Assessing the safety of cosmetic chemicals: Consideration of a flux decision tree to predict dermally delivered systemic dose for comparison with oral TTC (Threshold of Toxicological Concern)

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ABSTRACT

Threshold of Toxicological Concern (TTC) aids assessment of human health risks from exposure to low levels of chemicals when toxicity data are limited. The objective here was to explore the potential refinement of exposure for applying the oral TTC to chemicals found in cosmetic products, for which there are limited dermal absorption data. A decision tree was constructed to estimate the dermally absorbed amount of chemical, based on typical skin exposure scenarios. Dermal absorption was calculated using an established predictive algorithm to derive the maximum skin flux adjusted to the actual 'dose' applied. The predicted systemic availability (assuming no local metabolism), can then be ranked against the oral TTC for the relevant structural class. The predictive approach has been evaluated by deriving the experimental/prediction ratio for systemic availability for 22 cosmetic chemical exposure scenarios. These emphasise that estimation of skin penetration may be challenging for penetration enhancing formulations, short application times with incomplete rinse-off, or significant metabolism. While there were a few exceptions, the experiment-to-prediction ratios mostly fell within a factor of 10 of the ideal value of 1. It can be concluded therefore, that the approach is fit-for-purpose when used as a screening and prioritisation tool.

1. Introduction

1.1. Background to the Threshold of Toxicological Concern

The Threshold of Toxicological Concern (TTC) concept originates from the United States Food and Drug Administration's (U.S. FDA's) development and application of the Threshold of Regulation (US Food and Drug Administration 1982; 1993a; 1993b]. The approach built on Frawley's (1967) attempt to "determine a level of use of any food-packaging component which could be considered to be safe, regardless of its degree of toxicity" and was initially intended to deal with indirect food additives that pose a negligible risk. The TTC concept proposes that de minimis exposure values can be established for many chemicals, including those of unknown toxicity, based on comparison with the known toxicity of a wide range of compounds (Kroes et al., 2004). By assigning chemicals to

three structural classes following the decision tree method of Cramer et al. (1976) and plotting chronic oral no-observed-effect levels (NOELs) from animal data for the most sensitive systemic toxicological endpoint, Munro et al. (1996, 1999) demonstrated that toxicity varies significantly as a function of structural class. An uncertainty factor of 100 was applied to the 5th percentile NOEL of each Cramer class, and the resulting conservative TTC values are intended to represent exposure thresholds below which there is no appreciable risk to human health over a lifetime of daily exposure for chemicals of that class. TTC provides threshold toxicity values for oral dosing in structural categories as Cramer classes I, II and III (1800, 540, and 90 µg/d or 30, 9 and 1.5 µg/kg/d) respectively. In some cases the oral NOEL derived from animal data might fail to predict human toxicity for example where metabolism and uptake differ between species.

The TTC concept has the potential to provide a pragmatic, transparent, consistent and scientifically sound approach to the prioritisation of chemicals. TTC utilises different systemic endpoints, such as developmental and reproductive toxicity, immunotoxicity and neurotoxicity (Barlow, 2005; Mueller et al., 2008).

An expert group organised by the European cosmetic industry association studied the utility of the TTC approach in the safety evaluation of cosmetic ingredients and end products. They considered a number of issues related to the chemical nature and effects of ingredients and their exposure when used as cosmetics, including differences in metabolism between the dermal and oral routes of application, and default adjustment factors for topically applied cosmetics. The expert group concluded that, "overall the TTC approach provides a useful additional tool for the safety evaluation of cosmetic ingredients and impurities of known chemical structure in the absence of chemical-specific toxicology data" (Kroes et al., 2007).

Kroes et al. (2007) evaluated 58 Cramer class III chemicals with no observed adverse effect level (NOAEL) values of 1 mg/kg or less. Also, the data were evaluated to determine whether the oral-route toxicity could be used to predict dermal-route toxicity more accurately by including metabolism in the liver (systemic) and first pass oral versus dermal metabolism.

In 2012, three independent non-food Scientific Committees of the European Commission were jointly tasked with evaluating potential applications of the TTC approach for human health risk assessment of chemical substances (European Commission, 2012, 2013). Their opinion focused on the potential applications of the TTC concept for cosmetics and other consumer products in relation to their mandates. The Scientific Committees considered the TTC approach, in general, "scientifically acceptable for human health risk assessment of systemic toxic effects caused by chemicals present at very low levels, as based on sound exposure information". However, they emphasised the need for a high level of confidence in (1) the quality and completeness of the toxicity databases; (2) the reliability of the exposure data for the intended use of the chemical; and (3) the appropriateness of any extrapolations in order to apply the TTC approach in risk assessment. The safety of dermal exposure can be assessed with confidence by extrapolation from oral toxicity data with refinement of bioavailability rather than assuming 100% absorption, thus increasing both the reliability and regulatory acceptance of the TTC concept for cosmetic safety assessment. Recently TTC has been re-evaluated and recommended as a valid screening tool by the European Food Safety Authority & World Health Organization (2015).

1.2. Cosmetic use patterns

Cosmetic products are common to every household in most regions and cultures of the world and their use is driven mainly by our interest in personal appearance, hygiene, or feelings of well-being. Depending on the reason for which a cosmetic product is selected and used, the exposure profiles of its ingredients will generally be predictable and serve as the basis for quantifying the exposure to the cosmetic product. It is obvious that for cosmetics applied to skin the percutaneous absorption of ingredients needs to be considered, whereas for oral hygiene products such as mouth-rinses and lip care products, different considerations of the exposure routes are required to make a complete safety assessment.

Dermal exposure to cosmetic products can be categorised as leave-on, in which the product remains on the application site for its intended period of use, or rinse-off, where the products are applied and shortly thereafter, rinsed away. Examining general use patterns of cosmetic products reveals variable frequencies of use per day, ranges of daily applied amounts, and differing periods of a product's use, for example, seasonally for sunscreens, monthly for permanent hair colour products, weekly for exfoliating cosmetics, or daily for moisturisers. The use frequency is also influenced by other factors such as variation in consumer preference (daily use of day-creams containing UV filters), marketing (local versus global advertising), changes in personal preferences, or product discontinuations. While general use patterns can be shown, the exposure assessment will usually require a case-by-case approach to account for the factors pertinent to the specific cosmetic ingredient being evaluated.

Exposure estimation is a key part of the safety assessment for cosmetic products and their ingredients. Indeed, it is the exposure that is factored together with the hazard data to characterise the potential risk of an adverse outcome that might arise from using cosmetic ingredients. The advantage and principal basis for using the TTC approach for risk characterisation lies in its use for chemicals for which adequate systemic hazard (toxicity) data are not available.

1.3. Dermal absorption and oral-to-dermal extrapolation

The application of TTC has commonly assumed oral uptake and systemic exposure as the toxicity data are derived following oral dosing. The default assumption (comparison with the unadjusted oral TTC) assumes 100% oral bioavailability.

The different characteristics of dermal exposure and absorption from oral ingestion mean that consideration of route-to-route extrapolation offers an important refinement when applying the oral TTC for cosmetics which are topically applied. Following topical application, absorption is generally lower compared to oral administration, as a result of the barrier function of the skin's outer layer (the stratum corneum (SC)), relative to the efficient absorption which takes place through the epithelium of the gastrointestinal (GI) tract (e.g. Scheuplein, 1971; Karadzovska et al., 2013). In terms of systemic exposure, blood levels of a topically applied chemical are typically much lower and take a longer time to reach a maximum concentration than when orally dosed. Furthermore for lipophilic molecules, a reservoir may form in the SC from which the release is prolonged.

