Institution: Liverpool John Moores University (LJMU)

Unit of Assessment: UoA3 Allied Health Professions, Dentistry, Nursing and Pharmacy

Title of case study: Developing New Approaches for the Safety Assessment of Cosmetics to Replace the Use of Animal testing.

1. Summary of the impact

The European Union Cosmetics Directive (adopted in 2003) banned the use of animals for testing cosmetic ingredients and the final deadline for compliance was March 2013. The development of alternative methods of safety assessment was therefore essential to ensure both consumer protection and viability of the cosmetics industry. Our research has focussed on the development of computational alternatives to animal testing, including the identification of structural alerts that have been encoded into computational workflows to support toxicity prediction. These methods have delivered tools to the cosmetics industry in Europe and worldwide to enable them to comply with the directive and develop new products. Our findings have also been used to inform thinking and policy in Europe and to develop a new approach to the safety assessment of cosmetics.

2. Underpinning research

On March 11th 2013 the final phase of the European Cosmetics Directive came into force banning the use of animal testing on any cosmetic ingredient. In light of this, new methods were required by industry to replace the use of animals in the safety assurance of cosmetic ingredients. We have been at the forefront of developing essential, alternative (including computational) methods for toxicity prediction for over 25 years so were well placed to apply and build upon this research to advise the regulators and help provide a solution for the cosmetics and personal care industries. Our research (Cronin, Madden and Enoch) has focussed on the investigation of the relationship between physico-chemical and structural features of compounds and their associated activity/toxicity. Knowledge of these structure-activity relationships has been directed towards an investigation of the applicability, to cosmetics, of the Threshold of Toxicological Concern (TTC) approach to risk assessment and the development of Adverse Outcome Pathways (AOPs) to provide a mechanistically-based framework to capture information relating to toxicity pathways. Some of the projects that have contributed to this research are outlined below:

As a postdoctoral researcher (PDRA) at LJMU (1991-1994; funded by Unilever) Cronin developed (Quantitative) Structure-Activity Relationships ((Q)SARs) for human health endpoints relevant to the personal care industry with an emphasis on modelling skin absorption and sensitisation [3.1]. This research formed the basis of the IMAGETOX EU FP5 project (2000-2004; employing Netzeva as a PDRA), the outcome of which was a series of models, databases and techniques that were successful in predicting the toxicity of chemicals capable of binding covalently to proteins; a process crucial to skin sensitisation. This led to the CAESAR project (EU FP6, 2006-9) employing Enoch as a PDRA, who further developed the work on chemical reactivity, focussing on electrophilic mechanisms [3.2]. This research produced improved models for predictivity and applicability for skin sensitisation and, alongside other models generated via CAESAR; were incorporated into a suite of software for toxicity prediction currently distributed online via VEGA (Virtual models for property Evaluation of chemicals within a Global Architecture). Structural alerts developed for skin sensitisation were also coded into the freely available Toxtree software (http://toxtree.sourceforge.net/).

Concurrently, a DEFRA-Link project (2007-2010; employing Bajot as a PDRA) in collaboration with Unilever, Proctor & Gamble, Shell, Lhasa, Marks and Spencer and the University of Tennessee continued investigations into the role of electrophilic reactivity in skin sensitisation. Further fundamental research into reaction rate chemistry was performed [3.3] and a database of the reactivity of organic chemicals was developed [3.4]. Understanding the mechanistic chemistry behind toxic effects (such as skin sensitisation) is essential to toxicity prediction and has been a continuing theme in the development of our models. Unilever funded a further collaborative project (2008-2011; with Ledbetter as a PhD student) to develop HPLC and molecular fragment based approaches to model the uptake of molecules across biological membranes [3.5].

