***In Silico* Prediction of Skin Metabolism and its Implication in Toxicity Assessment**

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Abstract

Skin, being the largest organ of the body, represents an important route of exposure, not only for the abundance of chemicals present in the environment, but also for products designed for topical application such as drugs and personal care products. Determining whether such incidental or intentional exposure poses a risk to human health requires consideration of temporal concentration, both externally and internally, in addition to assessing the chemical's intrinsic hazard. In order to elicit a toxic response *in vivo* the chemical must reach its site of action in sufficient concentration, as determined by its absorption, distribution, metabolism and elimination (ADME) profile. Whilst absorption and distribution into and through skin layers have been studied for decades, only more recently has skin metabolism become a subject of intense research, now recognised as playing a key role in both toxification and detoxification processes. The majority of information on metabolic processes, however, has generally been acquired via

studies performed on the liver. This paper outlines strategies that may be used to leverage current knowledge, gained from liver metabolism studies, to inform predictions for skin metabolism through understanding the differences in the enzymatic landscapes between skin and liver. The strategies outlined demonstrate how an array of *in silico* tools may be used in concert to resolve a significant challenge in predicting toxicity following dermal exposure. The use of *in vitro* methods for determining skin metabolism, both to provide further experimental data for modelling and to verify predictions is also discussed. Herein, information on skin metabolism is placed within the context of toxicity prediction for risk assessment, which requires consideration of both exposure and hazard of parent chemicals and their metabolites.

Keywords

In silico, in vitro, skin metabolism, toxicity prediction

Introduction

Human skin is continually exposed to an abundance of diverse chemicals present in the environment, home, workplace or in products directly applied to the skin surface. Chemicals responsible for incidental exposure include industrial chemicals, pollutants, household or industrial cleaning and fragrancing products. Intentional exposure via skin occurs as a result of the application of personal care products, cosmetics or topical drug formulations. The ability of a chemical to elicit toxicity in humans, or indeed in any organism, is governed by three factors: (i) the intrinsic hazard of the chemical (or transformation product thereof); (ii) the potential for external exposure i.e. the presence of the chemical in the environment or in a topically applied product; and (iii) the ability of the chemical (or its transformation products) to reach its site of action in the body at adequate concentration. Knowledge of these three factors is essential in performing risk assessment, however, to obtain such information for all chemicals of interest via empirical testing would not be economically or practicably feasible nor would it be ethically responsible in terms of animal use. The application of alternative methods in evaluation of chemicals, or in risk assessment, is therefore essential. Whilst predictive toxicology has been used to address these issues for many years, metabolism has often proved to be a confounding factor that requires specific or inherent incorporation into the modelling process. Complications can arise in model building where it is the transformation product, rather than the parent molecule, that is responsible for the activity. Problems arising from metabolic activation and the presence of reactive metabolites, particularly following oral drug administration, are now well recognised and this has led to greater interest in predicting the identity of metabolites and their rate and extent of formation. Although it is known that the majority of organs possess metabolic capability, metabolism studies have predominantly focused on the liver - the main organ of metabolism and of key importance following oral exposure. As the skin is one of the most important routes of exposure, it is now recognised that predicting metabolism in skin is essential to obtaining accurate predictions of potential toxicity or activity (e.g. in the case of topical drug administration). There are several differences to consider between oral and dermal routes and incidental versus intentional exposure to chemicals. These factors include: frequency and duration of application or ingestion; concentration of the chemical; enzyme expression at the site of exposure or site of distribution; and the

ease of uptake or distribution from the site. Notably, skin has evolved to provide a barrier function whereas the gastro-intestinal tract is designed for the uptake of essential nutrients. Also, toxicity testing has traditionally involved (relatively) high concentration, acute, oral dosing, whereas use of personal care products is generally a low dose, long term application. A wealth of information now exists relating to oral absorption and liver metabolism, this includes information on uptake, rate and extent of metabolite formation, metabolite identity, enzymes responsible and their expression. It is now possible to leverage this important data and apply it to predictions of skin uptake and metabolism, providing appropriate adjustments are made. Such adjustments need to take account of differences in exposure scenarios, uptake potential and enzyme expression / activity levels. This paper identifies various sources of information and *in silico* tools that may be applied to predicting skin metabolism and potential toxicity following dermal exposure. How the knowledge acquired from the application of these *in silico* tools can be put together in an overall predictive strategy is discussed, as well as the importance of incorporating further data from *in vitro* studies for modelling and verification purposes.

Skin metabolism in the context of toxicity prediction

Many factors determine the likelihood of a chemical eliciting local or systemic toxicity following dermal exposure. The significance of skin metabolism has increasingly been recognised and whilst this forms the focus of this paper, other aspects must also be considered in order to place the role of metabolism in context. Figure 1 shows the numerous elements governing the potential to elicit toxicity and where skin metabolism fits within this overall scheme.

Figure 1 HERE

As illustrated in Figure 1, a wide range of data types are required in order to reach an informed decision. Fortunately, there are many *in silico* tools, and other data sources, that may be leveraged to fill the gaps in knowledge relating to uptake, metabolism and potential toxicity of chemicals following dermal exposure. Leveraging such information can also lead to the development of more robust models, particularly where experimental data can be used to develop improved models and verify predictions in an iterative process.

Historically, data have predominantly been accumulated following oral administration to test subjects or obtained from *in vitro* liver assays. This has led to a wealth of information being generated, albeit for an alternative exposure scenario. Table 1 lists the potential data sources that could be used to fill gaps in knowledge relating to skin metabolism, provided that appropriate adjustments are made to account for the differences between the oral and dermal routes.

Table 1 HERE

As alluded to above, it is not only skin metabolism *per se* that determines the potential for toxicity, it is also influenced by a plethora of additional, inter-related factors that require consideration. Table 2 (and references provided therein) provides useful sources of data for these additional factors, relevant to overall risk assessment.

Table 2 HERE

Tables 1 and 2 indicate the diverse resources available that can be utilised to make predictions for individual components within the process of assessing toxicity. The individual elements can then be rationally combined within an encompassing predictive model. In this manner, deconstructing the problem into individual components, allows greater use of different data types and more flexible adjustment of the individual factors that are relevant to the overall prediction. Each of the individual components, sources of information and adjustments necessary for the development of predictive models, as given in Tables 1 and 2, are discussed individually below.

1. The role of skin metabolism

Skin metabolism is an area that has attracted much recent interest due to its role in toxification or detoxification processes following dermal exposure. Improvements in analytical techniques have led to many metabolising enzymes being detected in skin. Although enzyme expression and activity levels are generally much lower than liver, cumulatively, given the large area of the skin, the net capacity for metabolism may be significant. It is recognised that many skin sensitisers require metabolic activation to

elicit their toxicity, conversely, minoxidil (a therapeutic agent used in treatment of hair loss) requires metabolic activation in skin in order to be effective [10]. Thus skin metabolism may be involved in toxification, detoxification or pro-drug activation processes. Identification and, where possible, quantitative estimation of metabolic capacity, is essential to determining the potential for chemicals to be activated or deactivated in the skin - a key factor in toxicity prediction and prioritisation of chemicals for further testing.

Factors relevant to skin metabolism may be subdivided into distinct categories, each of these is summarised and discussed in more detail below (see sections 1.1 – 1.7). *In silico* tools that are available to assist in the prediction of these individual factors, associated with skin metabolism, are presented in Table 3. Note that many of these tools have been based on liver metabolism studies, however, knowledge obtained from these can be usefully applied to the issue of skin metabolism provided appropriate adjustments are made. The information provided in Table 3 (and references therein) is indicative of the types of resources available and is not an exhaustive list. Tables 3 and 5, both pertaining to relevant software for metabolism prediction, incorporate information from the excellent overview provided by Kirchmair [12]. The "Click2Drug" website of the Swiss Institute of Bioinformatics (https://www.click2drug.org/index.html; accessed May 2017) is also noteworthy as it provides an updated and comprehensive listing, with brief description, of software, databases and webservices useful in drug design including a range of tools for ADME and toxicity prediction.