Differences in bioavailability between dermal and GI routes may also be influenced by differential first pass metabolism of the absorbed molecule and specific transport processes in the GI tract and skin. Quantitatively, first pass metabolism is generally less in the skin (basal membrane) than the GI epithelium (Williams, 2008). Xenobiotic metabolising enzymes for which differences in GI and skin levels may be significant and influence absorption of the parent molecule include esterases, conjugating enzymes, alcohol dehydrogenases and acetyl transferases. Cytochrome p450 enzymes present at low levels extra-hepatically generally do not reduce absorption of the parent molecule (Kroes et al., 2007;

Wilkinson and Williams, 2008). For cosmetics, physiologically based pharmacokinetic modelling with example molecules has indicated that at the oral NOEL exposure level systemic bioavailability may be greater following dermal exposure compared to oral for caffeine and hydroquinone (Gajewska et al., 2014). An advantage of transdermal drug delivery compared to oral is that it avoids pre-systemic metabolism and that differences in the metabolic profile, relative to that seen post-oral dosing can be anticipated (Wiedersberg and Guy, 2014). Targeting drugs through the skin can result in increased bioavailability with reduced metabolism and greater efficacy but this is not generally associated with toxicity. Systemic bioavailability following dermal exposure might be higher than following oral for some chemical molecules but there is limited evidence of increased toxicity. There could be higher systemic exposure to chemical molecules that are corrosive and damage the stratum corneum.

Available quantitative information on the uptake of cosmetic chemicals across the skin has most often been derived from *in vitro* studies where steady-state conditions often apply. Absorption information is usually given as the skin permeability coefficient, kp, (in $cm\,h^{-1}$ or $cm\,s^{-1}$) representing steady state flux normalised by the concentration gradient. Flux is also often quoted in $\mu g\,cm^{-2}\,h^{-1}$ or $mol\,cm^{-2}\,h^{-1}$ and the maximum flux, J_{max} can be calculated from knowledge of the k_p and the saturation solubility of the chemical in the vehicle used (the majority of measurements being in aqueous solution). Although less frequently reported than k_p, J_{max} is ultimately more useful for risk assessment and is independent of the vehicle, provided that the vehicle is saturated with the solute and therefore providing equivalent thermodynamic activity (Zhang et al., 2009). After a century of research, there is still incomplete validation of how to use *in vitro* derived dermal flux data to predict systemic bioavailability and therefore toxicity. In particular a number of points must be noted when using J_{max} to characterise dermal absorption of chemicals from cosmetics. Firstly, many cosmetics are applied as a finite dose of small volume with the concentration of chemical ingredient well below saturation in the vehicle. As a result, significant depletion of the chemical by skin penetration may occur and the actual flux (J) observed will be less than the J_{max}. For most rinse-off products, the short time for which they are left on the skin, up to a few hours, may be insufficient for steady state to be achieved. While the latter may be possible for leave-on products, this will depend on the 'loading' of the chemical in the formulation and whether this is enough to sustain the flux for the longer period of application. Inefficient washing reducing removal of cosmetics from the skin surface could potentially sustain the flux. Secondly, cosmetic formulations are complex and comprise multiple ingredients and there may be chemical to chemical interactions. The prediction of a chemical's flux from mixtures based on experimental data from a simple aqueous solution must be made with considerable care, therefore. A particularly important concern is that cosmetic products invariably contain, *inter alia*, surfactants (such as fatty acids and alcohols) and co-solvents (e.g., simple alcohols, propylene glycol), which are recognised as skin penetration enhancers and can promote dermal absorption of cosmetic ingredients (Osborne and Henke, 1997; Williams and Barry, 2004; Lane, 2013). Although there have been efforts to predict the impact of such compounds on the flux of pharmaceuticals across the skin (Moss et al., 2012; Santos et al., 2012), there is no generally-agreed or validated approach to do so at this time.

In the absence of acceptable experimental data, predictive quantitative structure-penetration relationship (QSPR) models have been used to estimate dermal uptake (e.g., Potts and Guy, 1992; Wang et al., 2006; Magnusson et al., 2004). Magnusson et al. (2004), also clustered chemicals into 'good', 'intermediate'

and 'bad' skin penetrators based on predictions of J_{max} using MW, log P, $C_{w,sat}$, melting point and hydrogen bonding ability. Similarly, Kroes et al. (2007) proposed that, if experimental data were not available, dose absorbed adjustments of the 100% default figure should be applied based on calculations of J_{max} (in $\mu g/cm^2/h$), specifically ranking penetration into 3 classes of availability: i.e., 80%, 40% and 10% for $J_{max} > 10$, $10 > J_{max} > 0.01$, and $J_{max} < 0.01$, respectively.

The model of Potts and Guy (1992) calculates k_p (in $cm\,h^{-1}$) from water based on permeant size (expressed as molecular weight (MW)) and lipophilicity (expressed as the logarithm of the octanol-water partition coefficient P (log P)). The model equation was derived from a database ($n = 93$) of experimental *in vitro* and *in vivo* dermal absorption data for diverse chemicals ($18 < MW < 750$; $-3 < \log P < +6$) (Flynn, 1990):

$$\log kp = (0.71 \times \log P) - (0.0061 \times MW) - 2.7 \qquad (1)$$

One limitation of the Potts and Guy model is that k_p is over-predicted for highly lipophilic compounds, the skin permeability of which can be (at least in part) controlled by the viable skin layers below the SC. Cleek and Bunge (1993) recognised this deficiency and derived a modified expression for the permeability coefficient ($k_{p,mod}$) that acknowledges that the skin is not a simple hydrophobic membrane:

$$kp,mod\left(cm\,hr^{-1}\right) = k_p \Big/ \left\{1 + \left(k_p * \sqrt{MW}\right)\Big/2.6\right\} \qquad (2)$$

The model is based upon measurements of steady state flux; as discussed above, such conditions do not always apply for cosmetic use. Because Eq. (2) predicts a chemical's permeability from a water vehicle, the calculation of J_{max} must involve the aqueous solubility of the compound ($C_{w,sat}$), i.e.,

$$J_{max} = k_p,mod \times C_{w,sat} \qquad (3)$$

Validation of this predictive approach has been reported for chemicals in the EDETOX database (Guy, 2010; Kroes et al., 2007), for fragrance chemicals (Guy, 2010; Shen et al., 2014), and for transdermally delivered drugs (Wiedersberg and Guy, 2014). In each case, the predicted values of J_{max}, based on Eq. (3), compared favourably with experimental measurements taken from the literature. Whenever possible, the calculated J_{max} were determined using experimental values of log P and $C_{w,sat}$. In some cases however, one or both of these physicochemical parameters had not been measured and they were therefore estimated from web-accessible algorithms from ChemSpider and the Virtual Computational Chemistry Laboratory. Using these estimated values introduced no obvious bias into the findings. Given the typical variability observed in experimental J_{max}, and given that the calculated values of maximum flux involve assumptions and approximations as detailed above, the previous application of the model has considered a prediction "successful" when the ratio of experimental to predicted results falls in the range 0.1–10 (i.e., an order of magnitude on either side of the 'ideal' value of 1). Generally speaking, this level of validation has been comfortably achieved, and when divergence has been found, it has been possible to identify plausible reasons for the lack of agreement between theory and experiment (e.g., presence of a penetration enhancer, very low dose of chemical applied precluding attainment of anything close to an experimental J_{max}).

The goal of the research described here, therefore, is to develop methods to incorporate dermal bioavailability into the use of TTC for cosmetics. A decision tree workflow has been derived and evaluated to determine and use estimates of dermal penetration parameters to define systemic dose. In addition, it has been

evaluated for cosmetic exposure scenarios. We have considered the issues influencing differences between prediction and experimental data.

