A1.22) \]

For a parent compound:
GI tract with 1 compartment: \( Fl_{\text{GIF}} = dA_{Abn,Gl} \)
GI tract with sub-compartments: \( Fl_{\text{GIF}} = f_{ra} \cdot f_{gat} \cdot \frac{C_{lim}}{PC_{lim}} + f_{rb} \cdot f_{gat} \cdot \frac{C_{skn}}{PC_{skn}} + f_{rin} \cdot f_{gat} \cdot \frac{C_{skn}}{PC_{skn}} \)
FORM\(_{liv} = 0 \) (rate of formation of metabolites)
Venous blood:
\[ \frac{dA_{ven}}{dt} = f_{liv} \cdot \frac{C_{liv}}{PC_{liv}} + f_{ppt} \cdot \frac{C_{ppt}}{PC_{ppt}} + f_{hpt} \cdot \frac{C_{hpt}}{PC_{hpt}} + f_{adp} \cdot \frac{C_{adp}}{PC_{adp}} \]
\[ + f_{kid} \cdot \frac{C_{kid}}{PC_{kid}} + Fl_{skn} - f_{cre} \cdot C_{ven}, \quad A_{ven}(t = 0) = 0, \quad C_{ven} = \frac{A_{ven}}{V_{ven}} \]

\[ (A1.23) \]

For dermal absorption only:
Skin with 1 compartment (PBTK1): \( Fl_{skn} = dA_{Abn,skn} \)
Skin with 2 compartments (PBTK2): \( Fl_{skn} = f_{skn} \cdot \frac{C_{skn}}{PC_{skn}} \)
Skin with sub-compartments (PBTK3): \( Fl_{skn} = f_{skn} \cdot \frac{C_{skn}}{PC_{skn}} + \frac{1}{4} \cdot f_{skn} \cdot \frac{C_{skn}}{PC_{skn}} \)

Plasma quantification: \( C_{ven,pl} = \frac{A_{ven}(0.55 \cdot V_{ven})}{K_{hf}} \)
where: RBP is blood-to-plasma partition coefficient.

Arterial blood and lungs:

\[
\frac{dC_{\text{Ing}}}{dt} = f_{\text{Ing}} \left( C_{\text{ven}} - \frac{C_{\text{Ing}}}{PC_{\text{Ing}}} \right), \quad C_{\text{Ing}}(t = 0) = 0, \quad C_{\text{Ing}} = \frac{A_{\text{Ing}}}{V_{\text{Ing}}} \tag{A1.24}
\]

\[
\frac{dA_{\text{art}}}{dt} = f_{\text{cref}} \left( \frac{C_{\text{Ing}}}{PC_{\text{Ing}}} - C_{\text{art}} \right), \quad A_{\text{art}}(t = 0) = 0 \tag{A1.25}
\]

**Appendix B. Supplementary data**

Supplementary data associated with this article can be found, in the online version, at [http://dx.doi.org/10.1016/j.toxlet.2014.03.013](http://dx.doi.org/10.1016/j.toxlet.2014.03.013).

**References**

Besems, J.G.M., Geraets, L., 2013. Proper knowledge on toxicokinetics improves human hazard testing and subsequent health risk characterisation. A case study approach. Regul. Toxicol. Pharmacol. 67, 325–334.

Bookout Jr., R.L., Quinn, D.W., McDougall, J.N., 1997. Parallel dermal subcompartmental modelling for chemical absorption. SAR QSAR Environ. Res. 7, 259–279.

Born, S.L., Caudill, D., Smith, B.J., Lehman-Mckeen, L.D., 2000. In vitro kinetics of coumarin 3.4-epoxidation: application to species differences in toxicity and carcinogenicity. Toxicol. Sci. 58, 23–31.

Brent, R.L., Christian, M.S., Diener, R.M., 2011. Evaluation of the reproductive and developmental risks of caffeine. Birth Defects Res. B: Dev. Reprod. Toxicol. 92, 152–187.

Brown, R.P., Delp, M.D., Lindstedt, S.L., Romberg, L.R., Beliles, R.P., 1997. Physiological parameter values for physiologically based pharmacokinetic models. Toxicol. Ind. Health 13, 407–484.

Brun, R., Reichert, P., Künisch, H.R., 2001. Practical identifiability analysis of large environmental simulation models. Water Resour. Res. 37, 1015–1030.

Chiu, W.A., White, P., 2006. Steady-state solutions to PBPK models and their applications to risk assessment I: Route-to-route extrapolation of volatile chemical. Risk Anal. 26, 769–780.

Corley, R.A., English, J.C., Hill, T.S., Fiorica, L.A., Moggert, D.A., 2000. Development of a physiologically based pharmacokinetic model for hydroquinone. Toxicol. Appl. Pharmacol. 165, 163–174.

Csaıá, C., Haller, C.A., Benowitz, N.L., Verotta, D., 2005. Mechanistic pharmacokinetic modelling of ephedrine, nor Ephedrine and caffeine in healthy subjects. Br. J. Clin. Pharmacol. 59, 335–345.

EFSA, 2004. Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contacts with food (AFC) on a request from the commission related to coumarin. Question number EFSA-Q-2003-118. EFSA J. 104, 1–36.

English, J.C., Deissinger, P.J., 2005. Metabolism and disposition of hydroquinone in Fischer 344 rats after oral or dermal administration. Food Chem. Toxicol. 43, 483–493.

Ford, R.A., Hawkins, D.R., Mayo, B.C., Api, A.M., 2001. In the vivo dermal absorption and metabolism of [4-14C]coumarin by rats and by human volunteers under simulated conditions of use in fragrances. Food Chem. Toxicol. 39, 153–162.

