New Journal and we have not received input yet 21 (2022) 100213

Contents lists available at ScienceDirect

Computational Toxicology

journal homepage: www.sciencedirect.com/journal/computational-toxicology

A review of *in silico* toxicology approaches to support the safety assessment of cosmetics-related materials

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ARTICLE INFO

Keywords:
Cosmetics
Risk assessment
In silico
Computational
Read-across
Quantitative structure-activity relationship

ABSTRACT

In silico tools and resources are now used commonly in toxicology and to support the "Next Generation Risk Assessment" (NGRA) of cosmetics ingredients or materials. This review provides an overview of the approaches that are applied to assess the exposure and hazard of a cosmetic ingredient. For both hazard and exposure, databases of existing information are used routinely. In addition, for exposure, *in silico* approaches include the use of rules of thumb for systemic bioavailability as well as physiologically-based kinetics (PBK) and multi-scale models for estimating internal exposure at the organ or tissue level. (Internal) Thresholds of Toxicological Concern are applicable for the safety assessment of ingredients at low concentrations. The use of structural rules, (Quantitative) Structure-Activity Relationships ((Q)SARs) and read-across are the most typically applied modelling approaches to predict hazard. Data from exposure and hazard assessment are increasingly being brought together in NGRA to provide an overall assessment of the safety of a cosmetic ingredient. All *in silico* approaches are reviewed in terms of their maturity and robustness for use.

1. Introduction: Need for *in silico* toxicology approaches for the safety assessment of cosmetics-related materials

The full, i.e. testing and marketing, ban on animal testing for cosmetic products came into force within the European Union on 11 March 2013 through the implementation of EU Regulation, EC N°1223/2009 [1], with the Regulation representing one of the key pieces of legislation governing the safety of cosmetics ingredients and products [2,3]. As such it stimulated an upsurge of interest in the development of alternatives to "traditional" animal testing [4,5]. The ban on testing has effectively meant new ways of thinking and approaches to the safety assessment of cosmetic ingredients are required [6]. For instance, whilst a full toxicological dossier may still be required by the Scientific Committee on Consumer Safety (SCCS), the Notes of Guidance [7] state in Section 3-4.1 that validated alternatives, as well as scientifically valid alternatives which have not necessarily gone through a formal validation process, may be accepted on a case-by-case basis. Additionally, Section 3-6.10 of the Notes of Guidance states that some studies can be

Abbreviations: AED, administered equivalent dose; AEL, Acceptable Exposure Level; AOP, Adverse Outcome Pathway; AUC, Area under concentration-time curve; CIR, Cosmetics Ingredients Review; Cmax, Maximum concentration; COSMOS NG, COSMOS Next Generation Database; CRED, Criteria for Reporting and Evaluating ecotoxicity Data; DB, Database; DST, Dermal Sensitisation Threshold; ECHA, European Chemicals Agency; GCMP, Good Computer Modelling Practice; GLP, Good Laboratory Practice; IATA, Integrated Approaches to Testing and Assessment; ICCR, International Cooperation on Cosmetic Regulation; ICE, Integrated Chemical Environment; ITS, Integrated Testing Strategies; Jmax, flux across skin; Kp, skin permeability coefficient; JRC, Joint Research Centre; LRSS, Long Range Science Strategy; MIE, Molecular Initiating Event; NAM(s), New Approach Methodology(ies); NGRA, Next Generation Risk Assessment; NESIL, No Expected Sensitisation Induction Level; NO(A)EL, No Observed (Adverse) Effect Level; NTP, National Toxicology Program; OCHEM, Online chemical database; OECD, Organisation for Economic Cooperation and Development; ODE, Ordinary Differential Equation; PBPK/PBTK, Physiologically-Based PharmacoKinetic/ToxicoKinetic; PBK, Physiologically-based kinetic; PCPC, United States Personal Care Product Council; QsarDB, QSAR DataBase; RAAF, Read-Across Assessment Framework; qAOP, quantitative Adverse Outcome Pathway; QRA, quantitative risk assessment; (Q)SAR, (quantitative) structure-activity relationship; RIFM, Research Institute for Fragrance Materials; SAR, structure-actvity relationship; SAFs, Safety Assessment Factors; SCCS, Scientific Committee on Consumer Safety; SciRAP, Science in Risk Assessment and Policy; SEURAT-1, Safety Evaluation Ultimately Replacing Animal Testing-1; TTC, Threshold of toxicological concern.

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https://doi.org/10.1016/j.comtox.2022.100213
Received 12 September 2021; Received in revised form 3 December 2021; Accepted 4 January 2022
Available online 7 January 2022

waived if systemic exposure via dermal absorption is expected to be minimal. The use of non-animal tests in the context of regulatory requirements for the assessment of cosmetic products is well reviewed by Pistollato et al. [3].

The requirements to meet the regulations for safety assessment of cosmetics ingredients in the EU and elsewhere have encouraged new ways of understanding what is meant by, and what is required from, toxicological testing to support chemical safety assessment, without animal testing. These concepts are being implemented at the same time as many other activities in toxicology, such as the paradigm of 21st Century Toxicology [8], new advances in computational methods and their implementation, molecular biology (-omics) approaches, application of the Adverse Outcome Pathway (AOP) framework and other legislative drivers for regulation, such as REACH, or animal welfare and ethical concerns [9].

Whilst a ban on testing was implemented, scientifically the world was ill-prepared to replace the traditional suite of animal tests on which safety assessment was previously determined [10]. In a comprehensive review of the state of the art of alternatives, Adler et al. [11] concluded that it may require decades of investment and research to replace fully the information provided by animal tests for the "complex" toxicological tests such as those for repeat dose and developmental and reproductive toxicity. In the years that have followed that review, the main conclusions have been substantiated, with little progress on development and acceptance of valid, efficient and effective alternatives to complex animal tests [9], albeit with a growing list of validated alternatives to animal testing applicable for cosmetic products and their ingredients provided by the International Cooperation on Cosmetics Regulation (ICCR) [12]. There is also a growing realisation, stimulated in no small part by the report of the National Research Council [13] on 21st Century Toxicology, that the time is ripe to capitalise on new approaches to perform "better science" i.e. to use updated technologies to replace methodologies that have changed little for decades to give more human-relevant and ethically acceptable information. Specifically, there are new opportunities to use *in vitro*, high throughput and content screening as well as computational approaches guided by mechanistic information such as that provided by AOPs [14].

Thus, with regard to cosmetics ingredients, finding new means of performing safety assessment of ingredients has passed from being desirable to being a necessity for businesses to operate and innovate [15]. Initiatives such as the SEURAT-1 (Safety Evaluation Ultimately Replacing Animal Testing-1) cluster of projects, from 2011 to 2015, co-funded by the European Commission and Cosmetics Europe (formerly Colipa) provided a stepping-stone to further research projects [4,5]. Following SEURAT-1, Cosmetics Europe has funded a series of targeted projects and case studies through the Long Range Science Strategy (LRSS) which have attempted to make the industry more able to apply non-animal methods to the safety assessment of cosmetics-related materials [16,17] as well as drawing on the expertise of the ICCR [18] and other groups [19]. These have been supplemented, and drawn upon for inspiration, by a large number of European funded projects in the area of alternatives to animal testing, some of these projects have been reviewed recently by [20].

Computational, or *in silico*, techniques are fundamental to non-animal chemical safety assessment [21,22]. Currently, these are applied to both internal exposure and hazard identification, hence they provide information to act as the cornerstones of risk assessment. Computational methods range widely in not only the techniques and approaches that may be applied, but also endpoints that are covered. Excellent and detailed recent reviews of *in silico* methods to predict toxicological endpoints exist and will not be repeated here [22–27]. The applicability of computational (*in silico*) approaches to predict endpoints that may assist in the safety assessment of cosmetics is established [7,27]. This paper intends to provide an overview of these approaches with a focus on the results of recent research demonstrating real-life applications and development of novel tools and integrated strategies for safety assessment.

2. Resources for exposure assessment

The first stage of the safety assessment of cosmetics materials is likely to be an evaluation of potential exposure to an ingredient in a product. Naturally compounds with low exposure, unless of high hazard, are likely to be of lower concern with regard to safety. With regard to resources for available to assess exposure, these range from tables of typical use cases for exposure to products and simplistic rules of thumb/QSARs to predict uptake, to Physiologically-based kinetic (PBK) and multilevel models that can estimate concentrations at the organ and tissue level. This section reviews, briefly, some of the resources and *in silico* approaches most relevant for the evaluation of exposure to a cosmetics ingredient. Table 1 provides a summary of the approaches [28–36], these are explored in greater detail in the accompanying narrative (refer to corresponding section numbers).