2. Methods

2.1. Application of the TTC concept

To apply the TTC concept to cosmetics applied to the skin, one can either develop a dermal TTC or adapt an oral TTC. The latter approach, which is based on external dose, has been used but with consideration of dermal exposure and absorption as recommended by Kroes et al. (2007). Derivation of dermal-specific thresholds was not pursued due to a lack of quality toxicity data following dermal dosing to support derivation of a dermal TTC. Since there is sufficient understanding of oral absorption and skin permeability, the oral-to-dermal extrapolation of TTC thresholds by combining the absorption, distribution, metabolism and excretion (ADME) knowledge and oral repeated-dose toxicity data was considered more pragmatic. As the existing dermal absorption/skin permeability databases did not contain sufficient cosmetics-related chemicals, a new resource based on the EDETOX database (www.newcastle.ac.uk/edetox) (Soyei and Williams, 2004) and the Samaras database (Samaras et al., 2012) enriched with cosmetics-related chemicals has been established (www.cosmostox.eu).

2.2. Evaluation of dermal TTC

There are two possible approaches that may be taken in applying the TTC concept to the dermal route of exposure. The first is route-to-route extrapolation, or the prediction of an equivalent dermal dose and dosing regimen which produce the same toxicological response as that obtained for a given oral dose and dosing regimen, while taking into account differences in metabolism and kinetics (Mueller et al., 2008). Alternatively, a database specific to dermal toxicity studies could be assembled and used to derive dermal-specific TTC values. Both approaches rely on grouping substances into structural classes based on a decision tree approach and using the resulting "Cramer classification" as an indicator of systemic toxicity (Cramer et al., 1976). However, the scheme devised by Cramer and colleagues aims to classify and rank chemicals according to their expected level of oral systemic toxicity. Whether these criteria are applicable to systemic toxicity via the dermal route of exposure is unknown.

Therefore, an attempt was made to assess the applicability of the Cramer classification scheme to systemic toxicity via the dermal route of exposure.

A reference database containing NO(A)EL values for systemic toxicity via the dermal route of administration in rats, mice and rabbits was compiled. Data for a total of 140 substances were harvested from public databases (echemportal.org, 102 entries) as well as the open peer-reviewed literature dating back to 1970 (38 entries). Only repeat dose studies where the NO(A)EL for systemic toxicity was lower than the LO(A)EL for local effects were included, which excluded primary irritants and corrosive substances. For studies retrieved via echemportal.org, only those experimental studies with a Klimisch score of 1 (reliable without restriction), or 2 (reliable with restrictions) were selected. For data extracted from the peer-reviewed literature, reliability was not formally assessed. Multiconstituent substances, UVCBs (substances of unknown or variable composition, complex reaction products, or biological materials) and those lacking the minimum data requirements were excluded. In cases where more than one NO(A)EL value was identified for the same substance, the most conservative value was retained. For many substances, the dermal NO(A)EL was the highest dose tested (i.e. no systemic toxicity was observed).

2.3. Application of oral TTC and prediction of systemic availability

To apply the oral TTC threshold values to cosmetic-related chemicals, the degree of absorption/permeability of compounds through the skin and the differences in systemic bioavailability between dermal and oral uptake must be considered. This can be achieved by determining the systemic availability following topical application either from experimental dermal absorption data, or from prediction of the dermal uptake. For molecules which are metabolised pre-systemically, oral-to-dermal differences in local metabolism will need to be considered and experimental data may need to be obtained. The Lipinsky rule of 5 can be used to predict whether a molecular structure will be orally available, but for some molecules local metabolism in the GI tract must be considered when applying the oral TTC.

The Potts and Guy (1992) model, with Cleek and Bunge's (1993) correction for lipophilic molecules, has been used here to generate k_p and J_{max} values, preferably whenever possible from experimental data for log P and $C_{w,sat}$ (as described above). It is recognised that predictive algorithms to estimate these physicochemical parameters are available too, but that the predictive values can sometimes differ substantially from one another (especially aqueous solubilities) (Guy, 2010). Shen et al. (2014) provided guidance on the selection of estimated physicochemical parameters and recommended use of the mean, or mean + 1SD, from several different models to estimate log P and water solubility, if experimental data were not available. It was concluded that these predictions (which are based on dermal uptake from aqueous solution) were relatively conservative compared to published experimental data and that the approach may have value therefore, for regulatory purposes.

To incorporate the effects of chemical absorption/permeability across the skin in the TTC evaluation process, a decision tree was designed using a prediction of J_{max} when no relevant absorption data were available. Obviously, if there are robust experimental data from an *in vitro* absorption study reproducing the *in vivo* exposure scenario to the product, it is appropriate to use this information to calculate the systemic dose. It seems reasonable to hypothesise that either of these approaches must represent an improvement on the current default assumption of 100% absorption.

When no useful measurements are available, as discussed above and later in Section 3.2, J_{max} can be estimated and the maximum amount (Q_{max}) of chemical entering the systemic circulation predicted. The calculation requires the permeability coefficient ($k_{p,mod}$) derived from the Potts and Guy equation and the aqueous solubility of the chemical ($C_{aq,sat}$) The Cleek and Bunge modified value is used for molecules with log p greater than 4.5:

$$J_{max}\left(mg\ cm^{-2}\ hr^{-1}\right) = k_p, mod\left(cm\ hr^{-1}\right) \times C_{aq,sat}\left(mg\ cm^{-3}\right)$$

$$(4)$$

$$Q_{max}(mg) = A\left(cm^2\right) \times J_{max}\left(mg\ cm^{-2}\ hr^{-1}\right) \times T_{exp}(hr) \quad (5)$$

In reality, the cosmetic product formulation may not be water, nor saturated with the active compound of interest and may contain ingredients that enhance skin penetration (e.g. surfactants, fatty alcohols etc.). These and other factors may impact on the actual skin flux result. Eq. (4) assumes that there is no diffusional lag time, no depletion of chemical from the skin surface (e.g., by evaporation or abrasion), and that penetration stops at the end of the exposure time. The validity of the non-depletion assumption

for both rinse-off and leave-on products was addressed earlier. Furthermore, it is widely accepted that uptake of chemical into the body does not cease immediately when the product is removed from the skin. The ability of (in particular) lipophilic compounds to form so-called 'reservoirs' in the skin, from which continued release may occur over a prolonged period, is well known. However, this under-estimate of the total amount absorbed is, in large part, compensated for by the assumption of no diffusional lag-time (i.e., that the flux equals J_{max} from the moment of product application).

The following **tiered decision tree approach** for systemic bioavailability depicted schematically in Fig. 1 has been developed with a number of sequential steps requiring yes/no decisions.

Step 1: Are there exposure/absorption data available for the compound of interest that allow the systemic dose to be estimated? If yes, the TTC paradigm can be applied. If no, then continue to step 2. If the absorption data available were obtained based on an *in vitro* study using an applied dose that differs greatly from that in a typical exposure scenario, then the decision tree approach should also be followed.

Step 2: Obtain chemical formula and structure. Does the cosmetic compound fall within the defined chemical space for TTC and can the appropriate Cramer class be assigned (e.g., using ToxTree) and can a predicted J max be derived? If yes, move to step 3. The approach has not been evaluated for metal salts, ionised compounds or high MW macromolecules or polymers.

Step 3: Define typical cosmetic exposure scenarios for the formulation; ascertain chemical concentration, formulation composition.