The expertise gained from the above research enabled us to participate in the Seurat-1 Cluster of projects, working towards the replacement of in vivo repeat dose systemic toxicity testing. Within this we co-ordinate the COSMOS Project (2011-2016; total funding of €6.7million) which aims to

develop methods and freely available computational tools to predict the effect of long term exposure to cosmetic ingredients and determine their safety in humans. At LJMU, (previously with Enoch as a PDRA and currently employing Richarz as project manager, Przybylak and Mellor as PDRAs and Steinmetz and Nelms as post-graduate students) we have developed models for predicting organ level toxicity following repeat-dose exposure. New data were harvested and curated within the COSMOS database from sources not previously available (e.g. US FDA legacy data) using database and quality control procedures developed within the project. Computational models developed to predict activity (toxicity) of chemicals based on their structure relationships were investigated in terms of the mechanistic chemistry involved in the interaction of a chemical and a biological target, and a database of reactive organic chemistry was compiled [3.6].

One of the new paradigms in toxicity assessment is the development of Adverse Outcome Pathways (AOPs). These provide a knowledge framework to support chemical risk assessment by enabling information on key steps in toxicity pathways to be logically organised. For example, fundamental knowledge of the relationships between structure and activity (e.g. the ability of a chemical to bind covalently to a protein) can be captured and linked to the potential to initiate an adverse effect (e.g. skin sensitisation). Using our previous research we developed structural alerts to provide information for AOPs and also devised a template for developing and assessing AOPs which formed the basis of the OECD AOP development program launched in 2012 [5.6]. The TTC approach has not yet been utilised in the cosmetics sector. As part of the COSMOS project, we have investigated the applicability of this method for cosmetics, performing an analysis of the chemical space of the COSMOS cosmetics inventory compared with the chemical space of an established TTC dataset.

3. References to the research

3.1 to 3.5 were all published in peer reviewed journals (citations from Web of Science)

[3.1] Cronin, MTD and Basketter, DA (1994) Multivariate QSAR analysis of a skin sensitization database, *SAR and QSAR in Environmental Research*, 2, 159-179.
DOI: 10.1080/10629369408029901. Citations: 75.

[3.2] Aptula AO, Enoch SJ and Roberts DW (2009) Chemical mechanisms for skin sensitization by aromatic compounds with hydroxy and amino groups. *Chemical Research In Toxicology*, 22, (9), 1541-1547. DOI: 10.1021/tx9000336. Citations: 9.

[3.3] Koleva, YK, Madden, JC and Cronin, MTD (2008) Formation of categories from structure-activity relationships to allow read-across for risk assessment: Toxicity of alpha,beta-unsaturated carbonyl compounds. *Chemical Research in Toxicology*, 21, 2300-2312. DOI: 10.1021/tx8002438. Citations: 25.

[3.4] Schwöbel JA, Koleva YK, Enoch SJ, Bajot F, Hewitt M, Madden JC, Roberts DW, Schultz TW and Cronin MTD (2011) Measurement and estimation of electrophilic reactivity for predictive toxicology. *Chemical Reviews*, 111, 2562-2596. DOI 10.1021/cr100098n. Citations: 17.

[3.5] Ledbetter, MR, Gutsell, S, Hodges, G, O'Connor, S, Madden, JC, Rowe, PH and Cronin, MTD (2013) Prediction of immobilised artificial membrane chromatography retention factors using theoretical molecular fragments and structural features. *SAR and QSAR in Environmental Research,* 24, 661-678. DOI: 10.1080/1062936X.2013.792872. Citations: 0.

[3.6] http://www.seurat-1.eu/pages/library/seurat-1-annual-report.php - Provides links to Seurat-1 annual reports volumes 1 and 2 detailing the work of the COSMOS consortium.

