Reprint

Soheila Anzali, Michael R. Berthold, Elena Fioravanzo, Daniel Neagu, Alexandre RR Péry, Andrew P. Worth, Chihae Yang, Mark T.D. Cronin, Andrea-Nicole Richarz

Development of Computational Models for the Risk Assessment of Cosmetic Ingredients
Development of Computational Models for the Risk Assessment of Cosmetic Ingredients

Soheila Anzali1, Michael R. Berthold2, Elena Fioravanzo3, Daniel Neagu4, Alexandre RR Péry5, Andrew P. Worth6, Chihae Yang7, Mark T.D. Cronin8, Andrea-Nicole Richarz8

1Merck KGaA, Darmstadt, Germany; 2KNIME.com AG, Zürich, Switzerland; 3Soluzioni Informatiche srl, Vicenza, Italy; 4School of Computing, Informatics and Media, University of Bradford, Bradford, England; 5Unit METO, INERIS, Verneuil-en-Halatte, France; 6European Commission Joint Research Centre, Institute for Health and Consumer Protection, Ispra, Italy; 7Altamira LLC, Columbus, USA; 8School of Pharmacy and Chemistry, Liverpool John Moores University, Liverpool, England

Keywords: Toxicity, Computational Models, Risk Assessment, Threshold of Toxicological Concern (TTC), Cosmetic Ingredients Inventory

This publication was presented as a keynote lecture at the 27th IFSCC Congress, October 15-18, 2012, Johannesburg, South Africa

INTRODUCTION

Cosmetic ingredients must be safe for human use. To ensure safety, risk assessment is performed; this is a process that makes the link between the exposure to a cosmetic and its intrinsic hazard. Exposure is a function of the route, duration and quantity of a cosmetic product. This is determined with knowledge of the use of a cosmetic. Hazard, however, is more difficult to assess. Traditionally, hazard has been identified through the use of animal testing or, occasionally, from clinical reports of adverse effects following human use. The identification of hazard, or the harmful effects to humans, of cosmetic ingredients will always be controversial. The use of animals in traditional toxicity testing to identify hazard will, at best, be highly restricted and more likely not possible due to legal and ethical concerns. Therefore, alternative methods are increasingly being applied to determine hazard. To understand the problem, we must appreciate the full range of potential harmful, or toxicological, effects that may be brought about. These range from dermal effects (irritation and dermatitis etc) to chronic effects at the organ level. There is a growing realization that there will never be a single alternative method that is appropriate to provide information to identify these different individual hazards, but this process will be an integration of computational and biological methods.

There is great effort in these areas, with biological data being derived in vitro, from molecular biology, stem cell and many other technologies. This also links well to

ABSTRACT

The COSMOS Project (Integrated In Silico Models for the Prediction of Human Repeated Dose Toxicity of COSmetics to Optimise Safety, www.cosmostox.eu) is a unique international collaboration addressing the safety assessment needs of the cosmetics industry, without the use of animals.

COSMOS is developing an integrated suite of computational workflows including models based on the threshold of toxicological concern (TTC) approach, innovative toxicity prediction strategies based on chemical categories, read-across and quantitative structure-activity relationships (QSARs) related to key events in adverse outcome pathways and multi-scale modeling based on physiologically-based pharmacokinetics to predict target organ concentrations and extrapolate from in vitro to in vivo exposure scenarios. The KNIME technology is being used to integrate access to databases and modeling approaches into flexible computational workflows that will be made publicly accessible and provide a transparent method for use in the safety assessment of cosmetics.

First results include establishing the COSMOS chemical inventory of cosmetic ingredients and their associated chemical structures; a new dataset for TTC analysis for assessment of the applicability of the TTC approach to cosmetics; developing the COSMOS database for repeated dose toxicity data; as well as KNIME workflows to identify structural rules, fragments and properties associated with particular mechanisms of toxicity.
the current state of the art of alternatives and the concepts of 21st Century Toxicology to identify hazard being developed [1]. However, a recent report [2] reviewed the state of the art of alternatives to toxicity testing and concluded that for endpoints relevant to human health, there are as yet no acceptable alternatives.