Step 4: Estimate exposure to the chemical in typical exposure scenarios, e.g. leave-on single dose, rinse-off, repeat doses, etc. Use authoritative sources for skin contact times and exposed areas (European Commission, 2012).
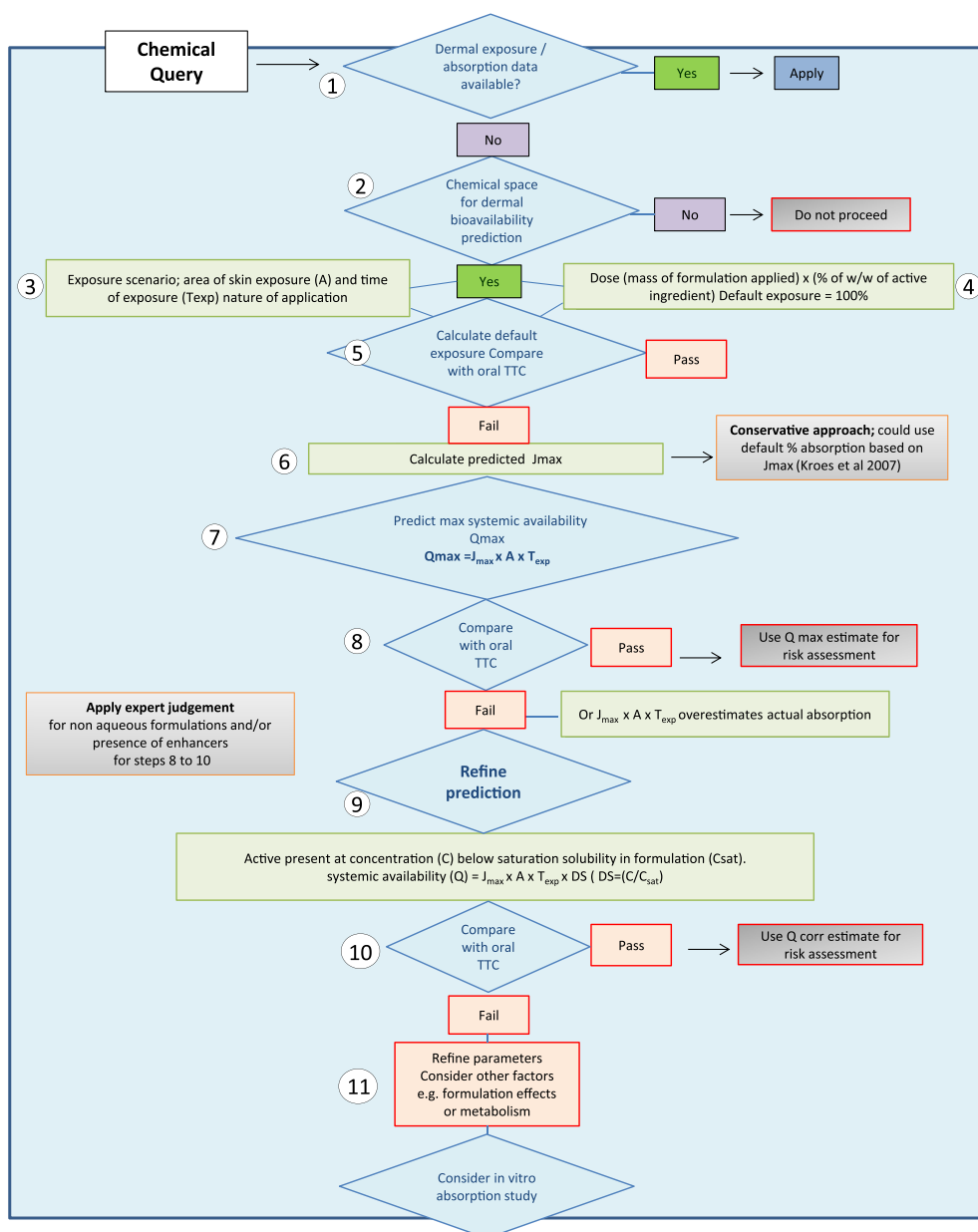

Fig. 1. Tiered decision tree approach for the prediction of systemic availability of a dermally applied cosmetic ingredient.

Step 5: Compare default exposure assuming 100% absorption, to oral TTC. If exposure exceeds appropriate TTC value, move to step 6.

Step 6: Obtain values of MW and where possible, experimentally determined measures of log P and aqueous solubility. If the latter are not available, then use available predictive algorithms (including EPISuite, Chemspider, Virtual Computational Chemistry Laboratory, ToxTree, OECD QSAR Toolbox) to calculate mean values of these parameters (eliminating outliers with an appropriate statistical test). Either obtain k_p or J_{max} needed for estimation from the COSMOS database or calculate using the Potts and Guy approach (Eqs. (2) and (3) (Guy, 2010). Use Jmax to calculate Qmax in step 7.

An alternative at this stage is to use the default assumption for % absorption based on Kroes et al. (2007) to obtain a conservative estimate of systemic availability by ranking Jmax into 3 classes of availability: i.e., 80%, 40% and 10% for $J_{max} > 10$, $10 > J_{max} > 0.01$, and $J_{max} < 0.01$, respectively to compare to TTC.

Step 7: Calculate maximum systemic availability (Q_{max}) from Eq. (4) in units of $\mu g/cm^2$ or $\mu g/cm^2/kg$ body weight.

Step 8: Compare the maximum systemic availability with oral TTC. If Q_{max} exceeds TTC, or even 100% exposure/absorption, then proceed to step 9. If not, use estimate in your assessment.

Step 9: Refine prediction by calculating a modified systemic availability (Qcorr) using DS (degree of saturation) the actual concentration of chemical in the formulation (C) relative to its solubility therein (C_{sat}), i.e.,

$$Qcorr = A \times J_{max} \times T_{exp} \times (C/C_{sat}) \tag{6}$$

The calculations in steps 6 to 9 are based on the assumptions that (i) a chemical's flux from any vehicle will be the same if the degree of its saturation within the formulation is kept constant, and (ii) the vehicle/formulation does not change the skin's barrier function or enhance penetration so actual flux relates to concentration (some molecules inappropriate to proceed). If Csat in formulation is not available use aqueous Csat as default.

Step 10: Compare Qcorr with oral TTC. If the TTC threshold is much greater than the estimated systemic availability, the assessment passes and there is no concern. In cases where the systemic availability exceeds or approaches the TTC, an expert assessment is warranted to evaluate the confidence in the estimate or re-evaluate conservatism of assumptions. Systemic exposure may be under predicted in cases of non-aqueous formulations, the presence of penetration enhancers or short residence times.

Step 11: If the assessment fails in Step 10, expert judgement will determine whether further information or experimental data may be required.

3. Results

3.1. Evaluation of dermal TTC

The 140 chemicals selected for inclusion in the reference database represent a range of industrial and consumer chemicals, with 52 of the substances (37%) being used in cosmetics currently marketed in Canada (Health Canada, 2013). An additional 28 substances (20%) had INCI names and/or were listed in CosIng, the European Commission's inventory of cosmetic ingredients, but were not presently identified in cosmetics on the Canadian market. Therefore, it can be surmised that roughly half these substances are

currently or may potentially be used in cosmetics. The allocation of chemicals to Cramer classes was performed with the extended version of the Cramer decision tree of the open source program Toxtree-v2.5.0 (Patlewicz et al., 2008)[1]. Of the 140 chemicals, 44 were assigned to Cramer class I, 5 to Cramer class II and 91 to Cramer class III. This distribution is similar to the Munro dataset (Munro et al., 1996) used to derive the oral TTC values (Table 1).

The cumulative distribution of the NO(A)ELs (animal derived data) of substances separated into Cramer structural classes appears in Fig. 2. As observed by Munro et al. (1996) for the oral route of exposure, there is a clear effect of chemical structure on systemic toxicity via the dermal route, as indicated by the distinct separation of the cumulative distributions for Cramer classes I and III. With only 5 substances falling into Cramer class II, this group was excluded from further analysis. The 50th percentiles of the distributions were 500 and 200 mg/kg bw/d for structural classes I and III, respectively, and the difference in means between the two groups was statistically significant (T-test for independent means, $T = 3.89$, $p = 0.000155$). The 5th percentiles of the distributions of NO(A)ELs were 35.3 and 5.0 mg/kg bw/d for class I and III, respectively. Therefore, although the number of substances is relatively small, these results strongly suggest that the Cramer classification scheme is still applicable to the ranking of chemicals according to their expected level of systemic toxicity, even when the route of exposure is dermal.