The following funds were awarded to the QSAR and modelling group with Prof. Mark Cronin as PI:

Title	Awarding body	Date	Value
IMAGETOX RTN	EU FP5 IMAGETOX RTN	2000-04	€190,000
ReProTect	EU FP6 ReProTect	2004-9	€167,000
CAESAR	EU FP6 CAESAR	2006-9	€150,000
DEFRA-LINK	DEFRA-LINK	2007-10	£323,000
Lhasa PhD studentship	Lhasa	2008-11	£50,000
Hydrophobicity PhD studentship	Unilever	2008-11	£66,000
COSMOS (Project co-ordinator)	EU FP7	2011-16	€1 million

4. Details of the impact

The cosmetics industry in Europe employs approximately 1.7 million people and is worth an estimated €70 billion. It is a high-profile industry with a need to continually develop innovative products to remain competitive within the market. This case study describes the impact of research undertaken at LJMU in the development of computational alternatives to animal testing and the provision of expert opinion on the use of these alternatives that has informed policy making at the European level. Our research has provided impact in two ways (i) providing highly curated databases and computational models that are of use to the cosmetics industry as alternative methods to animal testing and (ii) influencing the future direction in which safety assessment may be performed i.e. the application of AOPs and the TTC approach to cosmetic ingredients.

(ia) The outcome of the CAESAR project was a suite of freely available software for toxicity prediction (released 2011 and now distributed via the VEGA website (http://www.vega-qsar.eu/use-qsar.html). VEGA supports the correct use of in silico/QSAR models for the evaluation of chemical safety for regulatory purposes and to reduce the impact of chemicals on humans and the environment. The VEGA platform is a series of QSAR models (many developed within CAESAR) that predict a series of properties of environmental, ecotoxicological and toxicological interest for regulatory purposes. It is freely available both online and as a standalone application to download. Over 1000 users had downloaded this software by April 2013. Skin sensitisation is an important element of assessing the toxicity of cosmetics and personal care products and the alerts we developed for skin sensitisation, alongside other models developed within the CAESAR project, were incorporated into predictive models that showed substantial improvement on previous approaches both in predictivity and applicability and importantly are freely available to any user [5.1]. Structural alerts developed for skin sensitisation were also coded into the Toxtree software by Ideaconsult, now freely distributed within ToxPredict platforms; (https://www.ideaconsult.net/) [5.2], and over 19000 downloads of Toxtree have been recorded since January 2008 (http://toxtree.sourceforge.net/).

(ib) Our collaborations with Unilever, Proctor & Gamble, Shell, Lhasa, Marks and Spencer, the University of Tennessee and other academic institutions (as part of the DEFRA-Link, InSilicoTox projects and the Unilever-funded studentship), demonstrated the high level of cross-sector industry interest in developing and using these predictive techniques, particularly as the deadline for the Cosmetics Directive approached. Full compliance with the Directive required alternatives to be developed to predict more complex endpoints, such as repeat-dose toxicity and because traditional QSAR approaches were inadequate for these endpoints, industry recognised that a new way of approaching the problem needed to be developed. To address this problem the European Trade Association for Cosmetics Industries, Cosmetics Europe (formerly Colipa), entered into a unique partnership with the European Commission to fund the Seurat-1 Cluster of Projects. Funding (€50 million) was provided to seven projects and LJMU co-ordinated the COSMOS consortium of 15 partners including world leaders committed to donating software and algorithms to support open architecture workflows. Data from our research have been harvested and curated within the COSMOS database that links chemical structure to repeat dose toxicity [5.3] COSMOS combines new and current databases in a single state-of–the art, freely available public resource delivering a single, comprehensive resource for repeated dose toxicity data and although several other databases for chronic toxicity are available, other than COSMOS, there is no other single repository that is open, transparent and includes all aspects of the data available. Research has been carried out to assess the applicability of the TTC approach to cosmetics, including assessment of the chemical space of the cosmetics inventory (compiled by COSMOS) in relation to chemical space of an existing TTC database.

(iia) As a member of the European Centre for the Validation of Alternative Methods (ECVAM) and European Chemical Bureau working groups (2003 and 2010), Cronin had an advisory role in developing policy for alternatives to animals for skin sensitisation testing. These working groups provided realistic estimates of timescales for the replacement of animal testing by alternative methods and recommendations on how to achieve this. This resulted in an increase in efforts to accelerate the availability of alternative tests to enable industry to comply with the deadlines of the Cosmetics Directive (March 2009 and March 2013) [5.4]. Due to his considerable expertise in this area Cronin was also invited to attend the working group of an international panel of experts, organised by the European Commission, to present on-going research in the area. This resulted in

a publication used to inform policy development on the use of alternatives for cosmetic safety assessment [5.4]. In 2012 Madden also provided advice to the Scientific Committee on Consumer Safety (the European statutory committee that regulates cosmetic ingredients), the outcome of which is to serve as a basis for the SCCS guidance on dermal safety assessment [5.5].