2.1. Estimating realistic exposures of cosmetic Ingredients: Calculation methods and models

A number of assumptions are often made regarding exposure to cosmetic ingredients. For instance, the SCCS in its Notes of Guidance [7] provides standardised estimates for exposure in terms of the amount of product applied, frequency of use, retention and potential for uptake via the oral, dermal or inhalational routes [7]. These may be applied and adapted on an individual basis or an estimate could be made using appropriate software such as SpheraCosmolife [28] which allows input of the concentration and use case of a single ingredient as part of the risk assessment process (https://www.vegahub.eu/download/sphera-cosmolife-download/).

It is a more complex procedure to assess the aggregate exposure to an ingredient. The SCCS Notes of Guidance proposes methods to deal with aggregate exposures, considering whether this is by the same route or different routes and if there are potential differences in metabolism by the different routes. Another approach is the Creme RIFM model, this incorporates usage data from over 36,000 consumers in representative European and US populations (https://www.cremeglobal.com/creme-rifm/). The Creme RIFM model provides the opportunity to generate estimates of aggregate systemic and dermal exposure assessment for fragrance compounds and has been expanded and refined, since first published, to cover more cosmetics, personal care and hair care products [29,37]. Clearly this approach could be applied to broader types of cosmetics ingredients should the data become available. The approach of probabilistic aggregate exposure modelling has been demonstrated for fragrances and vitamin A arising from cosmetic product use, diet and food supplements [38,39].

The assessment of exposure can also be supplemented by existing information from the RIFM database capturing the frequency and combinations of products used at specific times during the day, which allows for the estimation of aggregate exposure for an individual consumer as based on samples from across Europe and the United States [40]. Further data on human exposure may be derived from human biomonitoring studies – the integration of such information into chemical safety assessment is only at a very initial stage but may provide a useful source of data for the future [41]. Human biomonitoring data are available for cosmetics ingredients [42] though their use is currently limited. Such data are becoming increasingly available, for instance through resources such as the Information Platform for Chemical Monitoring (IPCHEM), freely available from: https://ipchem.jrc.ec.europa.eu/. As well as providing information regarding exposure, human biomonitoring data offer the possibility of a "real-life" evaluation of exposure models. Aylward et al. [43], compared predictions from three screening exposure models (European Commission Scientific Commission on Consumer Safety [SCCS] algorithms, ConsExpo in deterministic mode, and RAIDAR-ICE) and two higher-tier probabilistic

Table 1
Summary of resources for assessing exposure to cosmetic ingredients.

Section	Type of Approach	Information available/output	Examples of Resources Including Software Guidance, Software or Other Tools	Strengths	Weaknesses
2.1	Calculation of exposure	Daily exposure to a product, per person on the basis of standard exposure scenarios	Scientific Committee on Consumer Safety (SCCS) Notes of Guidance (SCCS, 2021); SpheraCosmolife [28]	Accepted standards; Easy to calculate, accepted by SCCS; transparent	Gives no estimate of actual (systemic) exposure
2.1	Estimation of aggregate exposure	Estimated total exposure to an ingredient from all sources	Creme RIFM [29]	Large databases on human values, ease of use	Unknown applicability domain in terms of cosmetics ingredients applicable to (this is a relatively minor issue)
2.2	Rules of Thumb	An indication of possible low oral absorption or low dermal permeability	Oral – Lipinski Rule of 5 s [30]; Dermal [31]	Simple to apply, based on simple physico-chemical properties	Trivial rules to be used only as a guide
2.3	Databases or QSARs for dermal absorption	Measured/predicted values of skin permeability (Kp); flux across skin (Jmax); % absorbed	Edetox [32] or Huskin [33] databases of measured Kp values; Potts and Guy [34] model for Kp available in EpiSuite (https://www.epa.gov/tsca-screening-tools/epi-suitetm-estimation-program-interface), SWISSADME [35], SpheraCosmolife [28]	Provides a quantitative measure/estimate of properties related to dermal absorption, calculated from fundamental physico-chemical properties	High variability in data recorded and used in model development; often assumes infinite dose; vehicle/solvent effects not considered
2.4	PBK models	Maximum concentration (Cmax) or Area under concentration–time curve (AUC) in specific organs or tissues following specific exposure scenarios	pkSIM and Mobi (Open Systems Pharmacology, http://www.systems-biology.com/products/pk-sim/), QIVIVE tools; SimCYP (http://www.systems-biology.com/products/pk-sim/); PLETHEM (https://scitovation.com/plethem/); Integrated Chemical Environment (ICE) [36]	Adaptable and parameterisable for different exposure routes and scenarios; provides organ-level concentrations for risk assessment.	Models are data intensive and complex to develop/evaluate; lack of expertise; inconsistency in reporting

models (SHEDS-HT, and Creme Care & Cosmetics) to human biomonitoring data for average urinary excretion rates. The predictions were generally seen to be "realistic" with approximately 60–90% of model predictions for most models within a factor of 10 of the observed exposures (based the calculated minimum absorbed doses derived from the biomonitoring data – further details in Aylward et al. [43]). These results provide further evidence of the validity of the exposure models.

2.2. Rules of thumb for exposure estimates

Rules of thumb are usually derived from simple physico-chemical properties and counts of structural features and, mostly, aim to exclude compounds on the basis of probable poor bioavailablity. The most well-known rule thumb is Lipinski's rule of fives to predict poor oral absorption [30]. An analogous approach to the prediction of poor dermal absorption (hence more relevant to cosmetic ingredients) was proposed by Ates et al. [31] and cited by the SCCS [44]. Other rules for high and low permeability coefficients, based on log P and molecular weight have been available for over three decades and were originally described by Flynn [45]. These rules of thumb are easy to apply, especially the Lipinski rule of fives which is widely available from many software packages, although they must be considered as being rather crude means of determining poor bioavailability (and hence a low risk of toxicity) when used in safety assessment.

2.3. Properties relevant to dermal exposure of cosmetics materials

In order to make estimates of dermal exposure more quantitative, there are a number of usable QSARs for skin permeability, these are well reviewed by Tsakovska et al. [46]. Historical data on which QSARs for skin permeability are based are available via resources such as Edetox (https://research.ncl.ac.uk/edetox/theedetoxdatabase/) and HuskinDB (https://huskindb.drug-design.de/data/; [33]), however they are frequently characterised as being highly variable, which will be reflected in the quality of QSAR models [47]. Issues with data quality appear to be as a result of historical variations in methodology. A recent study of the measurement of the penetration of 56 cosmetic relevant chemicals into and through human skin used a standardised protocol and generally showed good reproducibility [48].

For the safety assessment of cosmetics ingredients, from the outset it may be possible to use the simplistic approaches of Ates et al. [31] and Flynn [45] as described above. Of the QSAR models available, the simplistic and transparent model presented by Potts and Guy [34], and all its variations, is as robust as most approaches. It is coded into many software applications including, SpheraCosmolife, EPISUITE etc. Likewise, Magnusson et al. [49] provided a simple model to predict maximal flux from molecular weight alone. Additionally, an *in silico* skin absorption model tailored for the safety assessment of fragrances which assigns absorption values of 10%, 40%, or 80% based on Jmax has been proposed by RIFM [50]. The model may be used for non-fragrance material provided they meet certain specified criteria. None of these models are able to provide definitive predictions of systemic bioavailability of cosmetic ingredients following dermal exposure and should be thought of as means as to identify compounds with potentially high or low dermal absorption. It is hoped that with access to more reliable data such as those published by Hewitt et al. [48] there will be a fairer evaluation of models for predicting parameters important to dermal uptake and an increase in the types of model available.

2.4. Physiologically-based kinetic (PBK) models

Physiologically-based kinetic (PBK) models, also referred to as Physiologically-Based PharmacoKinetic/ToxicoKinetic (PBPK/PBTK) models, are increasingly being used across various industrial and regulatory sectors to predict the organ level concentration of chemicals following exposure via different routes – oral, dermal and inhalation

[51]. This internal dose provides a more accurate indication of the potential of a chemical to elicit a toxic effect than considering only the externally applied dose. PBK models use chemical-specific information (e.g. solubility, hydrophobicity and plasma protein binding) in addition to physiological and anatomical information (e.g. organ volumes and blood flow) to determine concentration-time curves in different tissues. This is relevant for human exposure to cosmetic ingredients (as well as to food, drugs or environmental chemicals) and is increasingly used in translational research in the pharmaceutical industry as well for safety assessment in agrochemical and food industries [52]. The models can be adapted for different routes of exposure, species, age, race, gender, disease state etc. The aim of such models is to predict an appropriate dose metric – the dose measure causally linked to the toxic response (e.g. the maximum concentration that may be achieved in a particular organ or tissue (Cmax) or the area under the concentration-time curve (AUC)). The process of Next Generation Risk Assessment (NGRA) can utilise such dose metrics along with *in vitro* data to derive a Point of Departure [53].

