Institution: Liverpool John Moores University (LJMU)

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

Title of case study: New Computational Tools For The Improved Hazard Assessment of Chemicals.

1. Summary of the impact

The EU REACH regulation 2007 (Registration, Evaluation, Authorisation and restriction of Chemicals) requires producers and importers of chemicals to register them and provide information to ensure their safe use whilst minimising the use of animal testing. When the White Paper on REACH was published in 2001, over 90% of the 100,000+ chemicals in use had few or no toxicological data available. In order to address the deficiency in the ability for companies to comply with these regulations, LJMU has developed a suite of computational tools to predict toxicity of chemicals using only knowledge of chemical structure and properties. These models have been incorporated into predictive software including the OECD (Organisation for Economic Co-operation and Development) QSAR Toolbox which is promoted by the European Chemicals Agency (ECHA), as a useful means to provide information for REACH dossiers and has been taken up by industry internationally for this purpose.

2. Underpinning research

The traditional approach of using animals to assess the toxicity of chemicals has been criticised on the grounds of ethics, cost, time and lack of relevance to realistic exposure scenarios and species of interest. The need for alternative methods was reinforced in 2001 with the publication of the White Paper on the EU REACH regulation. To reduce the need for animal testing, much effort was devoted to promote the use, and regulatory acceptance, of alternative *in vitro* and *in silico* models to fill gaps in knowledge concerning toxicity of chemicals.

Since the 1970s the Quantitative Structure Activity Relationship (QSAR) and modelling group at LJMU has been at the forefront of research into the development and use of alternative methods to improve predictions for chemical activity/toxicity. This research has involved the investigation of the physico-chemical and structural properties of chemicals and the use of techniques to relate these properties to the activity (or toxicity) exhibited by the chemical. Recently, this research has been targeted at providing computational tools, for industry and regulators to aid toxicity prediction. However, uptake of these tools can only be assured by determining the validity of the models and promoting (regulatory) acceptance.

Dearden and Cronin contributed to the landmark industry-led workshop in Setubal (2002) which explored the applicability of computational methods to address these issues, assess the validity of QSARs and increase acceptance of their use by industry and the regulators [3.1]. The outcome of this collaboration was the formalisation of the OECD Principles for the Validation of QSARs [http://www.oecd.org/env/ehs/risk-assessment/validationofqsarmodels.htm] now internationally accepted as the standard for assessing QSAR models. This research contributed to the development of the OECD principles and the internationally accepted guidelines for assessing the validity of QSARs.

Since then researchers at LJMU (Cronin, Dearden, Madden and Enoch) have applied QSAR and other computational techniques to a range of REACH relevant endpoints with a focus on mechanistic interpretability. Some of the projects that have contributed to this research are outlined below:

In 2004 LJMU and FRAME (Fund for Replacement of Animals in Medical Experiments) collaborated in a Department for Environment, Food and Rural Affairs (DEFRA) sponsored project to develop a framework for assessing toxicity through integration of computational and *in vitro* data, an Integrated Testing Strategy (ITS). Cronin then co-authored a sequence of strategy papers relating to the use of ITS for REACH relevant endpoints including skin sensitisation, reproductive toxicity, eye and skin irritation [3.2]. Further research was undertaken on the ITS theme within the EU FP6 OSIRIS project (2007 - 2011; employing Hewitt as a post-doctoral researcher). This enabled the development of databases and models (such as ChemProp) for predicting REACH relevant endpoints.

The EU FP6 CAESAR project (2006 - 2009; employing Enoch as a post-doctoral researcher) then developed models for predicting skin sensitisation [3.3]. This research involved the collation of toxicological data and elucidation of the underlying mechanistic chemistry behind the process, enabling development of profilers (structural alerts and SMiles ARbitrary Target Specification [SMARTS] patterns) to allow relevant functional features of chemicals to be identified. In collaboration with partners in the CAESAR project, knowledge from this research was incorporated into a suite of freely available predictive software called VEGA.

Investigations into the mechanisms of skin sensitisation [3.4] provided knowledge subsequently used by Ideaconsult to further develop their freely available ToxTree and ToxMatch software which is now part of the ToxPredict and OChem software: http://apps.ideaconsult.net:8080/ToxPredict, http://opentox.org and https://ochem.eu//alerts/home.do?render-mode=full.