Table 3 HERE

1.1 Identification of the biotransformation pathway and enzyme(s) responsible

An initial step in predicting metabolism of a given chemical is identifying the relevant reaction pathway and which enzyme(s) may be involved in catalysing the process. Knowledge of the metabolic reactions of the parent chemical, or similar chemicals, may be available from the literature. For example, comprehensive reviews of biotransformation and bioactivation pathways have been published [13, 14]. As manual investigation of such literature sources may be time consuming *in silico* tools based on such acquired

knowledge are useful in providing more rapid predictions of potential interactions between a given substrate and a putative enzyme. Models have also been derived to classify which substrates are more likely to be metabolised by a particular cytochrome P450 (CYP) enzyme, such as the decision tree model of Zhang et al. [15]. Particular enzymes may have broad or narrow substrate specificity and more than one enzyme may catalyse the same metabolic process. Software is available to predict binding to enzymes (e.g. docking interactions / binding affinities), sites of metabolism, metabolic stability of parent chemicals and potential metabolic routes. Software for identifying potential interactions between chemicals of interest and putative enzyme(s) are given in Table 3.

1.2 Expression of enzymes in skin (and comparison to liver)

If the enzyme(s) relevant to the metabolic route identified above is known to be present in the skin then metabolism via that route is a realistic possibility following dermal exposure. True confirmation of the presence of a given enzyme requires experimental verification using skin-based experimental systems. Much of our understanding of the metabolic capability of skin has been based on *ex vivo* experiments using rodent and porcine skin. However, recent ethical and legislative changes have driven researchers to explore non-animal and more human-relevant *in vitro* models. Excised human skin usually obtained from abdominal or breast reduction surgery is an attractive *ex vivo* tool that offers a native tissue structure and mixed cell populations but has limitations as an experimental model due to availability, individual variability and the limited time for which the tissue can be used after excision. To overcome these limitations, the last decade has seen a dramatic increase in the use of tissue engineered, reconstructed skin equivalents, with researchers looking for control over tissue supply and experimental reproducibility. First developed in the early 1980s three-dimensional skin equivalents are produced by culturing primary dermal keratinocytes on top of a dermal fibroblast-containing matrix at an air-to-liquid interface [16, 17]. Morphologically, the models display a stratified squamous epithelium that is highly keratinised and so closely mimics the native structure and organisation of human skin. These skin equivalents offer an advantage over *ex vivo* tissue as they can be cultured on demand and for longer periods of time, allowing detailed studies on the molecular mechanisms of skin homeostatic and disease processes as well as xenobiotic metabolism. Their viability

over prolonged periods in culture enables enzyme kinetic assays to be performed either with freshly isolated tissue extracts or whole tissue. Skin equivalent models have been validated against native human tissue in terms of gene (microarray, qPCR) and protein (proteomic and immunoblot) expression. Furthermore, skin equivalent models are amenable to immunohistological examination to provide evidence of the specific expression of metabolising enzymes within the epidermis or dermis, as well as spectral analysis (Raman spectroscopy or mass spectrometry) to identify the distribution of metabolites within the tissue (see section 1.7). Such improvements in analytical methodology have led to more accurate identification and quantification of enzymes in skin. Van Eijl et al. reported a range of enzymes detected in human skin and skin models using proteomic analysis [18]. Enzymes detected in liver, but not in skin, and skin:liver expression ratios were also reported, enabling comparisons between the two organ systems: 36 enzymes were detected in both skin and liver; 46 enzymes, including 13 cytochrome P450 proteins were detected only in liver. Protein levels of enzymes involved in conjugation, hydrolysis, dehydrogenation, carbonyl reduction, oxidoreduction and oxidation were detected in skin at levels 4-10 fold lower than in liver, but levels of cytochrome P450 were reported as being 300-fold lower. This confirms the earlier suggestion that phase II metabolism predominates in skin with phase I reactions having a lesser role, although experimental processes (such as freezing and thawing) may affect quantification. Certain enzymes, such as alcohol dehydrogenase 1, epoxide hydrolases 1 and 2, hydroxyacyl-coenzyme A dehydrogenase and aldo-keto reductase 1C1 and 1C2 are expressed at higher levels in skin than in liver [19]. The review of available *in silico* and *in vitro* methods for assessing dermal bioavailability by Dumont et al. provides an excellent overview of the state-of-the-art in skin metabolism research, collated from an extensive range of literature studies [19]. The paper provides tables for enzyme detection in skin using protein expression and mRNA studies and provides skin:liver expression ratios where available. Further details such as experimental procedures, subsections of skin analysed, use of fresh or frozen samples (all of which may influence the outcome of the investigations) are available within the references provided. Discrepancies in results concerning presence or absence of specific enzymes may result from differences in analytical methods such as: anatomical differences in skin section used; freezing of samples (affecting integrity of the enzyme system), limits of detection etc. Variability inherent in such test systems was

highlighted by Manevski et al. [20]. These authors reported a proof-of-principle study using human skin explants to investigate the metabolism of 11 substrates via phase II reactions. The study confirmed formation of metabolites following glucuronidation, sulfation, N-acetylation, catechol methylation, or glutathione conjugation processes; inter-individual variability was reported at a level of 1.4 – 13 fold in the analysis. Activity of a given enzyme in skin can only be confirmed by experimental methods. Once the presence of a given enzyme has been confirmed, there are a number of software packages that can predict whether or not a given chemical is a likely substrate for that enzyme (as discussed in section 1.1 above). Developments in this area are heavily reliant on continued experimental verification of enzyme activity and improvement in *in silico* methods to predict enzyme:substrate interactions.

1.3 Reaction kinetics: predicting Vmax / Km / Kcat / CL_{int}

The rate at which metabolites are formed is another important factor in determining the time course of parent and metabolites in the skin and their potential to elicit toxicity. Rate of metabolite formation may be limited by the rate at which the parent molecule is presented to the metabolising enzymes (i.e. perfusion rate-limited for drugs that are readily metabolised) or may be limited by the capacity of the enzymes for poorly metabolised chemicals (i.e. low intrinsic clearance (Cl_{int})). For many chemicals, particularly drugs, intrinsic clearance of the compound by liver enzymes has been measured. Using these data quantitative structure-activity relationship (QSAR) models have been developed for the prediction of intrinsic clearance, as measured in hepatocytes or microsomes [21, 22, 23, 24] and for total clearance of drugs [25, 26]. The publication of Pirovano et al. reports QSARs developed for intrinsic clearance covering both drugs and environmental pollutants [27].

Clearance values can be used as an indication of the overall stability of the parent or conversely as the efficiency of metabolism by a specific enzyme. Adjustments are required when considering potential metabolism in skin versus liver as differences in enzyme expression and activity levels, as well as differences in perfusion between skin and liver, need to be taken into account. Inherent metabolic capability can be characterised in terms of Vmax (the maximum rate at which an enzyme catalyses a

reaction), Km (the substrate concentration at which half maximum rate of reaction is reached (i.e. indicating the affinity between enzyme and substrate) and Kcat (the number of substrate molecules each enzyme site converts to product per unit time, for a given enzyme concentration, when the enzyme is working at maximum efficiency). Measurements for Vmax and Km are intrinsically highly variable which complicates model development, however, it may be possible to develop local QSAR models for narrowly defined categories of chemicals. Resources such as the enzyme information system "Brenda" (http://www.brenda-enzymes.org/; accessed May 2017) provide an extensive database of Vmax, Km, Kcat and other values relating to enzyme kinetics from which further models may be developed, although it should be noted that there is very high variability in Km and Vmax values recorded, hence careful curation is required prior to selecting values for modelling. For certain enzymes there is a plethora of data but for other enzymes data are sparse. There are examples within the literature where QSAR models have been developed to predict relevant kinetic parameters using such data collations. For example, Pirovano et al. [28] provide models for prediction of Vmax and Km for compounds metabolised by four enzyme classes (alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH), flavin-containing monooxygenase (FMO), and cytochrome P450, using data obtained from Brenda, from the review of QSARs for P450 enzymes published by Hansch et al. [29] as well as other resources. Hybrid quantum mechanics / molecular mechanics (QM/MM) methods are now being applied to understanding the specific mechanisms involved in catalysis by enzymes such as cytochrome P450. Such advances in mechanistic understanding of enzyme-substrate interactions will provide further insight and more accurate computational models for predicting xenobiotic metabolism [30].

As data variability is high, one possibility is to predict a plausible range for the kinetic values rather finite values. Such an approach is analogous to that of Poulin and Krishnan who derived "theoretically plausible envelopes" of concentration in blood based on setting intrinsic clearance at theoretically possible minimum and maximum values [31]. This enables estimations to be made within a defined level of uncertainty. Software that may be used to predict Vmax, Km and intrinsic clearance are given in Table 3, although data on which such models are built are generally based on data from liver assays. *In vitro* verification of

predicted enzyme kinetics is invaluable for further model development and extension of the chemical space to which such models could be applied.