PBK models are considered to require expertise for proper application in the cosmetics industry. At the current time they are considered to be a speciality, however, it is inevitable that there will be increased use of these models to assist in the determination of exposure to ingredients with regard to NGRA. Previously, models have been developed using Ordinary Differential Equation (ODE) solvers such as those available in R (https://cran.r-project.org/web/packages/deSolve/index.html) or MATLAB (Mathsworks; https://uk.mathworks.com/). Another approach includes employing modelling packages such as SimCYP (https://www.certara.com/), Berkeley Madonna (https://berkeley-madonna.myshopify.com/), or pkSIM and Mobi (http://www.open-systems-pharmacology.org/). In addition, tools for quantitative *in-vitro*-to-*in-vivo* extrapolation (http://www.qivivetools.wur.nl/) have become freely available, increasing the accessibility of the models. The PLETHEM software (https://www.scitovation.com/plethem/) is also widely used and includes a variety of models [54]. A generic PBPK model has also been made freely available through the the National Toxicology Program (NTP) Integrated Chemical Environment (ICE) platform [36,55] (https://ice.ntp.niehs.nih.gov/).

To use PBK models successfully, the risk assessor needs to make important, and informed, decisions regarding the design of the model for the particular question asked (e.g. which compartments are relevant, appropriate exposure scenario etc) as well as considering the reliability of the input parameters (experimental or calculated properties) and the sensitivity of the model to the parameters selected. More detailed assessment of concentrations achieved in specific tissues or sub-compartments within organs may require multi-level modelling as described in Section 2.5. It should be noted that whilst consideration of potential exposure and toxic hazard herein has specifically focused on the parent ingredient, it is similarly possible to predict exposure and potential hazard of metabolites or abiotic transformation products of ingredients. Skin metabolism (of particular relevance to cosmetic ingredients) in comparison to liver metabolism, availability of *in silico* tools for prediction and implications in toxicity assessment has been reviewed elsewhere [56].

2.5. Multi-level models, virtual organs and (quantitative) systems toxicology

In silico models are being developed that provide more detail than "traditional" PBK models in terms of providing greater granularity of where a compound may be distributed to in an organ. The potential for this level of detail has particular relevance for organs which may potentially be exposed to high concentrations of cosmetics ingredients and where specific tissues may be prone to toxicological events. The so-called "multiscale" models are derivatives of PBK models and can be considered to be high-level descriptions of organ physiology and allow for the increase of resolution to the cell level [57].

With regard to the use of multiscale models, and similar approaches, for the evaluation of cosmetics ingredients and materials, there is limited uptake at the current time. Bois et al. [58] gave a general opinion of the use of such models, mainly focussing on PBPK models. Some examples were provided e.g. the visualisation of liver damage from acetaminophen comparing healthy and diseased subjects [59] but these remain chemical- and case-specific examples at this time. Thus, whilst progress has been made, practical and widespread implementation of multiscale models remains aspirational at this time due to the limited stage of development.

There has also been a number of attempts to develop virtual, or *in silico*, organs either as a result of, or in combination with, progress in systems toxicology [60]. There is considerable interest to utilise these methods to support risk assessment of chemicals, including cosmetics ingredients [61]. Systems toxicology itself offers the opportunity to translate the data from transcriptomics and other high content assays to meaningful outputs for risk assessment. Examples demonstrate how it can be applied, for instance, to test for cardiotoxicity using data from larval zebrafish [62]. Also, software such as DILIsym (https://www.simulations-plus.com/software/dilisym/) is available to make quantitative systems toxicology more readily available [63]. With regard to virtual organs, progress is being made towards a "virtual embryo" [64] and "virtual liver" [65], amongst others. The ability to produce virtual organs integrating, or not, quantitative systems toxicology approaches remains highly specialised and is labour intensive. There is no doubt it will provide useful information and usable models in the future, but at the current time it also remains aspirational for the routine safety assessment of cosmetics ingredients.

3. Threshold of Toxicological Concern (TTC) and internal Threshold of Toxicological Concern

The Threshold of Toxicological Concern (TTC) concept is a risk assessment paradigm founded originally for food contact substances and fragrances in the 1990s, with regard to those substances whose expected exposure is very low [66]. It has since found more widespread use for various other applications. It is based on the premise that at a particular low dose (the threshold), a chemical is deemed not have appreciable risk to human health [67]. This approach has been accepted by the SCCS as a usable tool that can be applied to the safety assessment of cosmetic materials [7]. The threshold is identified from the cumulative distribution of appropriate oral NO(A)EL values, with the 5th percentile being established and an uncertainty factor of 100 added [68]. Application of TTC values follows a decision framework where the structure of ingredients is checked step-wise. The chemicals are first screened as to whether they belong to "COC (Cohort of Concern)" classes which are out of domain (typically high potency carcinogens and highly bioaccumulating substances) as well as inorganics, metals, and metal containing compounds [67]. If the structure is genotoxic, the chemical enters the cancer TTC approach; if it is not genotoxic, the chemical enters the non-cancer TTC tree. Within the non-cancer tree, the Cramer Classification scheme [69] is applied to assign the chemical to one of three classes, depending on potential level of toxicity, with decreasing thresholds as potential toxicity increases. Despite the age of the Cramer Classes they are still widely applied through software applications such as Toxtree [70], although comparison of predictions from different software has identified problems that may occur [71,72]. At the time of writing of this review an update to the Cramer Classes is being considered, which extends the overall scheme to six classes [73].

A key aspect of TTC has been the underlying database to derive robust and reliable thresholds, which involves in-depth reviewing/assessing appropriate toxicological data. For cosmetic materials there was a need to extend the historical paradigm such that the data set from which thresholds are derived was enriched with cosmetics-related substances. Yang et al. [72] described the development of TTC specifically for cosmetics ingredients, requiring not only the use of a cosmetics inventory to determine coverage of chemicals, but compilation and expert-

review of data for repeat dose toxicity. The authors further established a "Federated Set" of data based on historical Munro and COSMOS TTC datasets to propose pragmatic and yet conservative thresholds. This COSMOS approach led to the SCCS's most recent decision on the thresholds of 2.3 and 46 μg/kg-bw/day for Cramer classes III and I, respectively, for use in relation to cosmetics-related substances [7]. This expansion of the data set demonstrated the stable nature of the historical thresholds. Further expansion of the data set has been made for Cramer Class II, e.g., from the RIFM database for which a total of 476 additional chemicals were identified (344 new additions beyond COSMOS TTC dataset) and added to the existing TTC databases. The expanded RIFM dataset provides 421, 111 and 795 chemicals in Cramer class I, II and III respectively [74]. In addition, the COSMOS TTC approach has been further applied to incorporate antimicrobials [75] in the "Federated Set" and to establish TTC thresholds for Japanese industrial chemicals [76].

The use of TTC for dermally applied cosmetics is still something in its infancy. It can variously be used as a direct estimation of risk, should the exposure and "dose" (i.e. concentration applied) be known, as well as part of a risk assessment strategy. A further issue of the application of TTC for the risk assessment of cosmetics ingredients is the use of thresholds based on oral repeat dose data for the assessment of dermally applied ingredients. Williams et al. [77] addressed this issue with a decision tree considering the potential exposure following dermal application, along with a series of case studies demonstrating how this could be utilised. As well as developing meaningful strategies to use TTC, there is increasing interest in to develop the Cramer Classes further, as they could be updated with current knowledge. One approach is to adapt and refresh the existing Cramer Classes with new understanding and interpretation [78]; a second, more aspirational, approach is to augment the method with a grouping-based method by developing potency-aware chemotypes [75]. This method was motivated by the fact that many of the antimicrobials are inorganics and organometallic compounds where the Cramer Classes do not cover. It is hoped that scientific progress and intuitive sense would allow for the greater uptake of class-based TTC in the near future.