3.2. Evaluation of the tiered decision tree approach for prediction of systemic availability

The tiered decision tree is outlined step by step in the methods and is shown schematically in Fig. 1. A worked case study example for acid orange 7 is shown in Table 2. Acid Orange 7 passed when related to TTC using the decision tree and new data from SCCS ($0.25 \, \mu g/cm^2$) will also clear the use of Acid Orange 7 in a cosmetics product at 0.5% in formulation if there are no compound-specific toxicity data (Scientific Committee on Consumer Safety, 2014). Cosmetic exposure scenarios for 19 cosmetic chemicals and 3 contaminants were used as examples for application of the decision tree to compare prediction with experimental data. Table 3 summarises the physicochemical properties of the chemicals, the J_{max} and Qcorr predictions from the decision tree and for comparison the experimental results. The molecules considered covered a range of log P values characteristic of the compounds from which the Potts and Guy algorithm was derived (with the possible exception of zinc pyrithione and resorcinol). Chemicals were mostly chosen from among those for which there is SCCS accepted experimental data. Toxicity data exist for all of the examples presented, meaning that a TTC would not be used for these chemicals in practice; however, they serve as useful cases for checking the validity of the approach.

The experimental/prediction ratios (Q_{expt}/Q_{corr}) are shown in Table 3 and graphically in Fig. 3 and mostly fall within the range $0.1-10$ with many close to the ideal value of 1. For the examples considered in this paper, the predicted fluxes are compared to experimental values obtained using a variety of vehicles. Results were supportive of the application of the approach even for cosmetic scenarios where formulations are not aqueous. The prediction underestimated availability for zinc pyrithione, but the Potts and Guy equation has not been evaluated for ionisable compounds. Predictions for kojic acid showed good agreement, despite

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[1] Additional information about the categorization of substances by Toxtree are available from the European Commission's Joint Research Centre (http://ihcp.jrc.ec.europa.eu/our_labs/predictive_toxicology/qsar_tools/toxtree).

Table 1
Allocation of substances by Cramer class for systemic toxicity following dermal dosing and oral toxicity from Munro et al. (1996).

	Cramer class	N (N%)
Repeat dose dermal −systemic effects (this study, N = 140)	I	44 (31%)
	II	5 (4%)
	III	91 (65%)
Chronic oral toxicity − original TTCs (Munro database, N = 631)	I	137 (22%)
	II	28 (5%)
	III	448 (73%)
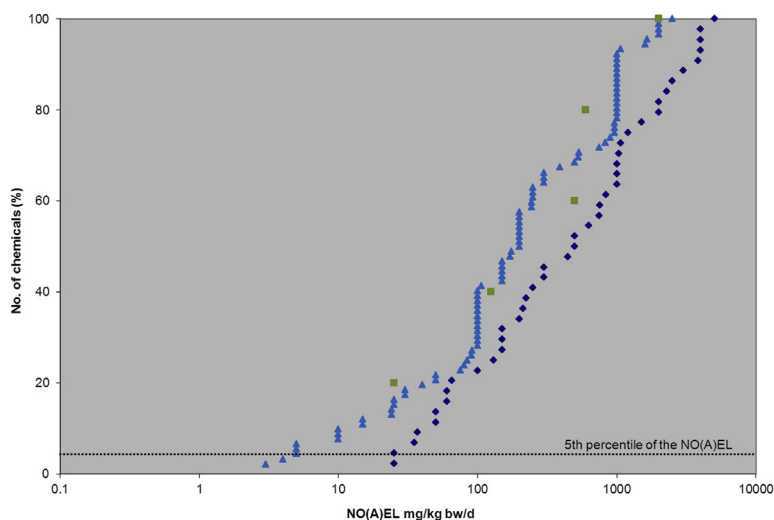
Fig. 2. Cumulative distribution of the most conservative NO(A)ELs for systemic toxicity following repeat dose dermal administration for compounds divided into Cramer structural classes I, II and III (Cramer et al., 1976).

Table 2
Example of application of the tiered decision tree to assessing exposure to acid orange 7.

Step	Acid orange 7:
	Rinse off exposure scenario of a hair dying client:
3	A(scalp) = 560 cm^2; (t) = 30 min/d; dose 20 mg/cm^2; concentration of 0.5% w/w 5 mg/mL
4	Maximum (default) daily exposure to chemical is 0.9 mg/kg/d
5 **FAIL**	Compare with oral TTC: Oral (1.5 µg/kg/d) < Dermal exposure
6	Do we have experimental absorption data in COSMOS skin permeability database? NO* - > use Potts-Guy to calculate.
7	Predict systemic availability Qmax • Jmax = 0.0144 mg/cm2/hr (exp. logP = 1.4, MW = 750.3, aq solubility = 110 mg/mL) • Qmax = 4.032 mg/60 kg = 0.068 mg/kg/day
8 **FAIL**	Compare with oral TTC: Oral (1.5 µg/kg/d) < Dermal exposure
9	Correct systemic Qmax for C/Csat: Qmax (corr) = 3.03 µg/kg/d
10 **PASS**	Oral TTC ~ dermal availability

the data being derived from skin absorption studies with non-aqueous formulations. Consideration of the extensive experimental data for different formulations and uses of DEGEE, illustrates that the prediction is acceptable except for formulations of DEGEE with short residence times (rinse-off) and in these cases, experimental exposure exceeded the estimate by factors of 3–18. Predictions for arbutin were low, compared to experimental data in some, but not all cases. For retinol, skin uptake was over-estimated, but this very lipophilic compound has a log P that falls outside the range encompassed by data that was used to develop the Potts &

Guy algorithm and is locally metabolised, underlining the importance of applying expert judgment to this approach. Methyl benzylidine camphor and benzophenone-3 formulations were saturated and a value of 1 used for DS (the concentration ratio), i.e. no correction was appropriate. The (Q$_{expt}$/Q$_{corr}$) ratio for triclocarban, acid orange 7, methylisothiazolinone and quercetin was between 0.1 and 10. For butyl paraben the experimental studies reported that skin penetration of butyl paraben was low, but significant amounts (more than 10-fold higher) of a degradation product (likely due to local hydrolysis in the skin) and not included

Table 3
Skin absorption values predicted derived from Potts and Guy equation and experimentally determined for 19 cosmetic chemicals in 54 formulations and for three impurities.