A key part of the approach to develop alternatives to identify the hazard of cosmetic ingredients (and for many other types of compounds) is the use of a battery of computational approaches to predict toxicity based on the chemical structure of the substances. Computational approaches can vary from the formation of robust inventories of structures and databases of toxicological information and data, through to the development of thresholds of exposure considered to be safe, as well as models and algorithms. These may ultimately be supplemented by relevant assays considering the pathways of toxicity, assessed in high throughput screening assays.

Current computational technologies are not yet suitable to replace the use of animals to identify the hazard of cosmetic ingredients. The main reasons are summarised in Fig. 1. In particular, modeling chronic, low dose exposure to chemicals is very complex due to the intricate series of biological effects that may result in toxicity, involving many different mechanisms, following repeated dose. The COSMOS project has been designed to specifically address a number of the shortfalls in the current state of the art of computational approaches. It is a multi-partner project bringing together interdisciplinary expertise from academia, industry, small/middle-sized companies and regulatory agencies from across the European Union and the USA. The project, coordinated by Liverpool John Moores University, England, is co-funded by the European Commission and Cosmetics Europe - The Personal Care Association and is part of the 50 Million euro SEURAT-1 (Safety Evaluation Ultimately Replacing Animal Testing) research initiative cluster of seven projects (www.seurat-1.eu).

COSMOS aims to support the development of computational models in a number of significant ways, including the development of a definitive inventory of cosmetic ingredients, reliable and comprehensive databases of toxicity values relevant to repeated dose exposure and models to assist in the estimation of effects directly and to the formation of relevant groups of molecules relating to biochemical pathways which, when perturbed, may result in toxicity. Another key aspect to the project is the modeling of physiological and pharmacokinetics processes that control the distribution of a substance in vivo and will enable the extrapolation of findings in pathways to the whole organism.

**An Inventory of Cosmetic Ingredients**

Currently there are two main sources of information for use in the EU and USA regarding ingredients that can be used in cosmetics, the CosIng [3] database and the list compiled by the US Personal Care Products Council (PCPC) [4]. CosIng (COSmetics INGredients) is an online database from the European Commission, launched in 2008, containing information on over 20,000 cosmetic substances and ingredients, which can be searched by International Nomenclature of Cosmetic Ingredients (INCI) name, CAS or EC inventory number and giving in-
formation such as the chemical name, function, possible regulation of the substance or links to opinions of the Scientific Committee on Consumer Safety (SCCS) [3]. The Personal Care Products Council is the leading US national trade association for the cosmetic and personal care products industry, which has established the globally used standard of the International Nomenclature of Cosmetic Ingredients (INCI).

There are also other lists of chemicals associated with cosmetics, e.g., from the United States Food and Drug Administration (US FDA). However, there is no comprehensive single inventory of cosmetic ingredients incorporating high quality and validated chemical structures which is openly available. Such an inventory is required to assist with the understanding of the chemical space of cosmetic materials and to enable chemical grouping and modeling. Therefore, within COSMOS a large comprehensive inventory of cosmetic ingredients was established. Chemoinformatics techniques have been applied to join information from the Cosing database and the PCPC list and to classify compounds into structural categories for reproducible and efficient classifications. The COSMOS Cosmetics Inventory v1.0 provides 9,883 unique CAS numbers and 20,598 unique INCI names and is used as a reference library of cosmetic ingredients. The overlap between the Cosing and PCPC inventories was used to define the COSMOS Cosmetics Inventory v1.0, a set of 4467 unique chemical structures to be used for chemical domain analysis. The Cosmetics Inventory contains a wide variety of substances. More than 100 use categories related to cosmetic ingredients and related chemicals are found in this diverse inventory. The population of the top seven categories is shown in Fig. 2. The inventory is further characterized by structural categories, i.e., chemical classes. These structural categories can be used to compare different datasets or inventories, see Fig. 4. To perform any computational analysis or modeling, the compounds have been transformed into a calculable form, which includes structure files. The chemical structures included were comprehensively quality controlled.