3.1. Internal TTC

As TTC moves forward, there is a desire to move to internal TTC i.e. the use and consideration of internal (e.g. plasma) concentrations of a substance. Currently, the development of internal TTC is in its infancy, with further work required to convert a NO(A)EL from an oral dose to an internal concentration. The process of deriving an internal TTC is well explained by Ellison et al [79], this will require the development of a database of internal blood concentrations derived from "external" (i.e. nominal) NO(A)EL values. Modelling approaches, such as PBK, will be required to achieve the internal doses both for the existing data and for the use of internal TTC in the safety assessment of new cosmetics ingredients and materials. Whilst this work is on-going, the internal TTC approach has been demonstrated for metabolites in a read-across scenario [80] and an interim internal TTC value has been proposed on the basis of ToxCast data [81].

At this time, thresholds from the internal TTC approach are highly desirable for the safety assessment of cosmetics ingredients and materials. However, its practical use and implementation will be minimal until there is greater agreement on the thresholds that are suitably protective, as well as practical means to calculate internal exposure simply, accurately and reliably.

3.2. Application of TTC

The use of TTC is a pragmatic solution to many safety assessment problems and, as described in later sections in this review, it has been incorporated in the so-called "*ab initio*" approach to the risk assessment of a (new) compound in a formulation [4,5]. Within the *ab initio* approach, TTC is applied as a first tier when chemical structure and exposure are known; if the envisaged exposure scenario is below the TTC, then minimal risk to human health may be assumed, if it is above, then compound-specific (*in silico/in vitro*) information is required [82]. Several tools are available to apply TTC, for instance the ToxTree software can provide an assessment of Cramer Class and COSMOS NG and SpheraCosmolife an assessment of whether a particular exposure would exceed TTC. New threshold values accepted by the SCCS [7] for cosmetics-related substances will be available in COSMOS NG.

Of relevance for assessing the skin sensitisation potential of a fragrance or cosmetic material is the combining of elements of the TTC approach and exposure. The Dermal Sensitisation Threshold (DST) has been established [83] and variously extended (see, for instance, Safford et al. [84]), below this value there is considered to be no appreciable risk of sensitisation. The application of the DST, in theory, negates the need for testing of sensitisation for ingredients where dermal exposure is sufficiently low. Specifically for fragrance materials, Api et al. [85] have described a process of quantitative risk assessment (QRA) for skin sensitisation. This QRA is proposed to use the No Expected Sensitisation Induction Level (NESIL) of the potential allergen (equivalent to an induction maximum no observed adverse effect level in a 100 subject Human Repeat Insult Patch Test). An Acceptable Exposure Level (AEL) is calculated from the NESIL and relevant Safety Assessment Factors (SAFs), where the SAFs account for uncertainties in determining the NESIL.

4. Databases to support safety assessment of cosmetics materials and the development of models

4.1. Databases of information relating to hazard

There is a need to store and provide access to data and information on the toxicology, use and exposure to cosmetics materials in an accurate and secure manner. This will enable existing information to be available for use in chemical safety assessment, where required. In addition, should the data be stored correctly, this will enable their use to develop models, allow for grouping and formation of categories (and hence for read-across) and will provide an opportunity to mine the information. With the growing need for non-animal approaches to safety assessment, it was recognised that there were specific needs to provide better access to, and understanding of, relevant information for cosmetics ingredients, specifically:

1. A more complete, high quality compilation of toxicological data and other properties relating to exposure e.g. skin permeability specifically focussing on data for cosmetics ingredients. The data could be utilised for existing molecules, analogues or for the development of models.
2. An understanding of cosmetics chemical space i.e. an inventory that incorporated as many of the single chemical structures associated with cosmetic ingredients as possible. This is required in order to support the analysis of cosmetics ingredients for concepts such as TTC, grouping and comparison with other chemical universes e.g. REACH, pharmaceuticals etc.

In order to address these needs and often serve as a first port-of-call for safety assessment, attention is drawn to the large, and increasing, number of data resources e.g. Pawar et al [86] listed nearly 1,000 publicly and commercially available databases covering many aspects of safety assessment. There is a number of significant databases that may provide a broad selection of toxicological information and data for cosmetics-related materials, amongst many other types of chemicals. Of the more significant, to find data directly for the ingredient in question, or to find data for "similar" substances (thus allowing for read-across), are the Organisation for Economic Cooperation and Development (OECD) eChemPortal database (https://www.echemportal.org/echemportal/) and the European Chemicals Agency (ECHA) resources

such as the listing of REACH registered substance data (https://echa.europa.eu/information-on-chemicals) which are included, at least partially, in the AMBIT cheminformatics data management system (http://cefic-lri.org/toolbox/ambit/). The OECD QSAR Toolbox (www.qsartoolbox.org) is a useful resource for such data, including those from ECHA, providing an opportunity to search multiple databases as well as identifying data to support read-across [87,88]. In addition, there are other sources of freely available data compilations with tens of millions of pieces of information for several million compounds, notable are ChEMBL (https://www.ebi.ac.uk/chembl/; [89,90]), ChemSpider (http://www.chemspider.com/; [91]) and Pubchem (https://pubchem.ncbi.nlm.nih.gov/; [92]). The US EPA Chemistry dashboard (https://comptox.epa.gov/dashboard; [93]) also provides access to a number of resources including data, models and information on use. Compared to what was available even a decade ago, these are truly remarkable compilations and resources, providing a meaningful starting place for the use of toxicological and other information to support the safety assessment of cosmetics related materials.

A number of key data resources are also available specifically for cosmetics related materials. COSMOS DB was instigated as part of the COSMOS Project (https://cosmostox.org/) within the SEURAT-1 Cluster to capture repeated dose toxicity information, as well as those for other toxicological endpoints. Since its first release, it has continued to compile data relating to cosmetics materials and make them freely available through the COSMOS DataShare Point initiative [94]. In addition, the COSMOS resources provide access to the COSMOS Cosmetics Inventory, developed by compiling chemical structures from the European Union's CosIng as well as the US Cosmetics Ingredients Review (CIR). Since 2021 COSMOS DB has been updated to COSMOS NG (https://www.ng.cosmosdb.eu/) which incorporates additional, freely available information and models. A full description of COSMOS NG and the former COSMOS DB, including numbers and sources of compounds, is provided by Yang et al. [95].

Other databases and information resources exist for cosmetics related materials. For instance, the Research Institute for Fragrance Materials Database (RIFM DB) is a source of toxicology data, literature and general information on fragrance and flavour raw materials, with information on more than 6,000 materials. The RIFM DB currently contains data from more than 135,000 human health and environmental studies as regulatory and compliance information (https://www.rifm.org/rifm-science-database.php#gsc.tab = 0).

The key to using historical toxicological, exposure or other information is to understand and appreciate their quality. There is no doubt that information and data from an existing toxicological study, that has been performed and recorded to a high standard, i.e. performed according to an OECD Test Guideline under Good Laboratory Practice (GLP) negates the requirement for further testing. Determining and classifying data quality for cosmetics related materials is extremely difficult, requiring expert knowledge [22,95]. There are several schemes available to classify data with the Klimisch score [96] being the most widely applied. This is undoubtedly used due to its simplicity, although it should always be remembered that it was originally developed for fish acute toxicity data. More comprehensive criteria for data quality have been established [97] which cover aspects of measurement and recording. Further, with a focus on toxicity data for cosmetics-related materials an update on the Klimisch scheme, based on fuzzy logic has been devised [98]. Recent approaches for the evaluation and reporting of toxicity data include a web-based tool from the Science in Risk Assessment and Policy (SciRAP) project has developed web-based tools (www.scirap.org) and the Criteria for Reporting and Evaluating ecotoxicity Data (CRED) provide a means to characterise the quality of data for ecotoxicological endpoints [99]. The OECD [100] has provided an overview and mapping of the guidance related to Integrated Approaches to Testing and Assessment (IATA) for risk assessment, including data quality. More generally, the GRADE methodology is useful for determining data and model quality [101] and there will undoubtedly be a continued increased use of systematic review to evaluate data and information sources [102].

4.2. *Mining databases of cosmetics materials – Relevant toxicology and "cosmetics space"*

Assuming toxicity data are well curated and of known quality, and they have been captured in a robust database platform, such as COSMOS NG, useful information can be derived from them. The process of data mining of toxicological information can be as trivial as knowing which tests, species and duration of tests have been undertaken. In addition, should toxicity data be captured appropriately then the detail regarding, for example, multiple target organs, and even effects can be identified. Analysis of the database of oral repeat dose toxicity data (oRepeatTOX DB), which had a heavy focus on data from SCCS reports and cosmetics ingredients, found the predominance of effects to the liver and kidney, followed to a lesser extent by effects to the forestomach and stomach as well as the spleen, most data being for the rat [103]. These results were updated for COSMOS NG by Yang et al. [95]. Further, Gustaffson et al. [104] assessed oral repeated dose toxicity data included in safety evaluation reports from 114 SCCS opinions (issued between 2009 and 2019) for 101 unique cosmetic ingredients. The analysis showed that the liver and the haematological system were potentially the most frequently affected organs following oral administration. Knowledge of relevant effects from e.g. the SCCS reports and opinions can also assist in the identification of the most important events for further research.