1.4 Stability / reactivity of metabolite

Once formed a metabolite itself may elicit desirable or toxicological effects, locally or remotely (following entry into the circulatory system) prior to further metabolism and/or its ultimate excretion from the body via renal, hepatic or other routes of elimination. Whilst metabolic activation is useful for administering pro-drugs, adverse effects of reactive metabolites are of significant concern in toxicology. Reactive metabolites may interact with a range of biological macromolecules (such as proteins or DNA), resulting in an array of effects including skin or respiratory sensitisation, mitochondrial toxicity or damage to DNA. The extent of damage elicited by a metabolite is a function of both the nature and the longevity of the metabolite i.e. whether the metabolite persists for sufficient time to cause a toxicological response or whether biochemical defences, or rapid clearance of the metabolite, obviate the response. Within drug discovery, drug candidates are routinely screened for potential reactive metabolite formation, using known structural alerts. Alerts for compounds with the potential to form reactive metabolites, in the context of drug discovery, have been reviewed by Stepan et al. [32]. Such alerts can be used to identify compounds associated with potential reactive metabolite formation following dermal exposure.

1.5 The likelihood of a given reaction occurring

Many potential reaction pathways to the formation of metabolites may be predicted from knowledge of organic reaction chemistry. However, the likelihood of any individual reaction occurring and leading to a given metabolite can be considered as a statistical question i.e. how often is the reaction actually observed in comparison to the number of instances it could be predicted to occur? Two sources of information are useful in predicting the overall likelihood of a reaction occurring. Firstly the overall extent of metabolism may be known as its converse (the fraction excreted unchanged in urine following oral administration) is often measured, particularly for drugs. Manga et al. developed a model to predict whether a drug would be

poorly or extensively metabolised following oral administration based on simple physico-chemical properties [33]. Secondly - assuming the chemical is subject to metabolism - then the more likely pathways can be predicted by statistical analysis of experimental databases of reactions. There are several empirical methods for ranking the most likely pathway for metabolism. PASS-BioTransfo gives the likelihood of a particular class of reactions occurring [34]; SPORCalc [35] and MetaPrint 2D (http://www-metaprint2d.ch.cam.ac.uk/; accessed May 2017) ranks the most likely sites for metabolism in a molecule; TIMES (http://oasis-lmc.org/products/software/times.aspx; accessed May 2017) and Metadrug (https://lsresearch.thomsonreuters.com/pages/solutions/18/metadrug; accessed May 2017) give a probability for formation of a given metabolite; SyGMa (Systematic Generation of potential Metabolites) ranks predicted metabolites according to an empirically derived probability score[36]. Meteor Nexus (metabolite predictor software from Lhasa Ltd; https://www.lhasalimited.org/products/meteor-nexus.htm; accessed May 2017) uses a static scoring methodology to order predict metabolites according to a pre-computed score of how predictive a particular biotransformation is, based on experimental data. A site of metabolism scoring function is also derived based on the static score but adapted appropriately using known data from similar compounds. Marchant et al. demonstrated that over-prediction of metabolites in Meteor Nexus could be reduced by incorporating a measure of the structural similarity of the query chemical to substrates with known experimental data [37]. As numerous metabolites are theoretically possible for any given chemical, it is important to rationalise those that are truly likely to be formed if a realistic safety assessment is to be performed. TIMES (TIssue MEtabolism Simulator) generates a metabolic tree where propagation of metabolites is constrained to those more likely to be formed using a defined mathematical formalism as described by Dimitrov et al. 2011 and Mekenyan et al 2012. [38, 39].

As with other information for metabolism the majority of data are derived from liver studies. Translating this to the probability of the reaction occurring in skin requires several other factors to be taken into consideration, for example are the same enzymes present in liver and skin or are there alternative enzymes that may also catalyse the same biotransformation? The ratios of different enzymes have been shown to be significantly different in skin and liver, hence consideration needs to be given as to how that would influence the metabolism of a specific chemical. Deciding on the most probable metabolites in skin, needs

to be informed by the aforementioned experimental studies to determine the actual levels of enzyme expression and activity in skin. Whilst predictions of the most likely metabolites are useful, *in vitro* or *in vivo* experimental verification as confirmation or disproval of metabolite formation would aid refinement of such statistical algorithms (refer to section 1.7 below).

1.6 Potential for induction / inhibition

Induction or inhibition of liver enzymes has been recognised as a significant factor in altering the amounts of parent or metabolite(s) present within the body, occasionally with serious or unpredicted consequences. For example the increase in unplanned pregnancies in women using hormonal contraceptives and co-medicating with St John's wort has been attributed to the induction of cytochrome P450 enzymes by St John's wort and the consequent increase in metabolism (hence reduction in circulating levels) of the hormonal contraceptive. Conversely, furanocoumarins in grapefruit juice have been shown to inhibit cytochrome P450s responsible for the metabolism of a wide variety of therapeutic agents (including anti-arrhythmic agents, anti-histamines, statins etc) leading to highly elevated levels of these drugs and resultant, significant toxicity. Hence determining the potential for induction or inhibition of enzymes is extremely important for the pharmaceutical industry and has led to a significant amount of research into possible induction or inhibition of enzymes by drugs, food and herbal products. For dermal exposure (e.g application of personal care products) a chemical may be applied every day over many years, for leave-on products in particular this leads to long term exposure with potential for induction of enzymes. Although cytochrome P450 enzymes have been shown to have low basal levels of activity in the skin these have been shown to be highly inducible, with potential consequences for repeated exposure [40, 41]. Information on substrates, inducers and inhibitors are available in the literature, some of which has been compiled into useful on-line resources for example:
https://static.medicine.iupui.edu/divisions/clinpharm/content/p450_Table_Oct_11_2009.pdf (accessed May 2017) provides a list of substrates, inhibitors and inducers of specific CYP1A2, 2B6, 2C8, 2C9, 2C19,

2D6, 2E1, 3A4, 3A5, and 3A7. Software for predicting the potential of a compound to act as an inhibitor of specific enzymes is given in Table 3.

1.7 The identity of the metabolites formed

Identifying the structure of metabolites that may be formed from a given parent structure has been a subject of intense research over many years, particularly following oral exposure. There are many literature-based sources providing details of metabolites of specific compounds [14] and on-line resources such as Drugbank (www.drugbank.ca; accessed May 2017) which lists key metabolites for drugs. The Drugbank database contains records for over 8,000 drugs including marketed pharmaceuticals, experimental compounds and drugs withdrawn from the market. The information having been collated for pharmaceuticals has a clear emphasis on the oral route and liver metabolism. In recent years, however, there have been an increasing number of publications relating to identification of metabolites in skin. Unlike data following oral exposure there is no single comprehensive, collation detailing all metabolites in skin, therefore data of this nature is highly variable and incomplete. Data are available within literature reports for either individual compounds or for a small number of compounds; these data often require extraction from text, rather than being in tabular format. The current literature provides a limited amount of skin-specific metabolism data and in some cases a comparison between skin and liver metabolism, although the data are not readily accessible. Table 4 (and references therein) provides examples of the types of data available for chemicals (drugs and non-drugs) and their metabolites that have been found in skin. The table also indicates where differences have been detected between liver and skin metabolism i.e. different enzymes involved and differences in metabolites, where these are known. Note that the table provides representative examples of the types of information available and is not intended to be an exhaustive list of available skin metabolism data.

Table 4 HERE

There are several software platforms available that enable users to predict potential metabolites, such as those listed in Table 3. Most data (and hence predictions) are focussed on liver, however, knowledge of relevant enzyme expression in skin enables appropriate adjustments to be made when comparing liver and skin metabolism. Note that many of the predictive software developed, being based on liver, has a significant focus on cytochrome P450 activity, within skin however, phase II metabolism is more predominant than phase I. The Organisation for Economic Co-operation and Development (OECD) QSAR Toolbox (http://www.oecd.org/chemicalsafety/risk-assessment/oecd-qsar-toolbox.htm; accessed May 2017) is useful as it possesses two metabolism simulators; one based on liver metabolism and the other based specifically on skin metabolism. This enables differential predictions to be made automatically for skin versus liver. The Toolbox simulates 203 transformations in skin compared to 345 in liver, based on existing knowledge. Meteor Nexus software (Lhasa Ltd) also possesses functionality that enables phase I and phase II metabolites to be generated individually.