Investigation of the chemical mechanisms underlying toxicity continued with the OECD QSAR Toolbox project (2008-2012; employing Enoch and Przybylak as post-doctoral researchers) with particular emphasis on skin sensitisation [3.4], respiratory sensitisation [3.5], protein binding and DNA binding [3.6]. The OECD QSAR Toolbox (developed by the Laboratory of Mathematical Chemistry (LMC), Bourgas in collaboration with LJMU, ECHA and OECD) is a unique software tool designed specifically to enable chemicals to be placed into categories allowing “read-across” predictions for chemicals for which toxicity data are lacking. The Toolbox uses “profilers” to identify chemicals that may act via the same mechanism. Structural alerts developed at LJMU have been encoded into profilers used in the Toolbox for protein and DNA binding (respiratory sensitisation and liver toxicity profilers are under development). Inclusion of profilers into the Toolbox (by encoding knowledge of structural alerts) can only occur following rigorous peer-review of the underlying research at the OECD; programming is carried out by Toolbox developers at LMC.

3. References to the research

3.1 to 3.6 were all published in peer reviewed journals (citations from Web of Science)

[3.1] Cronin MTD, Walker JD, Jaworska JS, Comber MHI, Watts CD and Worth AP (2003) Use of QSARs in international decision-making frameworks to predict health effects of chemical substances. *Environmental Health Perspectives*, 111(10), 1391-1401. DOI: 10.1289/ehp.5759. Citations: 114.

[3.2] Grindon C, Combes R, Cronin MTD, Roberts DW and Garrod JF (2006) Integrated testing strategies for use in the EU REACH system. *ATLA-Alternatives to Laboratory*, 34(4), 407-427. Citations: 34.

[3.3] Enoch SJ, Cronin MTD, Schultz TW and Madden JC (2008) Quantitative and mechanistic read-across for predicting the skin sensitisation potential of alkenes acting via Michael addition. *Chemical Research in Toxicology*, 21(2), 513-520. DOI: 10.1021/tx700322g. Citations: 44.

[3.4] Enoch SJ, Madden JC and Cronin MTD (2008) Identification of mechanisms of toxic action for skin sensitisation using a SMARTS pattern based approach. *SAR and QSAR in Environmental Research*, 19, (39), p555-578. DOI**:** 10.1080/10629360802348985. Citations: 45.

[3.5] Enoch SJ, Seed MJ, Roberts DW, Cronin MTD, Stocks SJ and Agius RM (2012) Development of mechanism-based structural alerts for respiratory sensitisation hazard identification. *Chemical Research in Toxicology*, 25(11), 2490-2498. DOI: 10.1021/tx3003092. Citations: 2.

[3.6] Enoch SJ and Cronin MTD (2012) Development of new structural alerts for chemical category formation for assigning covalent and non-covalent mechanisms relevant to DNA binding. *Mutation Research – Genetic Toxicology and Environmental Mutagenesis*, 743(1-2), 10-19. DOI: 10.1016/j.mrgentox.2011.12.029. Citations: 3.

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
Easyring	EU FP 5	2003-5	€232,000
CAESAR	EU FP6 CAESAR	2006-9	€150,000
In SilicoTox	Marie-Curie In SilicoTox	2006-10	€300,000
LJMU – FRAME Alternatives to Animals	DEFRA	2004- 5	£25,000
OSIRIS	EU FP6 OSIRIS	2007-11	€400,000
OECD QSAR Toolbox	OECD	2008-12	€230,000
IMI ETox	EU FP7	2010-16	€380,000
Alter REACH 2	Norwegian Research Council	2011-13	€20,000

4. Details of the impact

In 2011, world chemical sales were worth €2744 billion of which the EU has a 20% share employing 1.2 million workers and contributing over €5 billion to the EU economy (CEFIC). The introduction of the REACH regulation in 2007 meant that by 2018 this industry is required to present to ECHA, for approval, dossiers containing toxicological information on any chemical produced in or imported to the EU in quantities over one tonne. It was estimated that over 54 million animals (at a cost of €9.5 billion) would be required if animal testing alone were used but this could be reduced to 13 million animals if all available alternative methods were utilised where possible (Rovida and Harting, ALTEX 26, 3/09).

The QSAR group has developed models that can be used to predict activity / toxicity in both human health and environmental sciences and are used as an aid to hazard assessment of chemicals in the pharmaceutical, fine chemical and personal care product industries. This research has directly impacted on industry and regulators by (i) aiding the development of models and bespoke computational tools for prediction and (ii) promoting the uptake of alternative methods by contributing to the development of methods to assess their validity, hence increasing regulatory acceptance.

(a) As a partner in the EU CAESAR project (http://www.caesar-project.eu/) LJMU was part of an international effort that supported the development of computational models for predicting toxicity [5.1]. These models now form the basis of the VEGA platform (http://www.vega-qsar.eu/use-qsar.html) which provides online software, made freely available to industry, for predicting toxicity endpoints based on chemical structure relevant to the REACH regulation. Since April 2013 over 1000 copies of the free standalone version have also been downloaded.