Additionally, within an appropriate informatics structure, the chemistry underlying potential adverse effects can be investigated. Firman et al. [105] described the mining of toxicological databases for the development of structure–activity relationships (SARs) that could be relevant for a number of purposes including development of structural rules and identification of structural analogues for read-across relating to cholestasis, as described in Sections 5.1 and 5.2. Automated analysis and investigation can be undertaken using a variety of approaches and techniques such as ToxPrint chemotypes [106,107].

Mining of toxicological databases to retrieve usable knowledge is likely to remain a research tool at the current time, aiming to support development of approaches for chemical safety development. However, analysis of chemical structures associated with cosmetics ingredients will provide an indication of the spread of physico-chemical properties and descriptors that is represented by cosmetics ingredients. To this end, COSMOS NG has compiled the European Union CosIng inventory and US Personal Care Product Council (PCPC) inventory to gain an overview of so-called "cosmetic space". The analysis of cosmetics space enables a number of activities, which support safety assessment of ingredients in different ways:

i) Allowing identification of "similar" molecules or groups of molecules that may share similar properties [108].
ii) Allowing for an analysis of the property space occupied by cosmetic ingredients in comparison to, for instance, pharmaceuticals, biocides, other industrial classes [72].
iii) Providing a means to select chemicals for testing e.g. as part of an *in vitro* testing strategy [79].

Analysis of property space is a particularly valuable exercise and several points should be noted. Cosmetics space is broad in terms of physico-chemical properties (e.g. aqueous solubility) and the types of functional groups and molecular scaffolds covered. It has a very wide set of uses ranging, for instance, from fragrances, to hair dyes, preservatives and surfactants. An understanding of cosmetic space is important for the application of predictive computational models e.g. a model that is developed on pharmaceuticals, biocides or other industrial chemicals may not be applicable to cosmetic ingredients.

5. Computational and *in silico* methodologies for the prediction of effects

Computational models are useful to assess cosmetics ingredients for potential hazard, should there be insufficient data and TTC is unable to provide an estimate of risk. There are a great number of possibilities to develop and utilise a range of computational models to assess the potential hazard of cosmetics ingredients and hence inform their safety assessment [109]. However, with the possible exception of local topical toxicities (i.e. sensitisation, irritation and corrosion) they have seldom been developed specifically for cosmetics related ingredients. The reason for the upsurge in interest in computational methods to assess hazard is clearly founded in the desire to reduce and replace animal tests, but also as a part of the rapid and cost-efficient screening and hazard assessment of ingredients [110]. This latter point may become increasingly important for the assessment of multi-component formulations and a number of approaches are potentially usable, as summarised in this section.

5.1. Structural rules capturing structure-activity relationships

Computational tools for the identification of hazard fundamentally attempt to form a relationship between chemical structure, or properties thereof, and toxic effects. For instance, structural rules (the formalisation of SARs) have been developed for a number of endpoints where toxicity is driven by a definable and interpretable sub-molecular fragment [111]. A collection of structural rules with a common purpose, for instance being associated with a particular toxicological endpoint, is often termed a "profiler" and several tools, such as the OECD QSAR Toolbox are built around such profilers [88].

Structural rules can have a number of uses with regard to cosmetics materials. Specifically they can be used to:

i) Make a direct prediction of potential hazard, i.e. is a cosmetics related material likely to be associated with a particular hazard.
ii) Assist in grouping "similar" chemicals to support activities such as hazard i.e. grouping together of similar cosmetics materials on the basis of a common functional group and/or a mechanistic hypothesis.
iii) Provide "profiles of toxicity" that may support, or act as, New Approach Methodologies to confirm similarity or identify dissimilarity of molecules, i.e. for read-across of cosmetics materials that may be grouped together on the basis of functional group or mechanistic hypothesis.
iv) Act as descriptors, or fingerprints, for instance as descriptors in the development of QSARs, i.e. to act as the inputs to chemical similarity scores or machine learning types of QSARs.

The different uses of structural rules should be appreciated, also noting that different *in silico* profilers have usually been developed for different purposes. It is often assumed that collections of structural rules are equally applicable for all uses. However, some profilers, such as some of those in the OECD QSAR Toolbox are designed to be quite "general" and capture a large number of molecules, and hence may be over-predictive for the purpose of hazard identification.

Few structural rules or profilers have been developed solely for cosmetics-related materials, though there are some exceptions e.g. profilers by Nelms et al [112] developed for the mitochondrial toxicity of hair dyes. However, some rules have been derived with a good foundation in the chemical space of cosmetics related materials e.g. those for skin sensitisation [113]. This confirms the need to examine the basis and source of structural rules, whilst rules derived from non-cosmetics materials are likely to have some relevance to cosmetics-related materials, it may be a source of uncertainty.

Structural rules have been developed for many toxicities with a particular focus on mutagenicity [114] and skin sensitisation [115], both highly relevant to the risk assessment of cosmetics ingredients. COSMOS NG provides DNA and Protein Binders as "chemotype" profilers. Other well developed structural rules include those for phosopholipidosis [116] and hepatotoxicity [117]. The application of these rules has been automated through systems such as ToxTree [70], with several other systems being available and reviewed in Madden et al. [22]. A useful starting point are also the ToxAlerts, which form a database of structural rules [118], in the Online chemical database (OCHEM) available from https://ochem.eu [119]. Such approaches are useful to identify hazard and, when properly documented, provide a mechanistic rationale and insight to the prediction.

Whilst progress has been made for the prediction of several apical endpoints, there is concern relating to cosmetics ingredients with regard to organ level toxicity, especially to the liver [120]. To this end, a greater emphasis has been placed on the development of structural rules for mitochondrial toxicity [112,121] as well as nuclear receptor binding [122–124], both associated with specific effects to the liver such as steatosis. The new approaches to the development of structural rules are founded in a knowledge of the Molecular Initiating Events (MIEs) from an Adverse Outcome Pathway (AOP) [121] as well as utilising new sources of data and information, such as ChEMBL [123,124]. The role of *in silico* modelling is well explored [125] with knowledge being gained that can support evaluation of cosmetics materials against a wide range of MIEs including those for pharmacological endpoints [126].

5.2. Grouping and read-across

The application of structural rules is one of the key techniques in what is termed grouping, or category formation to allow for read-across. The premise to this is, in principle, relatively straightforward. Similar compounds are sought and should data be available for one (or more) of the compounds within the group, then the effect may be inferred for other compounds. A key step in read-across is the definition of similarity, with several approaches being taken, and its eventual justification [127]. The freely available OECD QSAR Toolbox has become the "industry standard" tool to develop groups of similar molecules and hence facilitate read-across. There is considerable interest in grouping and read-across for cosmetics materials, not only as it represents a potential method for the assessment of complex toxicity endpoints (e.g. repeat dose toxicity), but also due to the natural categories many cosmetics ingredients fall into such as those defined for fragrance materials [108].

Generic frameworks for the application of read-across [128] as well as information on resources are available [129]. There are a variety of use case scenarios, or approaches, to use read-across successfully for cosmetics related materials. These include:

i) Grouping of molecules according to similar functional group and performing read-across from one or more close structural analogue(s). This is a simple and straightforward approach. It is particularly useful for data rich classes of cosmetics-related materials where consistency can be proved within the class. The development of classes is an approach taken by RIFM in many of their safety assessments [108].
ii) Finding structurally "similar" analogues using chemical similarity measure in databases e.g. COSMOS NG. This is also a simple approach to execute, but may require further information and expertise to justify the predictions with expertise being required to interpret and utilise measures such as the Tanimoto index successfully [130]. The development of groups of similar molecules on the basis of "atom environment" similarity measures for the read-across of teratogenic effects is one such example [131]. One caveat is that the similarity coefficient/index can be widely different depending on the choice of a similarity method ("atom environment", "circular fingerprints", or pre-defined features like ToxPrints).

iii) Applying mechanistic knowledge to identify analogue(s). This is a more complex method to identify and justify an analogue for read-across, but has the advantage of being intrinsically robust and transparent particularly for complex toxicities e.g. chronic effects. The assumption is that the analogues will have a similar toxic effect which "drives" the NOAEL value [132]. The approach of deriving mechanistic analogues is also useful for reactive mechanisms of action, which may include skin sensitisation, and can facilitate read-across between mechanistically similar, albeit structurally varied, analogues and may even be extended across endpoints [133].

iv) Other types of similarity approaches can be identified in the guidance for read-across published by ECHA e.g. metabolic similarity [134]. These are valid for cosmetics materials given the correct conditions, with this concept being well illustrated with regard to caffeine [135]. Elsewhere property-based similarity may be considered.