Thus, the COSMOS Cosmetics Inventory with accurate chemical structure representation, in a format that links to chemoinformatics capabilities, allows us to map and search "chemical space." In this context the chemical space is a means of considering the chemicals within the inventory in terms of their structural and physicochemical properties. The extent of the cosmetic space can then be defined. This has a number of advantages: similar compounds can be identified; the full span of chemical space can be compared, e.g., with that for pharmaceuticals, so that the usability of models for different types of compounds can be considered; and it can even be used as a tool for chemical selection and identification for future testing. A further immediate benefit within the COSMOS project has been the ability to profile the inventory against known properties of toxic molecules, helping to direct the modeling effort.

**COSMOS Database**

Several databases for chronic toxicity data exist, e.g., the US EPA ToxRefDB (http://www.epa.gov/nct/toxrefdb/), the Fraunhofer RepDose ITEM database (http://www.fraunhofer-repdose.de/), the Japanese Repeat Dose Toxicity NEDO (New Energy and Industrial Technology Development Organisation) database and the eTox database (http://www.etoxproject.eu/) as well as data from submissions to the European Chemicals Agency (ECHA) available, for instance, through the OECD eChemPortal (http://www.echemportal.org/) and the OECD QSAR Toolbox (http://www.qsartoolbox.org/). However, no single repository of chronic toxicity data exists that is truly open and transparent as well as giving access to all aspects of the data, e.g., protocol details and main findings. Further, such a database needs to be linked to high quality and validated chemical structure and chemoinformatics tools to facilitate modeling. To predict chronic toxicity, it should not only provide the "raw data" to feed models, categories and read-across and the anchor to in vitro model development but also make available a potential basis for mechanistic understanding of, e.g., organ level effects. Furthermore, the quality of the chemical structures and data is crucial. Recently there has been a growing realization that many databases of chemical structures are riddled with errors, ranging from errors in structure to name, CAS number, etc. [5, 6]. The development of models from such erroneous information simply propagates errors and will lead to inaccurate
models. Therefore there is a need to check structures to ensure accuracy.

The COSMOS project includes a significant effort on data collection. The data model created for the COSMOS database to capture repeat dose toxicity data comprises two major areas which are interconnected – the chemical and the toxicological areas. The toxicological part of the data model reflects recent developments of ToxML [7]. A web interface for public access to the COSMOS database has been designed (Fig. 3). A data entry system (DES) is also being developed as a means to add new data into the COSMOS database. Data are entered first into the DES database and are checked by a quality control team before being released by the database administrator to the online production database. Furthermore, a specific data governance scheme is being applied to provide a new framework to integrate quality check and flexible data sharing functionalities. Data donations from the industry are welcome to complement the available toxicity data, and different levels of confidentiality can be accommodated.

To consider possible repeated dose toxicity effects via dermal use, bioavailability data are also needed. To this end, skin penetration and oral absorption data are also being compiled along with dermal metabolism information when available. These bioavailability data and physicochemical properties are essential to physiologically-based pharmacokinetics (PBPK) modeling and eventually used to address oral-to-dermal extrapolation when applying the threshold of toxicological concern approach.