Recent development in *in vitro* skin models linked to improvements in analytical methodology allows for more rapid identification of metabolites formed in skin that will help to refine existing predictive models. With the increased sensitivity and improved resolution of mass spectrometry analysers [48] the scope for detecting parent drugs and metabolites has greatly increased. A multitude of analytic techniques have been used to detect compounds from homogenates made from excised skin or human skin models including liquid chromatography coupled to atmospheric pressure chemical ionisation mass spectrometry (APCI-LC-MS/MS) [49], ultra-performance liquid chromatography-quadrupole time-of-flight (TOF) mass spectrometry (MS) and gas chromatography (TOF–MS) [50]. The growing development of mass spectrometry imaging (MSI) techniques has further introduced an additional dimension that not only allows metabolite detection but further informs on the spatial temporal localisation of a compound within the skin layers [51]. MSI has allowed the detection of drugs and metabolites from freshly frozen tissue sections [52, 53] and from formalin fixed tissue sections [54]. This information is being used to refine models for predicting skin metabolism, enabling comparison of predicted metabolites to those determined experimentally.

1.8 Development of skin metabolism simulators

Table 1 and sections 1.1 – 1.7 have identified key elements associated with metabolism. It has been established that the majority of data available are derived from liver metabolism studies, hence prediction of skin metabolism requires appropriate adjustments to be made. However, it is also recognised that due to developments in tissue engineering and analytical methodology more *in vitro* data are becoming available that have been directly measured in skin or skin equivalents (e.g. identity and rate of formation of metabolites). This presents an opportunity to develop *in silico* simulators of skin metabolism directly rather than relying on information derived from liver studies. As more information becomes available this can be used iteratively to improve such skin metabolism simulators, for example by confining predicted metabolites in skin to those derived from the most likely metabolic routes, as identified by analytical observations.

2. External exposure

Whilst the above has focussed on predicting skin metabolism, clearly there are other key factors to consider in an overall risk assessment for dermal exposure of chemicals. Although significant, these ancillary factors are not the focus of the current paper, hence are only briefly introduced - the reader is referred to the corresponding references and software resources presented in Table 5 for further information.

Table 5 HERE

In the case of incidental dermal exposure, predicted environmental concentrations, workplace exposure limits or typical use-case situations may be used to determine realistic or worst case scenario estimations of exposure. Typically separate exposure models are derived for workers or consumers [56]. For intentional, dermal application, levels of exposure are more closely controlled. For drugs, the specified dosing regimen determines the amount applied, the area and frequency of application and whether or not the site is

occluded. Similarly, for personal care products, standardised exposure scenarios have been published that take account of the intended use of the product, site and frequency of application, wash-off or leave-on scenarios etc, [2]. A discussion of models relating to exposure is beyond the scope of the current paper, however, indicative models and sources of information are given in Table 5.

3. Uptake and distribution within skin

Other significant factors relate to the rate and extent of uptake (absorption) and distribution of the chemical through the various skin layers and skin cells. Dermal absorption data are available from the reports of the Scientific Committee on Consumer Safety (SCCS) for cosmetic ingredients and skin permeability data are available for 470 test substances from COSMOS DB version 2 (https://cosmosdb.eu/cosmosdb.v2; accessed May 2017). Additionally, many models have been devised to predict dermal absorption or skin permeability. These models range from simple to complex including: simple discriminant functions (i.e. above particular cut-off values for molecular weight and/or lipophilicity, dermal absorption is less likely [57]; quantitative structure-property relationships that give a quantitative prediction of permeability based on correlation with physico-chemical descriptors [58, 59]; and more complex, complete kinetic models accounting for the rate and extent of diffusion into individual skin layers requiring more detailed input parameters [3, 6]. A full review of predictive models for skin uptake is beyond the scope of the work presented here, however, a detailed review of such models has been published by Mitragotri et al. [60]. Example software for the prediction of skin uptake is given in Table 5.

In terms of uptake via skin there are many sources of variability, the influences of which are poorly defined; these include ethnicity, the hydration status of the skin, presence of skin microflora, factors relating to mixture effects and choice of formulation or vehicle. Models have been developed to predict the influence of some of these factors e.g. vehicle, mixture and formulation effects [7-9], however, much more work is required in this area. Predictive models with defined levels of uncertainty (e.g. providing estimations of maximum/minimum uptake) that reflect the level of variability in the *in vivo* system may offer a more realistic solution than attempts to predict finite values for uptake.

4. **Potential to elicit toxicity**

The potential of a chemical to elicit a toxic effect *in vivo* is a consequence of both intrinsic hazard and the concentration-time profile of the chemical in the relevant organ or system. There is a vast amount of toxicity data currently available, much of which has been collated and curated into global repositories such as the OECD QSAR Toolbox (http://www.oecd.org/chemicalsafety/risk-assessment/theoecdqsartoolbox.htm; accessed May 2017), DSSTox (https://www.epa.gov/chemical-research/distributed-structure-searchable-toxicity-dsstox-database; accessed May 2017), AcTOR (https://actor.epa.gov/actor/home.xhtml; accessed May 2017), and eCHEMPortal (http://www.echemportal.org/echemportal/index?pageID=0&request_locale=en; accessed May 2017). Access to data for over 700, 000 chemicals is available via the interactive Chemical Safety for Sustainability (iCSS) CompTox dashboard (https://comptox.epa.gov/dashboard/; accessed May 2017) developed by the United States Environment Protection Agency (US EPA). This resource includes millions of predicted physico-chemical properties associated with the chemicals in the database. Where data are lacking, prediction of toxicity using both *in vitro* and *in silico* methods are well-established scientific tools. There are many software platforms available to predict a wide range of toxicities (or to identify structural features associated with toxicity i.e. structural alerts) as given in Table 5; prediction of skin sensitisation being of particular importance in terms of dermal exposure. Prediction of intrinsic hazard can be carried out for both the parent and metabolite(s); it is an inherent property determined by chemical structure. Whilst toxicity is an inherent property, overall predictions for toxicity may need to be adjusted to take account of local toxicity and differences in metabolites that may be produced when comparing oral versus dermal exposure. Physiologically-based pharmacokinetic (PBPK) models play an increasing role in toxicity prediction as they can be used to predict the concentration-time profiles of a chemical at any specific site within the body and are designed to be readily adapted for different routes of exposure.

A PBPK model is a mechanistic, multi-compartment mathematical model that describes the time-course dynamics and overall kinetics of a xenobiotic throughout the body. This is achieved by describing the

different physico-chemical properties of the xenobiotic and the specific physiology of the organism, such that the evolution of the ADME processes can be accurately simulated *in silico*. Xenobiotic properties include tissue affinity, membrane permeability, enzymatic stability etc., while the organism/system component includes such properties as organ mass/volume and blood flow [63]. The structure of PBPK models typically revolves around the anatomical structure of the organism with different organs and tissues of varying perfusion rates being separated into distinct compartments. In the simplest case, these tissue compartments are treated as being well mixed, which is based on the idea that there is a rapid equilibration of the xenobiotic once it enters the tissue [61].

Clearly, however, local tissue architecture could create spatially heterogeneous xenobiotic profiles within the tissue and, to enable capture of such spatial profiles, these local tissue features therefore should be incorporated into the PBPK framework. Mathematical modelling of these features in relation to transdermal drug transport has been a major area of research [62]. Seminal work by Higuchi (1960) [63], based on Fick's law for transport processes, laid the foundation of current theories of skin penetration. Since then, a large number of modelling papers have been written attempting to describe various aspects of transdermal permeation (for reviews see [64] and [65]). These models range in complexity but are typically based on simple assumptions, such as a single layer of skin or a two layer composite. The norm is to treat each skin layer as a homogeneous medium with no distinct intra- and extra-cellular compartments [67, 68] and xenobiotic modification via metabolising enzymes has received little attention to date.

Exceptions are the 'bricks and mortar' mechanistic models (e.g., [65] and [67]) which do account for multiple pathways (intercellular and transcellular) and metabolism has been included in several models [68-71]. These approaches provide useful qualitative information but still suffer from a number of over-simplifications of the skin structure, cellular xenobiotic transport and enzyme effects and are therefore limited in their quantitative predictive potential. To be able to move this work forward to a more predictive framework and one which can accurately predict spatial xenobiotic and metabolite skin profiles, ingredients such as cellular geometries, paracellular transport, transport of the xenobiotic across the cellular membrane as well as accurate enzyme kinetic information need to be properly measured and incorporated.