Whilst simple in concept, read-across for cosmetics ingredients is complex to perform and, especially, justify. The problem relates, in part, to the justification of similarity and the hypothesis of the read-across approach. To assist in resolving these problems Schultz et al. [136] developed a strategy and reporting template for read-across, building on much previous knowledge. Several case studies were undertaken [137] with a focus on issues such as compounds with low, or no, toxicity. These case studies extended the current paradigm for read-across by including New Approach Methodology (NAM) data, i.e. any *in silico*, *in chemico*, *in vitro*, high-throughput or content, or molecular biology information; the outcomes of these studies demonstrate the possibilities for read-across of repeat dose toxicity [138–142]. Assessment of these case studies revealed a number of common shortcomings that would frequently need to be addressed, namely the quality and relevance of the underlying data from which read-across is based, the role and confirmation of toxicokinetics and support of a mechanistic hypothesis [143]. Following on from these conclusions, Schultz et al. [144] proposed criteria with which to define and evaluate the uncertainties associated with a read-across. Escher et al. [145] demonstrated the use of NAM data from a variety of sources to support the read-across hypothesis. The evaluation of uncertainties in a read-across, along with the use of the ECHA's Read-Across Assessment Framework (RAAF), have been compared and applied to a read-across demonstrating how and where NAMs and other existing data can reduce the uncertainty [142]. More recent approaches to defining the applicability domains of read-across analogues and categories, based on chemistry, toxicodynamics and toxicokinetics, have been described [146].

Overall read-across has been, and will continue to be, a well-used technique in the hazard identification relating to cosmetic ingredients. With regard to hazard assessment, read-across has been extended to allow for grouping of molecules to allow for the estimation of NOAEL boundaries [132]. Being able to read-across potency should be a significant improvement in the utility of this approach. Read-across has been built into RIFM's safety evaluation process for fragrance ingredients as a fundamental tool to assist in filling data gaps, the reader is referred to Api et al. [147] for a description of the process and the many examples of how it is been applied are available from the RIFM Fragrance Material Safety Assessment Center (http://fragrancematerialsafetyresource.elsevier.com/). Recently Alexander-White et al. [148] have presented a practical ten-step framework for performing read-across for cosmetic safety assessment along with a worked example and illustration of the resources that may be applied. A case study demonstrating its practical applicability for the parabens is provided [149]. This approach provides a means of justifying a read-across and places the read-across in the context of a "Next Generation Risk Assessment" (NGRA) i.e. a broader tiered approach allowing for decisions to be made (described in more detail in Section 7). At the current time, the application and acceptance of this approach for regulatory use is being investigated. With regard to acceptance of read-across, it is noted that there are potential confounding factors in obtaining suitable data for an analogue, specifically if a new test is performed on an analogue to allow for a suitable read-across. Such animal testing to support read-across may not be acceptable for many purposes including under the cosmetics regulation.

In order to practically apply read-across within a safety assessment framework, the initial identification of analogues can be performed using simple approaches e.g. similar compounds in databases such as COSMOS NG, the US EPA Chemistry Dashboard (https://comptox.epa.gov/dashboard; [93]) or a variety of other databases (described in more detail in [22]) The OECD QSAR Toolbox is also widely used to identify analogues, predominantly on the basis of mechanistic, empirical or endpoint specific profilers [87,88]. The OECD QSAR Toolbox is highly applicable to cosmetics ingredients and materials. The ChemTunes•ToxGPS® (https://www.mn-am.com/products/chemtunestoxgps) informatics platform provides a means of undertaking and reporting read-across. A plethora of other tools exist to identify analogues for read-across and are well reviewed by Patlewicz et al [129], prominent amongst these tools are ToxRead [150]; https://www.vegahub.eu/portfolio-item/toxread/), ToxMatch [151]; http://toxmatch.sourceforge.net/), the ChemMine tools [152]; https://chemminetools.ucr.edu/) and the NTP ICE platform ([55]; https://ice.ntp.niehs.nih.gov/).

5.3. Quantitative structure-activity relationships (QSARs)

The logical extension to *in silico* methods (although they have been used for over five decades) are quantitative structure-activity relationships (QSARs) that attempt to form a statistical relationship between potency and descriptor(s) of a molecule's chemical structure or properties.

There is a wide range of QSARs that may be applied. The users can obtain QSARs some publicly available resources such as the "QSAR DataBase" (QsarDB) from https://qsardb.org/[153], OCHEM or the European Commission's Joint Research Centre (JRC) QSAR Model Database available from https://ec.europa.eu/jrc/en/scientific-tool/jrc-qsar-model-database [154]. All are useful resources covering a range of QSAR models for toxicology, ecotoxicology and physicochemical properties, many of which are relevant to cosmetics. At the current time, QsarDB is a good starting point for investigation. A further resource is the Danish (Q)SAR Database (https://qsar.food.dtu.dk/) which provides the predictions (but not necessarily access to the models) from over 200 (Q)SARs including a wide variety of endpoints and effects to various species for over 600,000 substances.

From the outset there should be an appreciation of the different types of knowledge or expertise required for success in this area of science, namely:

i) QSAR developers. These are generally modellers who will hopefully bring in knowledge of chemistry, mechanistic and apical toxicology and appropriate statistical methodology. Unless a cosmetics company has a specific interest, they will seldom develop their own QSARs, relying on third parties or existing resources to do so.

ii) Users of QSARs. The majority of users in the cosmetics and related industries will require "usable" QSARs to perform the safety assessment of these materials. They are likely to have a background in toxicology or risk assessment, but possibly less so in the development of QSARs.

iii) Evaluators of QSAR predictions. Should a prediction from a model be submitted for regulatory use, the prediction may be evaluated. The evaluator will assess the quality and robustness of the prediction to meet a number of scientific and legal criteria. Such expertise to perform this task will draw upon a variety of backgrounds including knowledge of QSAR development and

application, risk assessment, toxicology and chemistry associated with cosmetics-related materials.

There are few bespoke QSARs available for cosmetics materials and it may be that QSARs may need to be developed for the specific chemistries covered by these ingredients. As noted above, users of QSARs are likely to favour "off-the-shelf" software that will provide a prediction directly from the input of a chemical structure or identifier e.g. the VEGA software. Before using such software products it is recommended that the user becomes acquainted with the spectrum of QSARs. These range broadly in terms of the endpoints addressed, chemical descriptors used and statistical methodology. However, at the most fundamental level QSARs can be considered to range from being "local" to "global" models [155]. Local models are based on a small set of structurally or mechanistically related substances and are the historical basis of QSAR models. These have the advantage of often being mechanistically robust, transparent and can be related to the chemical space of cosmetics related materials. They have been demonstrated to be more applicable to hazard identification to inform risk assessment [156]. At the other end of the spectrum are the global models which are based on large datasets of chemicals and large numbers of descriptors. These will often cover many areas of chemical space, but may not include cosmetics related materials – the expert analysis of the applicability domain becomes essential at this point. Due to their potential greater global applicability they may find more use for screening and prioritisation for testing [156]. In terms of usability, there are many (relatively) easy to use pieces of software that allow rapid predictions from global QSARs (e.g. VEGA), local QSARs will usually be made "on-the-fly" and may require a level of expertise. In terms of regulatory acceptance, the transparency and mechanistic robustness of a local model with a restricted and clearly demonstrable applicability domain may be crucial.

QSARs have been developed for many endpoints, a large proportion of which will be relevant to cosmetics ingredients e.g. carcinogenicity, sensitisation etc. There are a number of caveats to the development of QSARs. The first is that it is a potentially data hungry modelling technique unless local models (which may be from grouping) are developed. Whilst, increasingly large data compilations are becoming available, for some of these there is, as yet, little effort to curate the dataset and even less to determine data quality [157]. A lack of appreciation of data quality and precision runs the quite real risk of overfitting models or finding spurious correlations. Extending the comprehension of the data, ideally, QSARs should be developed with a strong mechanistic basis – something that is often overlooked, or even impossible, for large datasets of complex mammalian toxicities (e.g. carcinogenicity or developmental toxicity). Thus, whilst there is the potential to use these approaches, care must be applied. Some models have been formalised in expert systems e.g. ChemTunes•ToxGPS® for the prediction of mutagenicity [158].