Fig. 4 Structural classes in the COSMOS Cosmetics Inventory (red), the COSMOS TTC (green) and the Munro dataset (blue)

Fig. 5 Use categories in the COSMOS Cosmetics Inventory (blue) and the COSMOS TTC dataset (red)

Threshold of Toxicological Concern (TTC) Adapted to Cosmetic Ingredients

The threshold of toxicological concern (TTC) is a widely applied risk assessment tool that establishes a human exposure threshold value for chemicals below which there is a low probability of an appreciable risk to human health. This approach is an extension of the threshold of regulation (TOR) adopted by the US FDA for substances used in food-contact articles [8]. It is basically a statistical analysis of the distribution of no observed effect level (NOEL) values to determine the 5th percentile value to which a correction factor is applied. The original TTC concept used a single threshold for all chemicals based on the conservative assumption that an untested chemical could pose a cancer hazard. It was subsequently expanded to include non-cancer endpoints by Munro et al [9]. The Munro dataset contains 613 diverse tested chemicals and their NOEL values from oral repeat dose toxicity studies. Transforming the data to chronic NOAELs (no observed adverse effect levels), Munro et al (1996) identified the 5th percentile of the cumulative distribution for each Cramer class and devised the current thresholds. The Cramer classification scheme categorizes chemical substances into three classes (I, II and III) depending on their expected level of oral systemic toxicity (low, medium, and high). This is determined using a decision tree which consists of 33 questions to rank and classify chemicals. It was originally proposed by Cramer et al in 1978 as a priority setting tool in the safety assessment of food additives [10]. The Cramer classes have historically provided a robust method to categorize compounds but are based on 1970s knowledge to group compounds according to structural features thought to be responsible for toxicity.

The advantage of the TTC concept is that it is a risk assessment process based purely on estimated levels of exposure (it should be noted that there is also criticism for exactly this reason as well – i.e., risk assessment without a full toxicological profile). It has been applied quite often unchanged for a considerable length of time. However, it was never specifically designed to be applied to cosmetics. More particularly,
the Cramer classifications which assign a compound to one of three classes are poorly parameterized. The COSMOS project is assisting in the development of TTC and providing some specific adaptations to cosmetics, including extrapolation from the oral to dermal route exposures. To assist TTC development, a dataset containing repeated dose toxicity data for cosmetic ingredients has been derived by matching cosmetic ingredients in the Cosmetics Inventory with publicly available oral repeated dose toxicity data. Of the compounds with data obtained, 660 were unique cosmetic ingredients found in the COSMOS Cosmetics Inventory. From this collection, a set of 558 unique chemical structures containing NOEL/NOAEL values is being utilized for TTC analysis. Extension of the dataset is ongoing.

The COSMOS Cosmetics Inventory and TTC dataset have been used to compare the chemical space of cosmetics with that of the traditional TTC database first published by Munro in 1996. The COSMOS datasets were characterized by employing structure and sub-graph features and physicochemical property descriptors, including intrinsic properties that are defined purely by chemical structure (e.g., size and shape) or derivative properties (e.g., chemical reactivity), as well as extrinsic and biologically relevant properties such as metabolic behavior. The chemical space was then visualized by chemoinformatics methods to compare the different datasets. The results showed that the Munro dataset is broadly representative of the chemical space of cosmetics, although certain structural classes are missing, notably organometallics, silicon-containing compounds, and certain types of surfactants (nonionic and cationic classes). While the COSMOS TTC dataset lacks information on steroids, the dataset populates all other CTFAs (Cosmetic, Toiletries and Fragrance Association) classes that Munro lacked. In particular, cosmetic space enriches the classes of long aliphatic chains, glycol ethers, ketones, and nonionic alcohol ethoxylate surfactants. The different structural classes of the Munro, Cosmetics Inventory, and COSMOS TTC datasets are shown in Fig. 4 [11]. Moreover, the COSMOS TTC dataset is also representative of all the substance use types found in the COSMOS Cosmetics Inventory. The most highly populated use types include skin care (conditioning/moisturizers), emulsifiers, perfuming (fragrances), hair dyes, colorants, and UV absorbers/filters, antimicrobials, vitamins, and plasticizers (Fig. 5). The analysis of physicochemical descriptors representing size (molecular weight), shape (molecular diameter, number of rotatable bonds), partitioning behavior (logarithm of the octanol-water partition coefficient, log P), aqueous solubility (S), general characteristics of the structures (number of hydrogen bond acceptors and donors) and reactivity (dipole moment, energies of the highest occupied and lowest unoccupied molecular orbital (HOMO and LUMO, respectively), electronegativity, hardness, softness and electrophilicity) showed that the Munro dataset and the COSMOS Cosmetics Inventory contain larger structures (higher molecular weight) than the COSMOS TTC dataset; the Cosmetics Inventory has a higher number of structures with long linear chains (higher number of rotatable bonds and diameter); the COSMOS dataset has a higher prevalence of hydrophilic chemicals (lower log P values); the Munro dataset has a slightly higher prevalence of reactive chemicals. The COSMOS TTC dataset overlaps with the Cosmetics Inventory, indicating that it
is representative of the chemical space of cosmetics in general. When plotting the 3D space of the Cosmetics Inventory defined by log S, dipole moment, and molar volume, several chemical clusters emerged as illustrated in Fig. 6. The combination of water solubility, polarity/reactivity, and molecular size (volume) seems to separate well-known cosmetic ingredients, including quaternary ammonium alkyl chains, sugar polyols, ethoxylated alcohols, carboxylic esters, aikens and retinoic acids clusters.