Training and validation of such a framework against spatial enzyme kinetic and metabolite data obtained from *in vitro* data will drive the generation of a software platform that will then have the ability to predict metabolite production and their rates of elimination for xenobiotic compounds in skin, which, when coupled to PBPK frameworks, will also then allow the exploration of systemic penetration and subsequent systemic effects. This is, of course, a significant task, but current advances in the *in vitro* and *in silico* work, such as that described above, is making great steps towards making such model development possible. Resources, such as the oCHEM database (https://ochem.eu/home/show.do; accessed May 2017) provide a range of experimental data serving as useful inputs for such PBPK models; note that data sharing in oCHEM is based on the wiki principle.

The combination of information concerning external exposure, internal exposure (concentration-time profiles) and intrinsic toxicity, as outlined above, is essential in order for risk assessment to be performed. This enables identification of substances of concern or of no concern in terms of human health. Such information can be used to prioritise chemicals for further testing, inform decisions on control measures to be introduced or identify where alternatives need to be sought.

Using the information in risk assessment

Grouping and read-across approaches are now well-recognised as methods to aid the prediction of toxicity. Recent publications have provided guidance on the use of Integrated Testing Strategies (ITS) or Integrated Approaches to Testing and Assessment (IATA) and the importance of incorporating metabolic information into such predictions. As the use of grouping and read-across has become more prevalent, justification of analogue selection is essential to ensuring confidence in the prediction. Wu et al. provide a framework for evaluating the suitability of analogues for read-across which explicitly assess factors relating to the potential metabolism of the analogue and target [72]. These factors include: the strength of evidence supporting the occurrence of the reaction (e.g. *in vivo* human/animal data); influence of the route of exposure and relevance of metabolism to the endpoint. Recently there have been several publications promoting the use of read-across and establishing a framework to support broader acceptance of the

methods. For example Patlewicz et al. present factors to be considered to improve consistency and acceptability of read across predictions [73]. Within the framework, specific reference is made to potential metabolism i.e. are differences expected between metabolic pathways (and/or rate) for the target and the analogue bearing in mind the route of exposure. Tollefsen et al. discuss Adverse Outcome Pathways (AOPs) and how they can support IATA again identifying where metabolic information can be incorporated when performing risk assessment [74]. These recent publications provide a useful framework for how information relating to different aspects of metabolism (likelihood of reaction occurrence, route of exposure etc) can be usefully integrated into toxicity assessment.

Conclusions

The skin is an important route for both incidental and intentional exposure to a wide range of chemicals including pollutants, drugs and personal care products. In terms of risk assessment, to ascertain whether a chemical is likely to be of concern or no concern following dermal exposure requires many factors to be considered in concert, particularly the influence of skin metabolism. Traditionally, metabolism studies have focussed on the liver as the main organ of metabolism, hence there are more data available concerning liver metabolism and the oral route of exposure. Research into skin metabolism has been a more recent endeavour driven, in part, by the advances in analytical methodology which enables detection and quantification of ever lower concentrations of enzymes and metabolites. There is an increasing body of evidence concerning which enzymes are expressed in skin and how enzyme activity varies between liver and skin. Additionally, existing data, based on liver metabolism studies, can be leveraged and applied to the question of skin metabolism, providing appropriate adjustments are made for differences in uptake, distribution and enzyme expression / activity levels. The re-purposing of data derived from liver, using appropriate adjustments and the battery of *in silico* tools that are available enables the prediction of many key factors relating to skin metabolism. Further data, currently being generated from *in vitro* skin / skin models will be invaluable in aiding *in silico* model development and refinement as well as verification of model suitability and the coverage of the models in terms of chemical space. Combining all of this

knowledge will enable more robust models to be developed and will engender greater confidence in risk assessments of chemicals following dermal exposure.

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Table 1. Summary of the information requirements for predicting skin metabolism and potential sources for leveraging data from existing studies

Information Required	**Potential Sources of Information***	**Applicability to dermal route or adjustments required**
Enzyme(s) responsible - consider affinity, selectivity and competing pathways	Existing data on biotransformations of parent or "similar" compounds available in literature or on-line databases. Software available to identify whether compounds are potential substrates for individual enzymes and to predict enzyme-substrate interactions (e.g. binding affinity / docking calculations).	Existing data largely derived from liver studies; differences in enzymes present in skin versus liver need to be accounted for.
Regional enzyme expression level	Increasing availability of qualitative and quantitative experimental data for regional enzyme expression and activity levels (e.g. detection in native skin or skin models, mRNA and protein analysis; liver:skin expression ratios). - *NB high variability in measurements between individuals and between anatomical sites of the same individual; significant inter-species differences.*	Many data generated for liver historically, however, recently, more focus on dermally expressed enzymes; comparison of expression ratios enables adjustments to be made comparing liver:skin activity.
Rate of reaction - Vmax; Km; Kcat	Data available in literature and on-line compilations (e.g http://www.brenda-enzymes.info/; accessed May 2017); limited number of QSAR models currently available (curation of existing data may enable more models to be developed); software packages available to predict Vmax and Km.	Relate to intrinsic properties of a given enzyme and substrate; differences in rate at which parent is presented to metabolisng enzymes via the different routes needs to be considered.
Likelihood of reaction - Overall potential to be metabolised - Reaction occurence ratio	Literature data available for overall likelihood of metabolism (i.e. fraction excreted unchanged / total clearance (mostly for drugs); QSAR models available for clearance and fraction excreted unchanged. Occurrence ratios can be statistically derived from known reactions and applied within predicitive software to rank more likely metabolic routes.	Most data and models generated using liver studies; differences between skin and liver need to be accounted for (e.g. the likelihood of a given metabolic route given differences in expression ratios between the two organs).

Potential induction / inhibition	Known for some enzymes (e.g. potential for induction / inhibition of enzymes following co-administration of drugs well documented); computer packages available that predict potential for enzyme inhibition.	Current data mostly dervied from liver studies; consider differences in exposure scenarios – e.g. application of personal care products may be at low dose over many years with potential to induce enzyme activity.
Identity of metabolite(s)	Considerable amount of data available from literature and on-line resources providing known metabolites of many chemicals (predominantly drugs). Wide range of software packages available to predict potential metabolites from a given chemical structure.	Majority of packages developed using data from liver studies; consider applicability to skin where expression of the relevant enzyme is reduced or absent. Note: OECD QSAR Toolbox has a specific skin metabolism simulator; Meteor Nexus (Lhasa Limited, Leeds) enables metabolites derived from phase I or phase II enzymes to be generated separately.
Stability of metabolite - potential for further metabolism - potential to form reactive metabolites	Complete metabolic trees may be predicted for compounds including phase I and phase II metabolism. Structural alerts developed to identify potential reactive metabolites.	Software to predict metabolic trees largely developed using data from liver – requires consideration of differential enzyme expression between skin and liver. Structural alerts are based on intrinsic structural features, however, potential toxicity is also dependent on site of metabolite formation (e.g. liver toxicity versus skin sensitisation).