As organ level toxicity is probed more thoroughly, it will require a greater appreciation of the role of the MIE and how this information can be captured through computational models. For instance, Tsakovska et al [46] demonstrated how modelling of the PPARα receptor could assist in the development of toxicophores. The study indicated how methods, normally applied in drug design and lead identification, could be applied to rationalise toxicological data, especially within the AOP framework. Such approaches will prove invaluable in the modelling of complex organ level toxicity, especially those brought about by receptor-mediated toxicity. Moving away from toxicity prediction, a more thorough analysis of exposure will require models for concentration at specific organs [159] and other biokinetics properties [58]. Cosmetics-related materials are likely to be modelled using a number of similar approaches. There is also a place for multilevel models, which bring together distribution and effects in an attempt to simulate what may happen at the whole organ level (as described in Section 2.5).

5.4. Machine learning methods

Machine learning methods to model toxicity provide an extension to classic QSAR approaches. Machine learning methods may be considered to include regression analysis (hence providing overlap with QSAR methods) as well as k-nearest neighbour analysis, decision trees, support vector machines, random forests and a variety of neural network analyses, amongst others [160]. As such, machine learning methods represent a wide spectrum of statistical methods that attempt to find patterns within data. When the data are properties relating to chemical structure, the resultant models can be used to make predictions of activity. Their main functionality has been to derive models for use with "big data" with widespread use across many areas of science, technology and engineering [161]. With regard to predicting biological activity, their main application has been as tools to support drug discovery [162–163]. Due to the success of these methods in the provision of models for drug discovery, it is little surprise that there is interest, and increasingly concerted effort, in their use to model toxicity [164].

The concept of big data is well established, with the "Big Vs" being used to identify and describe such data (the three major Vs are considered to be Volume, Velocity and Variety) [165]. Richarz [157] reported that list of Vs has been expanded to Ten Vs of big data and discussed their applicability in predictive toxicology. A key question remains, especially with regard to cosmetics ingredients, whether we truly have big data that are relevant for modelling. Such data are likely to be from omics analyses, high content/throughput screening (such as ToxCast) or possibly large data compilations of test results. At this time, it is not certain that we have sufficient data for them to be considered "big enough" to gain the full benefit of machine learning, although we appear to be moving in that direction. Wang et al. [160] have provided useful insight into the current status of machine learning concluding that data availability (and quality assurance) does influence the type of methods that may be applied. Further, other issues have been identified such as overfitting, interpretability and mechanistic relevance. Demonstrating the utility of machine learning, there are many examples of models being developed for drug-induced toxicities [166–167], mutagenicity [168], properties of natural products [169], nanotechnology [170] and many others. These studies (and the papers reviewed within) describe many machine learning approaches and types of data to which they have been applied.

It is clear that many machine learning models have been developed for the prediction of toxicity, however, with a clear focus on drug toxicity, where there may be greater availability of big data for modelling. Despite the focus on drug molecules, there is a obvious opportunity that such methods may be applicable to support the safety assessment of cosmetics ingredients. The advantages of using models derived from machine learning methods is that such models can often be developed rapidly. In addition, due to their reliance on big data, they may have global applicability and hence may not be restricted to individual chemical classes (depending on the applicability domain as defined by the training set). The limitations of machine learning models may be possible overfitting (although this can be alleviated by good practice in modelling), a lack of reproducibility and transparency and difficulties with assigning mechanistic relevance. Further, machine learning models are often "bespoke", i.e. the users may need to develop them themselves rather than being available "off-the-shelf". The majority of existing machine learning models are not for regulatory endpoints per se, but related effects, such as organ level toxicity, or for MIEs or key events in an AOP. As such, they are currently unlikely to be able to replace an animal test in the same manner and with the same level of confidence a robust read-across can do, but they are likely to provide a weight of evidence to support safety assessment of cosmetics ingredients.

6. Mechanistic anchoring and links to models – Including data from new approach methodologies (NAMs)

In order for alternatives to animal testing, including *in silico* models, to be fully useful, they require some type of link to a mode of action and/or mechanistic anchorage. This means that the model can be understood in terms of the mechanisms it "represents" and the domain can be described. Mechanistic interpretation and anchorage is also another valuable source of "validation" of the model, increasing confidence in output by ascertaining its relevance.

The use of fundamental biological information in risk assessment has been on-going for many years, the WHO's MOA framework exemplifies this [171]. Of greater relevance, and popular uptake, has been the very rapid integration of AOPs into the risk/safety assessment paradigm. Whilst AOPs were spawned from environmental science in the US [172], they are being used to stimulate research and thinking both in North America and in Europe, with other activities progressing globally. Much has been written about AOPs [173] but at their heart is the ability to organise mechanistic information into networks that help us understand and predict organ level toxicity. They can provide frameworks to support development of integrated schemes for risk assessment [174]. They can also provide a basis to *in silico* models by demonstrating a direct link from the Molecular Initiating Event, or other Key Events and/or Key Event Relationships, to the model [125,175].

Thus, for cosmetics-related materials there is an emphasis to understand the underlying and important mechanisms of action which may drive organ level toxicity. Focus placed on understanding mechanisms of liver toxicity with steatosis [120,122,176] and cholestasis [120,177], and their respective AOPs being good examples. Other work has been targeted towards mitochondrial toxicity [112,121] and interactions with nuclear receptors [120,122,176]. Another problem has also been identified, but by no means resolved, with regards to cosmetics-related materials; that is compounds of no or low toxicity. Since many cosmetics-related materials are designed to be inert, and safety may have been assured after many years of use, there is no mechanism of action driving toxicity. Thus, using repeat dose toxicity as an example, the no observed effect concentration (NOEC) may be relatively high and associated with non-specific effects such as weight loss. These are areas where the implementation of AOPs requires more thought i.e. how to prove the absence of a relevant specific toxicity and take into consideration that (high dose) unspecific effects will drive the safety assessment.

AOPs and NAMs data in themselves will not provide overwhelming evidence for the safety assessment of cosmetics ingredients and materials, but the information that may be derived from them is increasingly being viewed as of great use [178,179]. AOPs provide the insight into mechanism which can be proven or disproved using relevant NAMs data from appropriate assays. AOPs are being further developed into increasingly usable networks, whereby individual AOPs are combined, allowing for the most important Key Events to be identified [180]. Of relevance to cosmetics ingredients will be the recent effort to provide a network of AOPs relating to liver toxicity [181], this goes further than most current AOP networks by identifying the most relevant Key Events and identifying existing assays associated with them. AOPs are also being quantified (qAOPs); this enables a quantitative prediction of an adverse effect to be predicted from the knowledge of data for underlying Key Events [182].

The role of NAMs and their organisation into linear, networked and quantitative AOPs will ultimately revolutionise chemical safety assessment in all sectors, but with a particular relevance to cosmetics ingredients [183]. The information from NAMs will support NGRA (see Section 7) allowing for a more mechanistic and justifiable safety assessment decision to be made. At the current time, this process is not routine for cosmetics ingredients across the industry, but as more examples are provided and acceptance illustrated their use will certainly become more mainstream.

7. Integration of models and data streams into usable strategies

It is increasingly appreciated that no single model or approach will be able to provide the same information as a "complex" test such as those for repeat dose or developmental and reproductive toxicity [184,185]. With regard to the testing of cosmetics ingredients, two general approaches could be applied. The first is to use a "battery" of tests – a broad selection of *in vitro* or high throughput screens, the aim being that the tests will cover the variety of effects that may be identified by the animal test to be replaced. Such an approach is designed in many ways to replace the animal test, although the full coverage of the test may not be known. Of more possible use to determine the safety of cosmetics ingredients is the implementation of a tiered approach. There has been considerable interest for the past two decades in the development of tiered strategies of tests; these have been called variously tiered testing strategies, Integrated Testing Strategies (ITS) and IATA. The concepts behind these approaches are different, potentially incorporating separate lines of evidence. The basic flow of information, however, includes existing information (and test data where available), data for similar molecules, *in silico* predictions and then *in vitro* analyses. A weight of evidence approach may be required to draw a conclusion from these strategies; if applied correctly, there is the possibility to make an assessment and decision at any stage of the workflow. With regard to safety assessment of cosmetics, the SEURAT-1 Conceptual Framework provides a means of integrating various strands of information and has been demonstrated through the *ab initio* case study [4,5,82].