Chemical	MW	log P	Csat (mg/cm³)	Kp, *corr (cm/h)	Jmax (µg/cm²/h)	Product	Exposure time (h)	Dose applied (µg/cm²)	Area (cm²)	Qmax (µg/cm²)	Capp (mg/cm³)	DS = C/Capp	Qexp (µg/cm²)	Qcorr (µg/cm²)	Qexp/Qcorr
Basic blue 124[a]	305.8	−1.79	150	1.56E-06	0.23407	hair dye	0.5	100	560	0.11703	5	0.0333	0.017	0.0039	4.36
Zinc pyrithione[b]	317.7	0.9	0.015	1.08E-04	0.00161	water, CMC	8	100	500	0.01291	0.015	1	1.32	0.01291	102
Kojic acid[c]	142.1	−0.64	44	9.82E-05	4.32051	Cream	16	21	500	69.128	10.5	0.24	3.58	16.496	0.22
Arbutin[d]	272.3	−1.35	100	5.09E-06	0.509	Cream	24	346.5	500	12.22	63	0.63	6.31	7.7	0.82
Arbutin[d]	272.3	−1.35	100	5.09E-06	0.509	Cream	24	165	500	12.22	30	0.3	0.21	3.66	0.06
Arbutin[d]	272.3	−1.35	100	5.09E-06	0.509	Cream	24	346.5	500	12.22	63	0.63	0.49	7.7	0.06
Arbutin[d]	272.3	−1.35	100	5.09E-06	0.509	Cream	24	165	500	12.22	30	0.3	0.35	3.66	0.1
Arbutin[d]	272.3	−1.35	100	5.09E-06	0.509	Gel	24	346.5	560	12.22	63	0.63	0.49	7.7	0.06
Arbutin[d]	272.3	−1.35	100	5.09E-06	0.509	Gel	24	165	560	12.22	30	0.3	0.26	3.66	0.07
Butoxyethanol[e]	118.2	0.8	100	0.00144	143.11	hair dye	0.5	600	560	71.56	30	0.3	61	21.47	2.84
Butoxyethanol[e]	118.2	0.8	100	0.00144	143.11	hair dye	0.5	120	560	71.56	50	0.5	125	35.78	3.49
Benzophenone-3[f]	228.3	3.7	0.0037	*0.0298	0.11	sunscreen	24	200	500	2.65	0.0037	1	7.90	2.65	2.98
Benzophenone-3[f]	228.3	3.7	0.0037	*0.0298	0.11	sunscreen	24	600	500	2.65	0.0037	1	18.30	2.65	6.91
Benzophenone-3[f]	228.3	3.7	0.0037	*0.0298	0.11	sunscreen	24	200	560	2.65	0.0037	1	6.7	2.65	2.53
Benzophenone-3[f]	228.3	3.7	0.0037	*0.0298	0.11	sunscreen	24	600	500	2.65	0.0037	1	19.30	2.65	7.29
Me-Benzilidene camphor[g]	254.4	5.95	0.0002	*0.14	0.028	sunscreen	24	180	500	0.672	0	1	1.5	0.672	2.231
2-nitro-5-glyceryl-MeAniline[h]	242	1.14	1.43	0.000454	0.65	hair dye	0.5	180	560	0.32	1.43	1	0.51	0.32	1.58
Retinol[i]	286.5	6.72	0.0067	*1.44	0.963	Gel	24	6	500	23.12	0.0067	1	0.360	23.12	0.02
Retinol[i]	286.5	6.72	0.0067	*1.44	0.963	Emulsion	24	6	500	23.12	0.0067	1	0.612	23.12	0.03
Benzisothiazolinone[j]	151.2	0.7	1.1	0.000775	0.849	Cream	24	2	560	20.38	0.1	0.09	1.04	1.85	0.56
DEEGE[k]	134.2	−0.54	1000	0.000129	128.96	Shampoo	0.5	279	560	64.48	50	0.05	60.5	3.22	18.77
DEEGE[k]	134.2	−0.54	1000	0.000129	128.96	shampoo	0.5	530	560	64.48	100	0.1	92.2	6.45	14.3
DEEGE[k]	134.2	−0.54	1000	0.000129	128.96	gel	24	831	500	3095	150	0.15	425	464.25	0.92
DEEGE[k]	134.2	−0.54	1000	0.000129	128.96	gel (occlusive)	24	859	500	3095	150	0.15	385	464.25	0.83
DEEGE[k]	134.2	−0.54	1000	0.000129	128.96	repeated occl	24	890	500	3095	150	0.15	459	464.25	0.99
DEEGE[k]	134.2	−0.54	1000	0.000129	128.96	emulsified	24	100	500	3095	20	0.02	43.7	61.9	0.71
DEEGE[k]	134.2	−0.54	1000	0.000129	128.96	emulsified	24	287	500	3095	50	0.05	140	154.75	0.9
DEEGE[k]	134.2	−0.54	1000	0.000129	128.96	emulsified	24	570	500	3095	100	0.1	267	309.5	0.86
DEEGE[k]	134.2	−0.54	1000	0.000129	128.96	emulsified	24	100	500	3095	20	0.02	52.7	61.9	0.85
DEEGE[k]	134.2	−0.54	1000	0.000129	128.96	emulsified	24	285	500	3095	50	0.05	128	154.75	0.83
DEEGE[k]	134.2	−0.54	1000	0.000129	128.96	emulsified	24	570	500	3095	100	0.1	294	309.5	0.95
DEEGE[k]	134.2	−0.54	1000	0.000129	128.96	repeated occl	24	100	500	3095	20	0.02	59.5	61.9	0.96
DEEGE[k]	134.2	−0.54	1000	0.000129	128.96	repeated occl	24	285	500	3095	50	0.05	167	154.75	1.08
DEEGE[k]	134.2	−0.54	1000	0.000129	128.96	repeated occl	24	570	500	3095	100	0.1	319	309.5	1.03
DEEGE[k]	134.2	−0.54	1000	0.000129	128.96	hair dye	0.5	400	560	64.48	20	0.02	8.40	1.29	6.51
DEEGE[k]	134.2	−0.54	1000	0.000129	128.96	hair dye	0.5	700	560	64.48	35	0.04	13.80	2.26	6.11
DEEGE[k]	134.2	−0.54	1000	0.000129	128.96	hair dye	0.5	1400	560	64.48	70	0.07	34.20	4.51	7.58
DEEGE[k]	134.2	−0.54	1000	0.000129	128.96	hair dye	0.5	200	560	64.48	10	0.01	3.70	0.64	5.74
DEEGE[k]	134.2	−0.54	1000	0.000129	128.96	hair dye	0.5	600	560	64.48	30	0.03	8.30	1.93	4.29
DEEGE[k]	134.2	−0.54	1000	0.000129	128.96	hair dye	0.5	1000	560	64.48	50	0.05	9.90	3.22	3.07
Diethyl phthalate[l]	222.2	2.4	1	*0.00456	2.61	whole body	24	100	17,500	62.67	1	1.75	1008	109.36	9.22
Acid orange 7[m]	350.3	1.4	110	0.000155	17.08	hair client	0.5	100	560	8.54	8	0.07	0.04	0.621	0.06
Acid orange 7[m]	350.3	1.4	110	0.000155	17.08	Hair	0.5	20	560	8.54	5	0.05	0.07	0.39	0.18
Acid orange 7[m]	350.3	1.4	110	0.000155	17.08	hair client	0.5	20	560	8.54	5	0.05	0.95	0.39	2.45
Acid orange 7[m]	350.3	1.4	110	0.000155	17.08	hair client	0.5	10	560	8.54	2	0.02	0.04	0.16	0.26
Butyl paraben[n]	194.2	3.47	1	*0.0327	32.69	leave on	24	7.9	17500	784.57	1	1	3	784.57	0.0038
Triclocarban[o]	315.6	4.9	0.00065	*0.0504	0.03	shower wash off	0.07	0.9	560	0	0.15	230.77	1.5	0.53	2.84
Quercetin[p]	302.2	1.48	2472	0.000344	848.81	cosmetic leave on 2 × 1.3	12	0.366	500	10,185.71	1	0.00041	0.23	4.12	0.06
Methylisothiazolinone[q]	115.2	−0.83	748	0.000104	78.12	hair stylist wash off	1.3	215.5	860	101.56	0.1	0.00013	0.1	0.01	7.37

Table 3 (continued)

Chemical	MW	log P	Csat (mg/cm³)	Kp,*corr (cm/h)	Jmax (µg/cm²/h)	Product	Exposure time (h)	Dose applied (µg/cm²)	Area (cm²)	Qmax (µg/cm²)	Capp (mg/cm³)	DS = C/Capp	Qexp (µg/cm²)	Qcorr (µg/cm²)	Qexp/Qcorr
Methyleugenol[t]	178.2	3.03	0.503	0.0241	10.78	leave on	16		200	172.53	0.04	0.08	7.409	13.72	0.54
Resorcinol[s]	110.1	1.03	748	0.00235	1737.68	hair client	0.5		560	868.84	12.5	0.02	2.06	14.52	0.14
1,4-dioxane[t]	88.11	−0.27	1000	0.000379	378.84	Fragrance	24		225	9092.23	2	0.002	0.26	18.18	0.01
Hydrazine[u]	32.05	−2.07	1000	0.0000434	43.37	face cream	12		565	520.46	1000	1	480	520.46	0.92
Acrylamide[v]	71.08	−0.67	640	0.00025	159.59	body lotion	24		17500	3830.21	4.45	0.01	9.7	26.62	0.36

Foot-notes.