**Details of software are given in Table 3; literature resources, databases and existing (Q)SARs are detailed in Section 1*

Table 2. Summary of the additional information requirements relevant to risk assessment and potential sources of data

Information Required	**Potential Sources of Information***	**Applicability to dermal route or adjustments required**
	External Exposure Scenarios	
Extent of incidental exposure: *Overall exposure in the workplace, home or public areas relating to amount, duration, frequency of exposure and control measures.*	Several methods are available to measure or model dermal exposure at work, in the home or in public areas. A wide range of methodologies and tools (including DREAM, DERM, EASE, MEASE, Riskof Derm, ECOTOC TRA, BEAT, ConsExpo, Spray Expo and a range of pesticide-specific models) were reviewed in detail by an expert working group of the World Health Organisation [1].	Derived for dermal exposure
Extent of intentional exposure: *Exposure as a result of intentional application of pharmaceuticals or personal care products, relating to amount, duration, frequency of exposure, wash-off/leave-on scenarios and/or use of occlusion.*	Data for application of pharmaceuticals are available from the relevant prescribing information / dosing regimen. The Notes of Guidance from the Scientific Committee on Consumer Safety [2] provide details for estimating systemic exposure following dermal application of personal care products based on in-use scenarios. Includes tables for estimating areas of exposure and frequency of application based on use cases (e.g. hand wash, body lotion, hair dyes etc).	Derived for dermal exposure
	Distrbution Within Skin	
Dermal absorption Skin permeability	Exisiting collations within the literature; reports of the Scientific Committee in Consumer Safety (SCCS); databases (e.g. COSMOS db, http://www.cosmostox.eu; accessed May 2017); (Quantitative) Structure-Activity Relationship ((Q)SAR) models; computer packages.	Derived for dermal exposure

Rate and extent of uptake in skin layers	Spreadsheet-based compuational algorithms using simple physico-chemical properties that may be measured or predicted using freely available software [3, 4]; mathematical models for drug transport in skin [5-6]. Physiologically-based pharmacokinetic (PBPK) models; computer packages.	Derived for dermal exposure or, in the case of PBPK modelling, readily adapted to dermal route.
Effect of formulation, solvent, vehicle, mixture components, occlusion, etc	Limited number of QSAR models available relating to how the effect of formulation, choice of solvent or vehicle may affect dermal uptake [7-9].	Derived for dermal exposure
	Potential to Elicit Toxicity	
Prediction of intrinisc hazard (for both parent and metabolite)	Wide range of software available to predict toxicity or to identify presence of strucutral features associated with toxicity (structural alerts).	Inherent property of parent or metabolite, determined by its structure; intrinsic hazard is not dependent on route of administration, although resulting toxicity may be dependent on route of administration.
Distribution with organism; subsequent location of parent or metabolite	PBPK models can be used to predict the time-course of parents and metabolites in individual organs of the body.	PBPK models are designed to be flexible concerning route of administration, therefore are readily adjustable for dermal exposure.

**Details of computer packages given in Table 5; literature resources, databases and existing (Q)SARs are detailed in Section 1*

Table 3. *In silico* tools (predominantly based on liver metabolism) to assist in the prediction of skin metabolism

Software	Capability / Methodology	Availability	Key Reference or Website
Tools to identify biotransformation pathways, sites of metabolism and the enzyme(s) responsible			
ACD/Percepta Platform (Regioselectivity of metabolism module)	Uses probabilistic models to predict likely sites of metabolism for the main metabolic reactions mediated by human liver microsomes and five key individual CYPs (CYP3A4, CYP2D6, CYP2C9, CYP2C19 and CYP1A2). Provides a reliability score for predictions based on similarity to training set.	Commercial	http://www.acdlabs.com/products/percepta/predictors.php; accessed May 2017
ADMET Predictor (metabolism module) from Simulations Plus	Identifies likely sites of metabolic oxidation by CYP P450 enzymes: 1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4. Classifies whether a molecule is likely to be a substrate for these CYP isoforms. Uses a curated and updated version of the Accelrys Metabolite database, including additional literature datasets, to train models.	Commercial	http://www.simulations-plus.com/Default.aspx; accessed May 2017
ChemTunes BioPath - Metabolism Database	Provides liver metabolism information for nearly 500 xenobiotics (drugs) over 2000 enzymatic reactions. Also houses over 4,000 enzymatic reactions for nearly 3,000 molecules involved in endogenous metabolism. Searchable for reaction centres, types and pathways.	Commercial; Free evaluation possible upon request	https://www.mn-am.com/php/profile.php; accessed May 2017
CypScore	Predicts likely sites of metabolism for CYP 450-mediated metabolism; uses six models for key oxidation reactions. Models based on reactivity descriptors from surface-based properties (using Parasurf based on AM1 semi-empirical molecular orbital theory); trained using Bayer Schering in-house MajorMetabolite database.	CypScore Pipeline Pilot Components freely available (differential licensing /support for academia, government, industry)	http://www.cacheresearch.com/cepos.html; accessed May 2017
FAst MEtaboliser (FAME)	Predicts sites of metabolism – Phase I and II metabolism can be predicted, both global and species-specific (human, rat and dog) models are available; uses random forest methodology, based on seven chemical descriptors; trained on over 20,000 diverse molecules.	Freely available from authors for academia and non-profit organisations	[11]
IMPACTS	Predicts sites of metabolism for CYP mediated reactions using docking, transition state modelling and substrate reactivity prediction.	Commercial	http://www.molecularforecaster.com/products.html; accessed May 2017

MetaPred web server	Predicts CYP isoform responsible for metabolising drug molecules using a Support Vector Machine approach (considers substrates of CYP3A4, CYP2D6, CYP1A2, CYP2C9 and CYP2C19).	Freely available	http://crdd.osdd.net/raghava/metapred/; accessed May 2017
MetaPrint2D	Predicts sites of metabolism in human, rat and or dog based on knowledge derived from data mining and statistical analysis.	Freely available	http://www-metaprint2d.ch.cam.ac.uk/metaprint2d; accessed May 2017
MetaSite	Predicts metabolic transformations for CYP and flavin-containing monooxygenase mediated phase I reactions; takes account of enzyme substrate recognition and chemical transformations. Identifies likely sites of metabolism and potential metabolites (ranked based on site of metabolism).	Commercial	http://www.moldiscovery.com/software/metasite/; accessed May 2017
MEXAlert	A screening tool to predict sites on a molecule where phase II metabolism (i.e. conjugation reactions) may occur indicating high probability of first pass elimination from the body.	Commercial	http://www.compudrug.com/mexalert; accessed May 2017
SMARTCyp	Predicts site of metabolism for CYP mediated reactions (CYP1A2, CYP CYP1A2, CYP 2A6, CYP2B6, CYP2C8, CYP2C19, CYP2E1, CYP3A4) by matching fragments with those in a fragment library for which reactivities have been pre-computed using density functional theory, transition state calculations and solvent accessible surface area.	Freely available	http://www.farma.ku.dk/smartcyp/index.php; accessed May 2017
StarDrop P450 Metabolism Prediction module130	Predicts sites of metabolism and relative vulnerability of that site for CYP3A4, CYP2D6, CYP2C9, CYP1A2, CYP2C19, CYP2C8 and CYP2E1 mediated reactions, using quantum mechanical simulations of chemical reactions.	Commercial	http://www.optibrium.com/stardrop/stardrop-p450-models.php; accessed May 2017
VirtualToxLab	Models binding of small molecules to CYP1A2, CYP2D6, CYP2C9 and CYP3A4.	Freely available for universities, government agencies, regulatory bodies and non-profit organisations	http://www.biograf.ch/index.php?id=projects&subid=virtualtoxlab; accessed May 2017
WhichCyp	Predicts which CYP isoform may bind query drug-like molecules (considers CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4).	Freely available	http://130.225.252.198/whichcyp/index.php; accessed May 2017
	Tools to predict reaction kinetics (Vmax, Km, CL_{int})		
ADMET Predictor (*metabolism module*) from Simulations Plus	Predicts Km and Vmax values for hydroxylation catalysed by CYP P450 enzymes: 1A2, 2C9, 2C19, 2D6, 3A4; predicts CL_{int} values resulting from metabolic activity of these five enzymes. Uses artificial neural network ensembles and 2D molecular descriptors; trained using experimental literature data.	Commercial	http://www.simulations-plus.com/Default.aspx; accessed May 2017