(iib) Recently there has been much interest in the development of AOPs as a framework for organising chemical and biological information associated with toxic events. Knowledge acquired through our fundamental research into chemical-biological interactions is currently being used to inform the development of AOPs. In 2012 we produced a template and wrote guidance for the development and assessment of AOPs which has been incorporated into the framework adopted by the OECD for the development of AOPs [5.6].

(iic) One of the major impacts of our research has been on the thinking behind a new way towards risk assessment as demonstrated by the influence of COSMOS (led by LJMU) in raising the possibility of extending to cosmetics the TTC approach used by regulators for assessing food contact substances. This is now being considered at the European level via expert groups co-ordinated by ILSI-EU which addresses major scientific issues relating to public heath [5.7]. In 2012, a joint report (SCCP/1171/08) by the Scientific Committee on Consumer Safety (SCCS), Scientific Committee on Health and Environmental Risks (SCHER) and Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) published the opinion that the TTC approach "in itself is scientifically acceptable for human health risk assessment of systemic toxic effects caused by chemicals present at very low levels''. The report acknowledged the work of COSMOS in developing a new TTC dataset more relevant to cosmetics and the characterisation of the COSMOS cosmetics inventory developed within the project [5.8].

In summary, research from LJMU has provided databases and computational models for skin sensitisation that have been incorporated into freely available predictive software for use by regulators and the cosmetics and personal care industry for safety assessment of cosmetic ingredients. It has also influenced debate at the European level concerning the possibility of extending the current regulatory TTC approach to cosmetic ingredients.

5. Sources to corroborate the impact

[5.1] Personal comments on the use of Toxtree and CAESAR for *in silico* consultancy and regulatory purposes can be obtained from the Managing Director (1) and/or Research Scientist (2) at S-IN.

[5.2] http://toxtree.sourceforge.net/skinsensitisation.html
http://toxtree.sourceforge.net/proteinbinding.html
Confirms our contribution to the development of the protein binding and skin sensitisation rules.

[5.3] http://www.seurat-1.eu/pages/library/seurat-1-annual-report.php Provides links to Seurat-1 annual reports which details the work of the COSMOS consortium within the Seurat-1 cluster.

[5.4] Adler, S et al (2011) Alternative (non-animal) methods for cosmetics testing: current status and future prospects-2010. *Archives of Toxicology,* 85, 367-485. DOI: 10.1007/s00204-011-0693-2. Citations: 52 (from Web of Science).

[5.5] http://ec.europa.eu/health/scientific_committees/newsletters/index_en.htm - December 2012 newsletter (p1) confirms outcome of this meeting (minutes can be supplied to confirm attendance)

[5.6] http://www.oecd.org/env/ehs/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm#Documents.Guidance document and template for developing and assessing AOP Series on Testing and Assessment No 184 (2013). Authorship is not acknowledged in the report but the associated report from the OECD (available on request) quotes *"The guidance document on developing an AOP and a glossary of terms associated with AOPs by Liverpool John Moores University (LJMU) are complete. The guidance on developing an AOP and the glossary of terms associated with AOPs are to be combined and moved forward at OECD for declassification and will serve as the basis for the work flow at the Adverse Outcome Pathways Programme (AOPP) at OECD."*

[5.7] http://www.ilsi.org/Europe/Pages/HomePage.aspx - Links to 2013 ILSI Europe Activity Document confirming role of ILSI within the working group

[5.8] http://www.bibra-information.co.uk/news_story-525.html - Links to the joint committees report sccs_0_092.pdf (SCCP/1171/08) confirming the contribution of COSMOS in developing the TTC approach.