In summary, the Munro dataset is broadly representative of the chemical space of cosmetics, although some differences were highlighted. Compared with the COSMOS Cosmetics Inventory, the Munro dataset has a slightly higher prevalence of reactive chemicals and a lower prevalence of larger, long linear chain structures. The COSMOS TTC dataset, comprising repeated dose toxicity data for cosmetic ingredients, showed a good representation of the Cosmetics Inventory in terms of physicochemical property ranges, structural features and chemical use categories. Thus, it was considered to be suitable for investigating the applicability of the TTC approach to cosmetics [11].

A clear advantage of the COSMOS database is that it can be directed to the development of TTC for cosmetics. Combining the information from the COSMOS Cosmetics Inventory with the database, i.e. the COSMOS TTC dataset, raises the possibility of developing a cosmetic ingredient specific TTC. This analysis is still ongoing; it may require the involvement of other stakeholders and specifically could provide a platform for the cosmetic industry to coordinate this work with the relevant regulatory agencies. In particular, from the database, NOELs are being compiled and quality controlled. The intention is to make this database of NOEL values suitable for TTC analysis freely available, in addition to undertaking a TTC analysis of these data. Further work to support the application of TTC will also consider better methods to split chemicals, formerly performed with the Cramer classes. COSMOS will update this categorization process, possibly through the use of mode of action information. More toxicologically relevant classes of compounds could be compiled, the NOELs analyzed and TTC values derived.

Prediction of Toxicity: From Mechanism to Model

In the absence of reliable, or any, in vivo data for toxicity, computational models will form the first of a battery of approaches to predict the toxicity of cosmetic ingredients. There is a long history to the computational modeling of toxicity. It usually revolves around finding some feature, property or descriptor of the molecule that is related to the toxic effect and then applying this knowledge to new molecules. For instance, 85 fragments of molecules have been identified that could be responsible for the binding of a molecule to DNA [12]. These molecular fragments have been developed from an understanding of the chemistry associated with DNA binding and coded to allow them to be used computationally, using SMARTS strings. SMARTS is a language for specifying substructural patterns in molecules, extending the Simplified Molecular-Input Line-Entry System (SMILES). As part of the COSMOS project, freely available computational tools are being developed, using the open source KNIME software [13], to allow a user to enter a molecular structure and then assess it against such SMARTS strings to identify if it has the potential for toxicity. These tools can be conceived as "computational workflows" and will also be available through a web interface.