Tools to predict potential for enzyme inhibition			
Biovia Pipeline pilot ADME-Tox	Predicts CYP 2D6 enzyme inhibition.	Commercial	http://accelrys.com/products/datasheets/qsar-admet-and-predictive-toxicology-with-ds.pdf; accessed May 2017
oCHEM (online chemical database with modelling environment)	Predicts potential of a compound to inhibit CYP3A4, CYP2D6, CYPC19, CYP2C9 and CYP1A2.	Freely available	https://ochem.eu/home/show.do
Tools to predict the identity of the metabolites formed (likelihood of a particular reaction occurring or metabolite forming)			
ChemTunes/ToxGPS Liver BioPath workflow	Generates metabolites based on the reaction rules learned from the ChemTunes BioPath Database using reaction chemotype rules to identify reactive sites; gives prioritised metabolites	Commercial	https://www.mn-am.com/products/toxgps; accessed May 2017
META Ultra	Uses a database of 15, 000 to predict sites of metabolism and metabolite trees for query chemicals human metabolite transformations.	Commercial	http://www.multicase.com/meta-ultra; accessed May 2017
Metabolexpert	A rule-based system for predicting potential metabolites in humans, animals or plants; presents results as a metabolic tree.	Commercial	http://www.compudrug.com/metabolexpert; accessed May 2017
MetaSite	Predicts structures of the most likely metabolites of a compound, ranking is derived from the site of metabolism prediction (see above).	Commercial	http://www.moldiscovery.com/soft_metasite.php; accessed May 2017
Meteor Nexus	Uses expert knowledge-based rules to predict metabolites; results are presented as an interactive tree with supporting data. Scoring can be applied to ascertain the relative likelihood of a metabolite being observed.	Lhasa is a “not-for-profit” organisation	https://www.lhasalimited.org/meteor/; accessed May 2017
MetaPrint 2D-react	Highlights potential sites of metabolism and indicates relative likelihood of metabolism occurring at these sites; identifies potential reactions and depicts metabolites. Uses data mining of Metabolite database and probabilistic scoring.	Freely available	http://www-metaprint2d.ch.cam.ac.uk/metaprint2d-react; accessed May 2017
OECD QSAR Application Toolbox	Predicts metabolites following skin or liver metabolism of a compound of interest; contains a database of known biotransformations.	Freely available	https://www.qsartoolbox.org; accessed May 2017
TIMES	Predicts metabolic maps using a library of biotransformations and abiotic reactions; transformations can be prioritised based on probability of occurrence.	Commercial	http://oasis-lmc.org/products/software/times.asp; accessed May 2017

Table 4. Representative examples of the types of data available relating to skin (versus liver) metabolism

Parent compounds	Information available relating to skin / liver metabolism	Reference
2-butoxyethanol; ethanol	Rat skin demonstrated to metabolise 2-butoxyethanol and ethanol in presence of NAD+ suggesting aldehyde dehydrogenase (ALDH) and alcohol dehydrogenase (ADH) activity; relative expressions of isoforms of ADH between skin and liver influence capacity to metabolise alcohols of differing chain length. Rat skin predominantly expresses ADH4 whereas in liver cytosol ADH1 predominates.	[42]
Methylsalicylate; PABA (p-aminobenzoic acid); dapsone; sulfamethoxazole; minoxidil; betamethasone 17-valerate; propranolol; capsaicin	Provides evidence for expression of a range cytochromes P450, flavin monooxygenases, glutathione S-transferases, N-acetyl transferases and sulfotransferases (at mRNA and / or protein level of expression) in skin. The following biotransformations are reported for skin: methylsalicylate metabolised to salicylate; PABA, dapsone and sulfamethoxazole metabolised to N-acetyl metabolites. Dapsone and sulfamethoxazole undergo N-hydroxylation - in skin flavin containing monooxygenase 3 (FMO3) and peroxidases are likely to be responsible for this transformation, although in liver this is accomplished by CYP2C9 (lacking in skin); minoxidil is metabolised to minoxidil sulphate; betamethasone 17-valerate metabolised to active betamethasone; oxidative metabolites of propranolol observed; capsaicin shown to undergo hydrolysis and oxidation.	[10]
4-amino-2-hydroxytoluene (AHT)	N-acetyl AHT; AHT sulphate and AHT glucuronide detected; differential metabolism reported depending on route of administration (intravenous, oral or dermal).	[43]
PABA, benzocaine, azo colour reduction products; testosterone; estradiol	Compounds containing primary amino group were substrates for N-acetyltransferase activity in skin; reference provides evidence for differential expression of a range of enzymes between skin and liver; metabolism detected by loss of parent in some cases where metabolites could not be identified; skin preferentially forms 5α-hydroxy metabolites of testosterone whereas liver forms both α and β isomers; also formed metabolites that co-chromatographed with 5x-androstane-3,17-diol; 4-androstane- 3,17-dione; and 5α-dihydrotestosterone.	[44]
Benzoic acid, benzocaine, PABA; methylsalicylate; benzyl alcohol	Approximately 7% of the absorbed dose of benzoic acid formed hippuric acid (glycine conjugate of benzoic acid); 80% of absorbed benzocaine underwent N-acetylation with <10% undergoing ester hydrolysis; PABA also metabolised to N-acetyl derivative; methylsalicylate hydrolysed by esterases to salicylic acid and 21% further metabolised via glycine conjugation to salicyluric acid; benzyl alcohol oxidised to benzoic acid; aryl hydrocarbon hydroxylase detected at >10:1 ratio between liver:skin.	[44]
Benzo[a]pyrene; trinitrobenzene; phenanthrene	Benzo[a]pyrene hydrolysed to benzo[a]pyrene 7,8,9,10-tetrahydrobenzo[a]pyrene, nitro groups on trinitrobenzene reduced to amino groups which may be further acetylated to acetamide derivative – 1,3,5-benzene triacetamide and 3,5-dinitroaniline detected; phenanthrene metabolised to 9,10-dihydrodiol, 3,4-dihydrodiol, 1,2-dihydrodiol and traces of hydroxyl phenanthrenes.	[45]
Butylated hydroxytoluene (BHT)	4-hydroxy derivative of BHT detected in skin.	[46]

Trans cinnamic alcohol; trans-cinnamaldehyde;	Formation of trans-cinnamic acid and cinnamic alcohol via alcohol dehydrogenase and aldehyde dehydrogenases.	[47]

Table 5. *In silico* tools to assist in the prediction of additional factors relevant to risk assessment

Software	Capability / Methodology	Availability	Key Reference or Website
	Tools to predict dermal exposure		
ConsExpo Web	A mathematical model used to assess exposure to chemicals from everyday consumer products (e.g. household cleaning products and personal care products (provided by the National Institute for Public Health and the Environment, Netherlands), considers inhalational, oral and dermal exposure.	Freely available (after registration)	http://www.rivm.nl/en/Topics/C/ConsExpo; accessed May 2017
ECETOC TRA	A tool for Targeted Risk Assessment (TRA) provided by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC). Calculates risk of exposure from chemicals to workers, consumers and the environment.	Freely available (after registration)	http://www.ecetoc.org/tools/targeted-risk-assessment-tra/; accessed May 2017
RISKOFDERM	The outcome of a 5th Framework Programme of the European Community, providing a Toolkit for predicting dermal exposure.	Freely available (after registration)	http://www.eurofins.com/consumer-product-testing/services/research-development/projects-on-skin-exposure-and-protection/riskofderm-skin-exposure-and-risk-assessment/; accessed May 2017
Stoffenmanager (substance manager)	Web-based quantitative exposure modelling tool for both respiratory and dermal exposure.	Freely available (after registration)	https://stoffenmanager.nl/; accessed May 2017
	Tools to predict uptake in skin		
DermWin	Predicts dermal permeability coefficient (Kp); part of the Estimation Programs Interface (EPI) Suite software, developed by the US Environment Protection Agency.	Free	http://www.epa.gov/tsca-screening-tools/download-epi-suitetm-estimation-program-interface; accessed May 2017
Excel spreadsheet-based model	A spreadsheet-based model to estimate bioavailability following dermal exposure, predicts transient skin absorption through stratum corneum, viable epidermis and dermis.	Free	[3]
Skin-in-Silico	Predicts absorption and permeation of chemicals and formulations into and through skin.	Commercial	https://www.xemet.com/en/products/#skin; accessed 2017
	Tools to predict toxicity or to identify structural features associated with toxicity		