* The asterisk indicates the use of Kp, corr value rather than Kp.

Data expressed as mean. Experimentally determined values for water solubility were used where possible. Source of experimental data a) SCCS/1542/14 b) SCCS/1512/13 c) SCCP/1182/08 d) SCCP/1158/08 e) SCCP/1045/06 f) SCCP/1201/08 g) SCCP/1184/08 h) SCCP/1477/12 i) Yourick et al., 2008 j) SCCS/1482/12 k) SCCP/1044/06 l) Frasch et al., 2007, Guy 2010, SCCS/1016/06 m) SCCS/1536/14 n) SCCS/1514/13 o) SCCS/0851/04 p) Da Belo et al., 2009, Lin et al., 2012 q) Roper et al., 2010 r) Guy, 2010, Schmitt et al., 2010 s) SCCS/1270/09 t) Marzulli et al., 1981 u) Keller et al., 1981 v) Kraeling and Bronaugh 2005.

in the experimental measurement were observed in the receptor compartment of the *in vitro* diffusion cell. The predicted absorption would include both butyl paraben and its degradation product and would be within an order of magnitude of that observed experimentally. Appropriate experimental data were not available in some cases and assumptions have been made, for example for diethyl phthalate. When considering potential impurities, the prediction for dioxane was poor and largely over-estimated, most likely because the chemical is highly volatile and a substantial fraction of the 'dose' evaporates before absorption can occur. The predictions for hydrazine and acrylamide, on the other hand, aligned well with experimental data.

Two cosmetic impurities provide illustrations of how the decision tree approach might be used in conjunction with oral TTC to determine whether a chemical in a cosmetic may or may not raise a potential exposure risk alert. Hydrazine (which is, in fact, prohibited as an ingredient in cosmetics) may occur as a residual in polyvinylpyrrolidone (PVP), a polymer typically used in cosmetics at concentrations of 0.3−10% w/w. If hydrazine was present as a residual in PVP at 1 ppm, this would equate to a level of 3−100 ng/g. In Table 4, the J_{max} and Q_{corr} predictions from Table 3 for hydrazine are used to calculate a daily exposure to the chemical based on the use of a leave-on face cream product. This predicted systemic 'dose' is then compared with the relevant TTC value and it is found that the two differ only by a factor of about 3. If such case occurred with a substance lacking repeated dose toxicity data, careful evaluation would be necessary to determine if the estimate of dermal availability is reliable. Acrylamide monomers represent a common residual of several polycationic polymers (so-called "polyquaterniums") used in personal care products. Table 2 compares the oral TTC with the decision tree derived predicted daily exposure when acrylamide is present at 10 ppm in a leave-on body lotion (containing 3% of a polyquaternium) over the entire torso, and excluding the head. In this case, the ratio of TTC divided by predicted exposure is greater than 5.

The alternative approach proposed by Kroes et al. (2007) for prediction of dermal bioavailability extrapolated from Jmax to a % absorbed which could then be applied to the actual exposure scenario. Kroes proposed ranking penetration into 3 classes of availability: i.e., 80%, 40% and 10% for Jmax >10, 10 > Jmax >0.01, and Jmax <0.01, respectively although gave no reason for the cut off values used. Shen et al. (2014) demonstrated that this conservative approach could be applied to fine fragrance materials. The results of this approach for the cosmetic scenarios considered with the decision tree are shown in Fig. 4 for comparison with Fig. 3. Fig. 4 contains the ratio Q experimental to Q derived from the Kroes binning. The predictions were conservative and higher than the decision tree approach and for many of the molecules the ratio was less than 0.1.

As highlighted at step 9 of the decision tree there are limitations of the decision tree approach. First, the default starting point is to estimate a chemical's potential maximum flux across the skin, assuming that it is saturated in the formulation. If this is not the case (and usually it is not), then the estimated flux must be corrected to a smaller and more realistic value by multiplying J_{max} by the degree of saturation (DS) of the chemical in the formulation. This is simple when the formulation is water-based, but may require experimental measurement when the formulation is oil, for example, and the solubility of the chemical therein is not known. Second, while the maximum flux is the key predictor used here, the calculation assumes that the formulation has no effect on skin barrier function − that is, the vehicle is considered benign with no skin penetration enhancing components. With respect to the example chemicals considered here, with the exception of butyl paraben (see above), extensive metabolism in the GI tract or skin
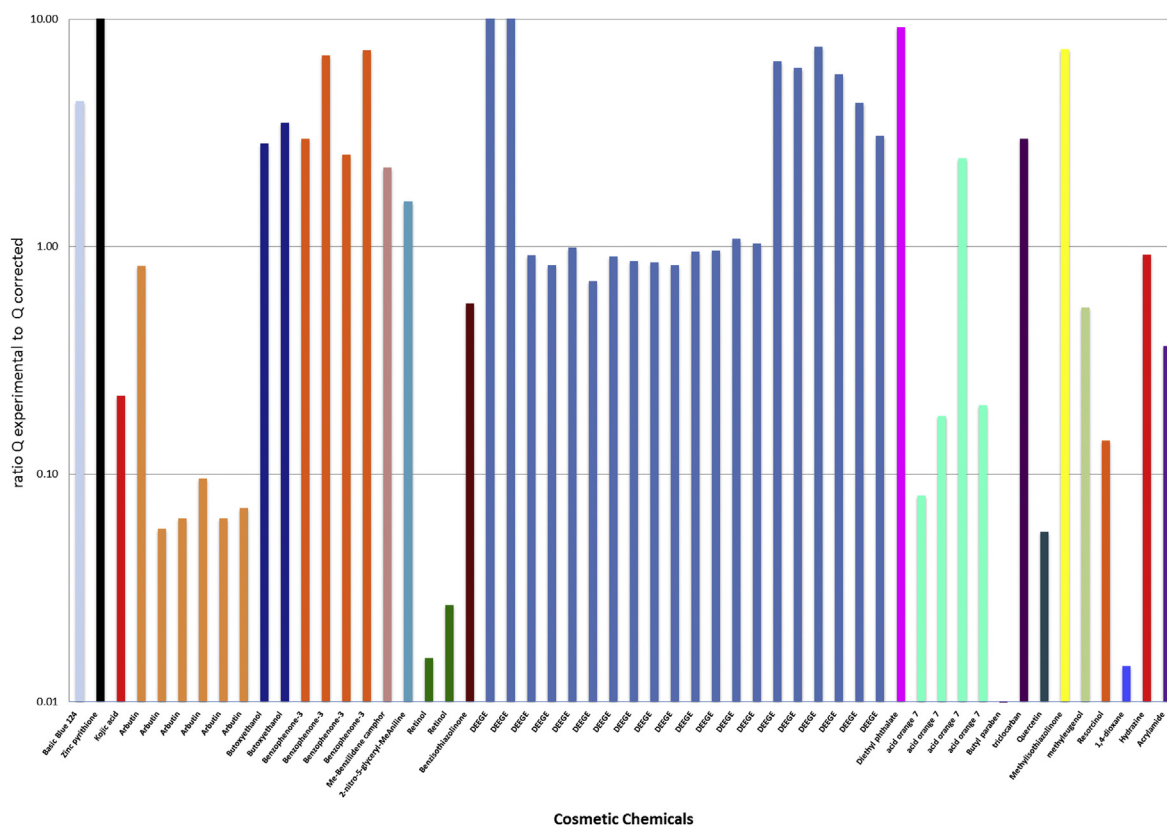
Fig. 3. Comparison of the experimentally determined Q exp and theoretically predicted Qcorr dermal absorption for 19 cosmetic chemicals in 54 formulations and for three impurities. The ratio of Qexp to Qcorr is shown. The optimum ratio would be 1. Values refer to chemicals as in Table 3.