There are a number of desirable features for computational models of toxicity, depending on their intended use. For risk assessment purposes, the most stringent features will be required to ensure, or define, the level of confidence in a prediction. For instance, it is highly desirable to give transparency to a prediction, so the user can determine from where it has been derived and its likely verification. The area of chemical space for which the model is valid, its so-called application domain, should also be defined and available as well as the availability of the data set and its quality assessment, a full description of the model and a firm foundation in mechanistic toxicology.

The interest in providing the mechanistic basis to predictions is being taken up by COSMOS. This is, of course, an ambitious aim for any modeling approach. It requires an understanding and definition of mechanisms of action. COSMOS is addressing this by considering organ level toxicity and identifying key mechanisms and clinical effects. Thus, for liver toxicity, these can be broadly considered to be related to effects such as reactive hepatotoxicity and non-reactive effects. Reactive hepatotoxicity is well understood for some chemicals, e.g., acetaminophen, and is amenable to computational prediction if the molecular fragments associated with this toxicity can be identified. Effort is ongoing in COSMOS to improve methods for the prediction of reactive metabolites, one of the key limitations of current modeling approaches.

While many liver toxicants have been shown to be reactive, research within COSMOS has shown that the majority of compounds shown to elicit liver toxicity in humans are not associated with reactive mechanisms. There are many other means by which these compounds may cause toxicity, including necrosis, phospholipidosis, cholestasis, steatosis and many others. Within COSMOS, chemoinformatic methods are being applied to gain information about "groups" of molecules that may cause these effects. These groupings are then supported by mechanistic understanding. The mechanistic understanding is being put within the adverse outcome pathway (AOP) concept. The AOPs are frameworks that link together the molecular initiating event (i.e., the interaction between the compound and the biological target) and the adverse outcome or apical effect [14]. This provides an opportunity to define the chemistry associated with toxicity initiating events, i.e., structural characteristics, and link it directly with the effect. The pathway is the series of biochemical events that occur at the cellular and organ level which may ultimately produce a response at the organism (or even population or ecosystem) level. The "key events" within such a pathway can be defined such that in vitro or other assays may be identified to assist in the verification of the pathway. The advantage of the AOP approach is that it provides a transparent link from chemistry to toxicological effect. COSMOS is developing a number of AOPs for liver toxicity and will provide these as freely available tools within KNIME workflows.
CONCLUSION

Modeling chronic, low dose exposure to chemicals is very complex due to the complex series of biological effects involving many different mechanisms. Therefore, the aim of the COSMOS project is to provide an alternative assessment strategy by developing computational workflows for the prediction of repeated dose toxicity to humans for cosmetics. The computational, open source and/or open access workflows will combine models based on the TTC approach, innovative predictive toxicology chemistry and physico-chemically-based pharmacokinetics.

First results from COSMOS include a database to capture repeat dose toxicity data, including a strategy for data quality assessment and quality control both of the chemical structures and toxicity data. A comprehensive inventory of cosmetics ingredients has been compiled with well-defined, unique chemical structures. To assist TTC development, a dataset with repeat dose toxicity data for cosmetic ingredients is being derived with unique chemical structures containing NOEL/NOAEL values. The analysis of the chemical space has shown that the COSMOS TTC dataset is a good representation of the COSMOS Cosmetics Inventory in terms of physicochemical property ranges, structural features and chemical use categories. Computational workflows coded in KNIME, linked to AOPS, have been developed to identify fragments and properties associated with particular toxicity mechanisms, form categories and allow for read-across to predict toxicity.

Acknowledgements

The research leading to these results has received funding from the European Community’s Seventh Framework Program (FP7/2007-2013) under grant agreement n° 266835 and from Cosmetics Europe. More information is available at www.cosmostox.eu and from info@cosmostox.eu.

References


Corresponding author
Andrea-Nicole Richarz
School of Pharmacy and Chemistry
Liverpool John Moores University
Byrom Street, L3 3AF Liverpool, England
a.richarz@ljmu.ac.uk