The SEURAT-1 Conceptual Framework provided the springboard, in part at least, for the ICCR framework for the safety assessment of cosmetics ingredients [18]. The purpose of the ICCR "Principles" for incorporating NAM into risk assessments for cosmetic ingredients is to support a process of NGRA. This is defined as an exposure-led, hypothesis driven risk assessment approach that integrates *in silico, in chemico* and *in vitro* approaches, providing an opportunity to make safety based decisions at various "Tiers" representing different levels of information. Pham et al. [186] demonstrated that data from NAMs, for compounds including cosmetics ingredients, could be utilised for safety assessment. Specifically, the inclusion of high-throughput toxicokinetic data enabled the calculation of administered equivalent doses (AEDs) for the *in vitro* NAM data. This suggested that NAM data relating to bioactivity may allow for conservative estimations of PoDs which can be used subsequently in safety assessment.

The fundamental principle behind data integration is, following problem formulation, the ability to make a decision based on the appropriate application of the NAMs forming the basis of the framework. This concept has been investigated, for instance, through the development of a mode-of-action ontology to provide the basis for the four key elements, namely chemistry, kinetics, mechanisms and toxicological observations [17]. The practical implementation of the ICCR principles has been further supported by considerations of the NAMs described and their integration in the risk assessment [19,187] and exemplified by a number of case studies. The best developed at this time are those from Baltazar et al. [178] who applied various NAM data in an integrated strategy to assure safety in a hypothetical safety assessment of 0.1% coumarin in face cream and body lotion and Reynolds et al. [179] who used NAM data to evaluate the skin sensitisation potential of coumarin products. Other case studies demonstrate different aspects of read-across [148], context-specific safety assessment of parabens [149] and caffeine [135] as discussed previously.

For cosmetics ingredients, it has become clear that the future will be an integration of various streams of data, a proportion of which will be *in silico* and supported by *in vitro* data from NAMs giving further confidence on toxicokinetics and toxicodynamics [149]. Thus, in a safety assessment context, no single *in silico* approach will be standalone but individual predictions will contribute to an overall, context-dependent, consensus. At the current time, these applications are relatively hypothetical, but it is anticipated that they will soon become widespread

[188]. There are various limiting factors to the acceptance of such approaches, not least standardisation, validation and acceptance [9], but none would seem to be insurmountable. The concept of NGRA has now been recognised with inclusion in recent SCCS Notes of Guidance [7].

8. Outlook: Striving for regulatory acceptance of *in silico* tools for the safety assessment of cosmetic materials

Acceptance of the non-animal safety assessment of cosmetics and ingredients is essential for their use. Acceptance will be required by the manufacturer/producer, consumer and, crucially, the relevant regulatory authority. Definite signs of change in the European Union are becoming apparent with greater recognition by the SCCS [7] and progress towards acceptance of alternatives, notably based around NAMs and NGRA, coming to the fore [189]. The importance of the use of non-animal methods brings the delicate question of what is to be accepted, i.e. a prediction from a read-across or an *in silico* model or the whole process of NGRA, and indeed will acceptance for one regulatory purpose imply acceptance for others. Clearly, acceptance of individual *in silico* predictions is an easier, and more achievable, goal. Whilst this review focusses on *in silico* predictions, the challenges of acceptance of an integrated NGRA should not be overlooked.

When using an *in silico* toxicology approach, whether it is the simplest structural rule or most complex machine learning model, there are three fundamental questions that the user must ask:

1. Is the model appropriate to make the prediction?
2. Is the prediction relevant for the intended purpose?
3. Is the cosmetic ingredient to be assessed within the "applicability domain" of the model?

Overall, QSARs form valuable tools for the safety assessment of cosmetics ingredients. It is alluring to use a freely available and easy-to-use QSAR system but the user must bear in mind the validity of the system and whether the molecule fits within the applicability domain of the model. Assessing these factors is not a trivial task, but it is vital given the heterogeneity of cosmetics ingredients. These, and other issues, form the basis of the challenges for *in silico* models to gain acceptance for the prediction of effects associated with cosmetics ingredients and materials [190].

Frameworks to assess the validity of *in silico* predictions in a regulatory context are widely used, these include the OECD QSAR Principles for the Validation of (Q)SARs [191] and the ECHA's RAAF [192]. These are supported by copious amounts of guidance [22]. As well as means of gaining acceptance for models and their predictions, there are recommendations for the development of (Q)SARs such Good Computer Modelling Practice (GCMP) [193]. In addition, a set of generic *in silico* modelling protocols have been created which aim to give the model (or applications integrating *in silico* approaches) more confidence [26] as well as those for specific endpoints of relevance to cosmetics ingredients and materials such as skin sensitisation [25], liver toxicity [23] and genetic toxicity [24]. The method of assessing the validity of *in silico* models is being updated with a focus on understanding the uncertainties associated with a model, particularly with regard to read-across [144] and QSAR [194]. The concept of using uncertainty assessment to evaluate mathematical models for risk assessment, and the prediction of toxicity in particular has become well established [195–197] and an *in silico* protocol has been developed to address confidence in models and predictions [198]. The advantage of applying approaches that identify areas of uncertainty, variability and bias within a modelling approach is that it allows for improvement of the model, i.e. the collection of further information, if uncertainty is too high; but also lead to quantifiable levels of uncertainty in a model that may be acceptable for a particular purpose [156]. For cosmetics materials and ingredients, these approaches will increase understanding of the models in terms of their strengths and weaknesses and may allow for greater acceptability.

For a multitude of legislative, scientific, commercial and ethical reasons, it is undesirable to return to the *in vivo* testing of cosmetics-related materials. This means that the industry requires robust and reliable alternatives to the traditional animal testing not only to comply with their own needs but also to satisfy regulatory needs, such as those stipulated by the SCCS. There has been progress in the years since Adler et al. [11] suggested that it would be decades of research, however the presumption of a longer-term solution to replacement of traditional animal tests is still valid. What progress has been made is in the growing acceptance of the need for integration of all approaches and methodologies to assess safety of a particular exposure scenario, rather than a direct replacement of a (potentially outdated) experimental assay. Behind the progress, there remains in Europe, however, a relatively uncomfortable intersection between the requirements of the Cosmetics Regulation and other chemicals regulation, most notably the protection of workers' health through REACH. For instance, recent decisions by ECHA's Board of Appeal (case numbers A-009-2018 [199] and A-010-2018 [200]) for substances used exclusively as cosmetic ingredients, have demonstrated that the animal testing ban for cosmetics products does not prevent testing to comply with the information requirements of REACH [3,6].

8. Conclusions

There is a very broad range of computational techniques and tools that will assist in the safety assessment, both for hazard identification and evaluation of exposure, of cosmetics-related materials. Much progress has been made in the creation of high quality and well curated databases of toxicological information. Repeat-dose data from these databases have been used to derive TTC values from cosmetics-enriched datasets. Recent advances in mechanistic understanding and application, especially through the AOP framework has allowed for more insightful *in silico* prediction of organ level toxicity using many approaches, ranging from structural rules to toxicophore models. Read-across, as a means to compile information from a mechanistic point of view allows us to bring NAM data to life. Lastly, as a means of implementing computational approaches, workflows and strategies are being developed, but with significant problems appreciated – such as proving the absence, or low, toxicity. Overall, a range of computational techniques will continue to be used, with increasing confidence, to assess the toxicity of cosmetics-related materials and will continue the trend started by being acknowledged by bodies such as the SCCS.

CRediT authorship contribution statement

Mark T.D. Cronin: Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Visualization. **Steven J. Enoch:** Writing – review & editing. **Judith C. Madden:** Writing – review & editing. **James F. Rathman:** Writing – review & editing. **Andrea-Nicole Richarz:** Conceptualization, Writing – review & editing. **Chihae Yang:** Conceptualization, Writing – review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Mark Cronin reports a relationship with MN-AM that includes: consulting or advisory.

Acknowledgements

This work would have been impossible without the input of the many scientists, too numerous to list, who worked on, contributed to and advised the COSMOS Project (2011-2015). The funding provided by European Community's Seventh Framework Program (FP7/2007–2013) COSMOS project under grant agreement number 266835 and from

Cosmetics Europe and is gratefully acknowledged.

Declaration of Interest

Mark Cronin receives research funding from MN-AM which maintains the COSMOS NG knowledgebase and Cosmetics Europe for the development of a chemoinformatics solution to next generation risk assessment.

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