4. Discussion

A decision tree based on the Potts and Guy equation, has been developed to predict systemic availability derived from k_p and J_{max}, and J_{max} adjusted for the actual exposure scenario/concentration. This decision tree offers a quantitative approach which has been established on scientific principles and an understanding of dermal absorption processes. The approach allows systemic availability to be estimated within an order of magnitude of the experimental results for many but not all cosmetic chemicals. The results of this study indicate that it is possible to evaluate cosmetic substances and their use scenarios and relate dermal exposure/systemic bioavailability to TTC. Substances should be considered on a case-by-case basis and for low level contaminants, it may be possible to refine parameters used, or apply SAR assessment rather than requiring experimental studies. In particular, expert judgement may be required when using the predictions for short residence time products.

Consideration of a limited number of examples, like creams, sunscreen preparations, oil-in-water emulsions, and hair dye formulations, with various degrees of saturation, a variety of vehicles and presence of enhancers, indicates that our approach yields predictions within an order of magnitude of experimental data for most of the cases considered and there were few outliers. This is despite as highlighted earlier, the Potts and Guy approach which has some limitations for extrapolation to cosmetic formulation, as the degree of saturation is often not known, or the vehicle can change rapidly (e.g. volatility), that the rules do not apply and the impact of enhancers cannot currently be modelled. For molecules for which the experimental/predicted ratio was an outlier, it was necessary to consider these confounding factors or first pass metabolism. The potential for local skin metabolism during absorption and the influence on relative oral to dermal bioavailability may be important particularly for molecules with high dermal absorption. In the case studies, local metabolism led to the experimental availability being lower than the estimated availability, and therefore an overly conservative prediction for butyl paraben. It is generally believed for transdermal pharmaceuticals and cosmetics that the low level of dermal metabolism will increase availability of the parent compound either in the skin or systemically compared to oral dosing. Differences in bioavailability between dermal and oral exposures, if significant, could affect the application of TTC and

Table 4
Predicted dermal systemic availability Qcorr expressed as μg/kg/d compared to oral TTC (μg/kg/d) for three potential cosmetic impurities.

Chemical	Jmax (μg/cm²/h)	Cosmetic use scenario	Exposure time (h)	Area cm²	TTC class & value μg/kg/d	Qcorr μg/cm²	Qcorr μg/kg/d	TTC/Qcorr
1,4-Dioxane	23.39	Baby shampoo	0.1	225	3 or 1.5	2.34E-04	0.0081	185.25
Hydrazine	1041.56	Face cream	24.0	565	geno 0.0025	1.04E-04	0.0008	2.97
Acrylamide	3830.21	Body lotion	24.0	17500	geno 0.0025	1.80E-06	0.0004	6.25

Fig. 4. Comparison of the ratio of Q exp to Q derived from Jmax and ranked percentage absorption. The ratio Q exp (μg/cm2) to Q derived (μg/cm2) is shown. The optimum ratio would be 1.

substance-specific toxicity data to dermal exposures. Therefore it may be appropriate to consider the relative oral and dermal bioavailabilities within the decision tree and define guidelines for whether metabolism should be incorporated (IGHRC, 2006). Currently for extrapolation of the oral TTC, an assumption is made that oral bioavailability of chemicals in the database is high but this is not necessarily the case for molecules which are substrates for pre-systemically located enzymes.

There are currently limited experimental data defining the rate of absorption of cosmetics through the gut, there is information from human tissue models such as Caco-2 cells, and from the parallel artificial membrane permeability assay (PAMPA) (e.g., Thörn et al., 2005; Cubitt et al., 2011; Rothe et al., 2014). The evaluation becomes even more complicated when considering that either the parent or metabolites could act with different potency on the same or different endpoints. This aspect is seldom well enough characterised to be included in risk assessments based on either specific toxicity data or on TTC. Further studies are required to evaluate the influence of pre-systemic metabolism in skin and GI tract on systemic availability for chemicals for which data are available before proposing guidelines for these.

When using experimental data it is important to look critically at the experimental data to see that the dose and time of application are relevant to the cosmetic exposure scenario under consideration. There is always uncertainty in the experimental data which is reflected when mean and standard deviation are reported for multiple cell experiments and for regulatory purposes, a conservative value of mean plus one or two SD is often used (Navidi and Bunge, 2002). Significant inter-laboratory and inter-assay variation has been defined for model chemicals in *in vitro* experiments using human skin (van de Sandt et al., 2004). For example, *in vitro* studies have shown a ten-fold range in penetration through different samples of human skin (Lee et al., 2001) and inter-individual and intra-individual variability in human skin barrier function has been shown to be high (Meidan and Roper, 2008).

Toxicokinetic (TK) modelling, or even physiologically-based (PB) TK modelling, can be used to generate absorption and metabolism parameters to fill some gaps for route-to-route extrapolation. PBTK modelling requires an extensive amount of physiological and toxicokinetic data on route-specific ADME processes for appropriate calculations (Geraets et al., 2014). Furthermore, PBTK models need to be carefully calibrated to ensure that the results are consistent with toxicokinetic measurements, and validated against human biomonitoring data in order to disentangle the biologically real variability in the inter-individual toxicokinetic behaviour from the uncertainty in the model parameters and input data. This results in a constant process of refining the PBTK model, thereby making this a labour-intensive and time-consuming approach. However, a number of derived models have been constructed to define the ADME and systemic distribution kinetics of cosmetic molecules which can be used with the decision tree to read across approaches to apply the TTC (Gajewska et al., 2014).

The TTC is intended to be a cautious, conservative exposure level to be used as a pragmatic tool for screening and prioritisation as a first step in risk assessment. It is only appropriate in cases where no compound-specific toxicity data are available or required by regulation. To be acceptable for use with TTC, predictions of systemic availability following dermal exposure should be reliable and similar to experimental measurements where these are available. Recently, Partosch et al. (2015) have highlighted the usefulness of systemic TTC values and have derived these for three datasets. A systemic TTC value used together with the flux based decision tree to predict bioavailability may remove some of the inconsistencies that have been seen here with example chemicals.74].

5. Conclusions

Cosmetic chemicals lend themselves to the application of oral TTC for risk assessment where toxicity data are not available. There is however a requirement for reliable dermal systemic availability

data derived from an experimental absorption study or from a prediction. We have developed a decision tree to predict flux and systemic availability based on the Potts and Guy equation with adjustment for actual exposure. Our approach has been evaluated for cosmetic chemicals and specific dermal exposure scenarios and the experimental to predicted ratios were within the range of 0.1—10, which indicates 'fitness for purpose' of the model.

For dermal exposure to low level impurities, the decision tree can generate a prediction of bioavailability which could be used with TTC. Our approach will need to be applied on a case by case basis with expert judgement and in depth knowledge. Case studies demonstrated that the model in most cases over predicts dermal availability, but under some circumstances also under predicts. If the TTC threshold is close to the estimated systemic bioavailability, specific considerations and expert judgement are necessary.

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