(b) LJMU research also led to new rules being implemented in the Toxtree and Toxmatch software. These new rule bases incorporated alerts developed at LJMU for skin sensitisation, protein binding, DNA binding and revisions to the Verhaar rules [5.2]. Updated versions of the software are now available via the ToxPredict interface http://apps.ideaconsult.net:8080/ToxPredict for use by industry for initial hazard assessment of chemicals and over 19000 downloads have been recorded since 2008. The software is particularly useful for toxicity prediction as it is freely available and was originally commissioned by the European Commission (through the Joint Research Centre, Ispra).

(c) One of the most significant impacts of the research at LJMU has been its contribution to the development of the OECD QSAR Toolbox [5.3]. Research carried out at LJMU identified structural alerts associated with protein and DNA binding and these alerts have been coded into “profilers” in the Toolbox. This software (developed at LMC, Bourgas) is a unique tool designed specifically to aid industry with hazard assessment in light of the EU REACH regulation. The “read-across” methodology is transparent and therefore more acceptable to regulators. Reliable and justifiable predictions for toxicity can be made reducing reliance on animal experiments so industrial users are able to use the software to predict toxicity and fill the knowledge gaps in existing toxicity data required for the submission of their REACH dossiers The first version of the Toolbox was released in November 2008 with subsequent versions being released in 2010, 2012 and 2013. There has been a wide uptake across the industrial sector with an estimated 1000-2000 downloads [5.4]. It

has become a standard tool and industry has been supportive of further development [5.5, 5.6] Its use is strongly promoted by the European Chemicals Agency (ECHA), the agency responsible for ensuring safe use of chemicals in Europe and they have stated it is *"A valuable tool to consider when building categories or considering read across is the OECD QSAR Toolbox"* ECHA [5.7] In 2011, ECHA reported that already 20-30% of submitted dossiers contained read-across predictions and OECD QSAR Toolbox was reported as being the most frequently used QSAR model in an EC funded project (ORCHESTRA) to determine the use of QSAR for REACH purposes [5.8].

QSAR and modelling research undertaken at LJMU over several decades has resulted in many models and tools being developed for the prediction of toxicity. These tools are demonstrably important to industry and their uptake has been assured by additional activities aimed at ensuring developers and users of the models have confidence in the validity of the approaches.

5. Sources to corroborate the impact

[5.1] Confirmation of LJMU involvement in CAESAR

http://www.caesar-project.eu/index.php?page=participants

[5.2] Confirmation that research from LJMU was used in updating Toxtree/Toxmatch

http://toxtree.sourceforge.net/skinsensitisation.html
http://toxtree.sourceforge.net/proteinbinding.html
http://toxtree.sourceforge.net/dnabinding.html
http://toxtree.sourceforge.net/verhaar2.html

[5.3] Confirmation of the specific contribution of LJMU to the development of profilers is available at

http://www.oecd.org/env/ehs/risk-assessment/guidancedocumentsandreportsrelatedtoqsars.htm

(a) Report of the Expert Consultation on Scientific and Regulatory Evaluation of Organic Chemistry Mechanism-Based Structural Alerts for the Identification of DNA-Binding Chemicals; Series on Testing and Assessment, No. 120, Parts 1 and 2, (2010).

(b) Report of the Expert Consultation on Scientific and Regulatory Evaluation of Organic Chemistry Mechanism-Based Structural Alerts for the Identification of Protein-Binding Chemicals. No139 (2011).

[5.4] Confirmation of meeting and recommendations of industry user forum

http://newsletter.echa.europa.eu/home/-/newsletter/entry/6_11-qsar-toolbox;jsessionid=1BF0B7A5146B500876163480414AEE15.live2

[5.5] Personal comment on use of the OECD QSAR Toolbox in the personal care products industry may be sought from; Senior Scientist, Unilever.

[5.6] Links outlining industrial experience of using the toolbox, industry forum user's meetings etc. documentation on saving time, cost, and animals.

http://echa.europa.eu/en/web/guest/search?p_p_id=echasearch_WAR_echaportlet&p_p_lifecycle=0&p_p_state=normal&p_p_mode=view&p_p_col_id=column-1&p_p_col_count=1&_echasearch_WAR_echaportlet_doSearch=true&_echasearch_WAR_echaportlet_forceAdvanced=true

[5.7] http://echa.europa.eu/web/guest/support/information-toolkit

[5.8] Mays C, Benfenati E and Pardoe S (2012) Use and perceived benefits and barriers of QSAR models for REACH; findings from a questionnaire to stakeholders. *Chemistry Central Journal*, 6:159. DOI: 10.1186/1752-153X-6-159.