Biovia Discovery Studio (incorporating TOxicity Prediction by Komputer Assisted Technology - TOPKAT)	Predicts a range of ADMET properties including hepatotoxicity and identifies undesirable features using published SMARTS. TOPKAT predicts many toxicity endpoints (such as mutagenicity, carcinogenicity, developmental toxicity, LC50 (rat, fish, daphnia), skin and eye irritancy.	Commercial	http://accelrys.com/products/collaborative-science/biovia-discovery-studio/qsar-admet-and-predictive-toxicology.html; accessed May 2017
Case Ultra Models	Provides a collection of toxicity model bundles for endpoints including hepatotoxicity, renal toxicity, developmental and reproductive toxicity, skin and eye toxicity etc.	Commercial	http://www.multicase.com/case-ultra-models; accessed May 2017
ChemTunes ToxGPS	A knowledgebase for toxicity predictions to support safety evaluation and risk assessment of chemicals. Provides toxicity outcomes by using both QSAR and rule-based approaches. Statistical QSAR models are stratified across the mechanism of action pathways and the structure rules, developed by domain experts are enhanced by chemoinformatics approaches. Both QSAR and rule-based outcomes are then combined to reflect the weight of evidence of all information. The predictions are linked directly to large/high quality ChemTunes toxicity database through nearest neighbours.	Commercial	https://www.mn-am.com/products; accessed May 2017
Chemotyper	Identifies chemical chemotypes (substructures or subgraphs) within a dataset of chemicals that may be used to search for structural alerts for toxicity.	Freely available	https:chemotyper.org; accessed May 2017
DEREK Nexus	Uses rules derived from expert knowledge to predict toxicity endpoints including carcinogenicity, mutagenicity, genotoxicty, skin sensitisation, teratogenicity, irritation respiratory sensitisation and reproductive toxicity; provides a reasoned prediction of the likelihood of the toxicity.	Lhasa is a “not-for-profit” organisation	https://www.lhasalimited.org/products/derek-nexus.htm; accessed May 2017
HazardExpert Pro	Uses a rule-based system to predict oncogenicity, mutagenicity, teratogenicity, membrane irritation, sensitivity, immunotoxicity and neurotoxicity.	Commercial	http://www.compudrug.com/hazardexpertpro; accessed May 2017
Leadscope QSAR Models	Provides a series of QSAR models to predict endpoints including (non-human) developmental, genetic, reproductive and neurotoxicity and human cardiac, hepatobiliary and urinary tract toxicity. Also offers a rule-based system for genetic toxicity alerts based on publically-available alerts and a toxicity database of over 180,000 chemical records.	Commercial	http://www.leadscope.com/model_appliers/; accessed May 2017

oCHEM (online chemical database with modelling environment)	Possesses a range of predictive models (including AhR activation, AMES mutagenicity etc) and structural alerts that can be used to screen chemicals for molecular features associated with toxicity.	Freely available	https://ochem.eu/home/show.do; accessed May 2017
OECD QSAR Toolbox	The Toolbox uses existing data to fill gaps in knowledge for a range of (eco)toxicity endpoints. It identifies relevant structural features for a "target" compound that may be associated with a particular mechanism of toxicity (for example structural alerts associated with skin sensitisation, mutagenicity, carcinogenicity etc). Other compounds within its databases possessing the same characteristics as the "target" are identified enabling a read-across prediction. The Toolbox includes biotic and abiotic metabolism simulators enabling information regarding metabolites to be incorporated in read-across predictions.	Freely available	http://www.oecd.org/chemicalsafety/risk-assessment/theoecdqsartoolbox.htm; accessed May 2017 [55]
(Prediction of Activity Spectra for Substances) PASS online	Predicts over 3,500 types of biological activity (including pharmacology, toxicity and interaction with enzymes or transporters) using chemical structure alone. Prediction is based on analysis of structure activity relationships for >250,000 substances.	Freely available (after registration)	http://www.way2drug.com/passonline/; accessed May 2017
ToxPredict	Estimates hazard from chemical structure, provides 16 models for 14 toxicity endpoints.	Freely available	https://apps.ideaconsult.net/ToxPredict; accessed May 2017
Toxtree	Estimates toxic hazard for a range of endpoints (human health and environmental) based on a decision tree approach; encodes structural alerts for skin sensitisation, activity in micronucleus assay, predicts skin and eye irritation, biodegradation etc.	Freely available	http://toxtree.sourceforge.net/; accessed May 2017
VEGA	Virtual models for property Evaluation of chemicals within a Global Architecture (VEGA) provides a platform for *in silico* models to support safety evaluation of chemicals. VEGA is a combination of QSAR and read-across providing models for toxicity endpoints (including skin sensitisation, carcinogenicity, mutagenicity etc) and a tool enables evaluation of the result by consideration of the applicability domain of the model.	Freely available	http://www.vega-qsar.eu/; accessed May 2017
VirtualToxLab	Simulates and quantifies the interaction between a chemical of interest and biological target proteins known to trigger adverse effects (androgen, aryl hydrocarbon, estrogen α, estrogen β, glucocorticoid, hERG, liver X, mineralocorticoid, progesterone, thyroid α, thyroid β and peroxisome proliferator-activated receptors), uses docking combined with QSAR approaches.	Freely available for universities, government agencies, regulatory bodies and non-profit organisations	http://www.biograf.ch/index.php?id=projects&subid=virtualtoxlab; accessed May 2017

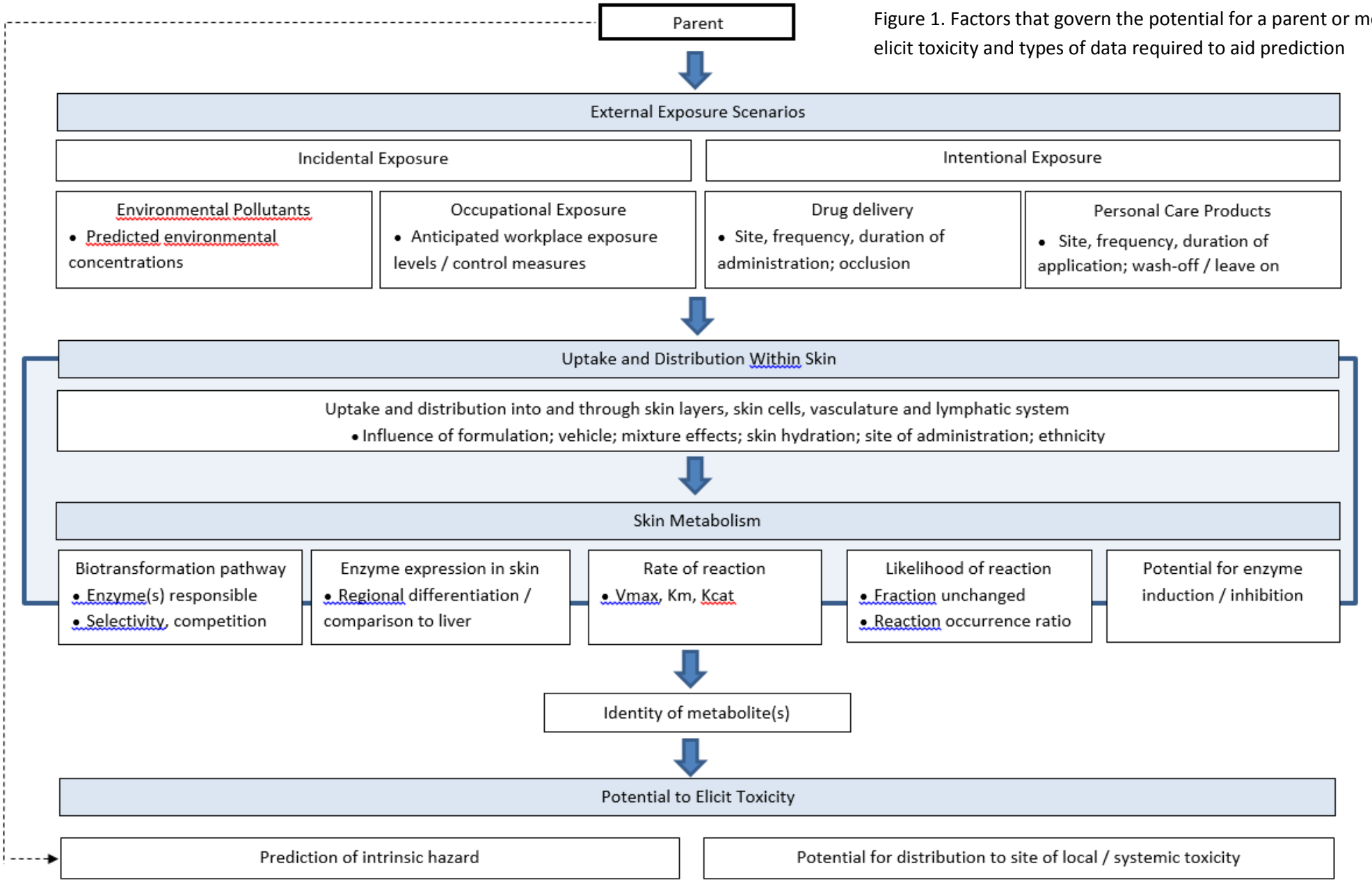

Figure 1. Factors that govern the potential for a parent or metabolite to elicit toxicity and types of data required to aid prediction