Ginsberg, G., Hattis, D., Russ, A., Sonawane, B., 2004. Physiologically based pharmacokinetic (PBPK) modelling of caffeine and theophylline in neonates and adults: Implications for assessing children’s risk from environmental agents. J. Toxicol. Environ. Heal. A 67, 297–329.

Hansen, S., Henning, A., Naege, A., Hesse, M., Wittum, G., Neumann, D., Kostka, K.H., Zbytovska, J., Lehr, C.M., Schaefer, U.F., 2008. In silico model of skin penetration based on experimentally determined input parameters. Part I. Determination of partition and diffusion coefficients. Eur. J. Pharm. Biopharm. 68, 352–367.

Kenyon, E.M., 2012. Interspecies extrapolation. Methods Mol. Biol. 929, 501–520.

Klouft, P., Glätting, G., 2009. Model selection for time-activity curves: the corrected (EC) test and the information criterion and the pseudo-F (pF) test. Int. J. Med. Phys. 19, 200–206.

Lelo, A., Birkett, D.J., Robson, R.A., Minors, J.O., 1986. Comparative pharmacokinetics of caffeine and its primary demethylated metabolites paraxanthine, theobromine and theophylline in man. Br. J. Clin. Pharmacol. 22, 177–182.

Loizou, G.D., Spendifft, M., 2004. A human PBPK model for ethanol describing inhibiti on of gastric motility. J. Mol. Histol. 35, 687–696.

McGregor, D., 2001. Hydroquinone: an evaluation of the human risks from its carcinogenic and mutagenic properties. Crit. Rev. Toxicol. 37, 887–914.

Mielke, H., Abraham, K., Götz, M., Vieth, B., Lampen, A., Luch, A., Gandert-Remy, U., 2011. Physiologically based toxicokinetic modelling as a tool to assess target organ toxicity in route-to-route extrapolation – the case of coumarin. Toxicol. Lett. 202, 100–110.

Moré, J.J., 1978. The Levenberg–Marquardt algorithm: implementation and theory. Lect. Notes Math. 630, 105–116.

Newton, R., Broughton, L.J., Lind, M.J., Morrison, P.J., Rogers, H.J., Bradbrook, I.D., 1981. Plasma and salivary pharmacokinetics of caffeine in man. Eur. J. Clin. Pharmacol. 21, 45–52.

OECD SIDS, 1996. Hydroquinone.

OECD SIDS, 2002. Caffeine: SIDS Initial assessment report for SIAM 14.

Omlin, M., Reichert, P., Forster, R., 2001. Biogeochemical model of Lake Zürich: model equations and results. Ecol. Model. 141, 77–103.

Otberg, N., Patzelt, A., Rasulev, U., Hagemeister, T., Linscheid, M., Sinkgraven, R., Sterry, W., Lademann, J., 2008. The role of hair follicles in the percutaneous absorption of caffeine. Br. J. Clin. Pharmacol. 65, 488–492.

The European Parliament and the Council of the European Union, 2009. REGULATION (EC) No 1223/2009 on cosmetic products. Off. J. Eur. Union.

Poet, T.S., Carlton, B.D., Deyo, J.A., Hinderliter, P.M., 2010. Hydroquinone PBPK model refinement and application to dermal exposure. Food Chem. Toxicol. 48, 3085–3092.

Reagan-Shaw, S., Nihal, M., Ahmad, N., 2008. Dose translation from animal to human studies revisited. FASEB J. 22, 659–661.

Rietjens, I.M.C.M., Boersma, M.G., Zaleska, M., Punt, A., 2008. Differences in simulated liver concentrations of toxic coumarin metabolites in rats and different human populations evaluated through physiologically based biokinetic (PBPK) modeling. Toxicol. Vitr. 22, 1890–1901.

Ritschel, W.A., Brady, M.E., Tan, H.S.I., 1979. First-pass effect of coumarin in man. Int. J. Clin. Pharmacol. Ther. Toxicol. 17, 95–103.

Ritschel, W.A., Brady, M.E., Tan, H.S.I., Hoffmann, K.A., Yu, I.M., Grummeck, K.W., 1977. Pharmacokinetics of coumarin and its 7-hydroxy-metabolites upon intravenous and peroral administration of coumarin in man. Eur. J. Clin. Pharmacol. 12, 457–461.

Schmitt, W., 2008. General approach for the calculation of tissue to plasma partition coefficients. Toxicol. Vitr. 22, 457–467.

Sofaert, K., 2010. R package FME: in vitro modelling, sensitivity, Monte Carlo – applied to a dynamic simulation model.

Sofaert, K., Petzoldt, T., 2010. Inverse modelling, sensitivity and Monte Carlo analysis in R using package FME: J. Stat. Softw. 33, 1–28.

Thorn, C.F., Akilili, E., McDonagh, E.M., Klein, T.E., Altman, R.B., 2012. PharmGKB summary: Caffeine pathway. Pharmacogenet. Genomics 22, 389–395.

Tibaldi, R., Ten Berge, W., Drolet, D., 2011. IH SkinPerm Help Manual.

Wester, R.C., Melendres, J., Hui, X., Cox, R., Serranzana, S., Zhai, H., Quan, D., Maibach, H.I., 1998. Human in vivo and in vitro hydroquinone topical bioavailability, metabolism, and disposition. J. Toxicol. Environ. Heal. A 54, 301–317.

Zandenvelt, A.S., Huitema, A.D.R., De Jonge, M.E., Den Hoed, R., Sparidans, R.W., Hendriks, V.M., Van Den Brink, W., Van Ree, J.M., Beijnen, J.H., 2005. Population pharmacokinetics of caffeine and its metabolites theobromine, paraxanthine and theophylline after inhalation in combination with diacetylmorphine. Basic Clin. Pharmacol. Toxicol. 96, 71–79.