Workshop report

Two Good Read-Across Practice Workshops. Making It Work For You!

Alexandra Maertens [1], Bruno Hubesch [2] and Costanza Rovida [3]

[1]Center for Alternatives to Animal Testing (CAAT), Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA; [2]European Chemical Industry Council (CEFIC) – LRI Programme, Brussels, Belgium; [3]CAAT-Europe, University of Konstanz, Germany

http://dx.doi.org/10.14573/altex.1605301

Read-across is an innovative approach that can be considered an alternative to animal testing – and at the moment it is probably the most effective method of reducing the use of lab animals. In fact, the latest ECHA report on the use of alternative methods revealed that 85% of REACH registration dossiers waived *in vivo* test requirements by using the read-across option (ECHA, 2014). However, the applicability of the read-across principle goes far beyond REACH, and experience gained in this field will help to clarify what "Good Read-across Practice" involves (Ball et al., 2016; Zhu et al., 2016).

Following recent publications on read-across (Hartung, 2016; Luechtefeld et al., 2016a-d), CAAT-Europe in collaboration with the integrated project EU-ToxRisk (www.eu-toxrisk.eu) and CEFIC-LRI (European Chemical Industry Council – Long Range Research Initiative, http://www.cefic-lri.org) convened a workshop to learn the opinions of main stakeholders in the field. This workshop took place in Brussels on February 26, 2016. About 100 individuals, representing toxicologists, industry, regulators, academia and other associations, took part. Full details and copies of the presentations are available at http://bit.ly/25NqtFf. A further workshop was held in March 2016 at the U.S. Food and Drug Administration in College Park, MD, also well attended by representatives from academia, regulators and industry, and webcasted to many more.

In 2015, the European Chemicals Agency (ECHA) published a document showing how to present a read-across strategy (RAAF, Read Across Assessment Framework; ECHA, 2015). While this document does not claim to demonstrate the scientific basis of the read-across principle, it does explain how to present data to regulators through a robust scientific justification. It considers two cases: i) different substances that give rise to (the same) common compounds to which the organism is exposed (biotic or abiotic transformation to common compounds) or ii) different compounds that cause the same type of effects on the organism as a result of structural similarity. This means that the justification for a read-across or category approach is the result of a complex assessment of both the chemical structure and biological behavior of the substances; chemical similarity is the basis, with homogeneous physical-chemical properties that may change with a regular trend within the category, but it is not sufficient for a proper justification. For some simple homologue substances similarity may be straightforward, but with more complex molecules or isomers this is not trivial. In fact, one of the main arguments against read-across is the presence of activity cliffs: Many chemicals that are identical in terms of 2- but not 3-dimensional structure – thalidomide being a well-known example – markedly differ in terms of bioactivity. Therefore, the grouping of similar molecules has to be based on more than just *chemically* similar molecules. Biological similarity approaches can take many forms, i.e., using several bioassays to identify common molecular targets of toxicity, narrowing the applicability domain to identify areas of "local validity," or identifying Adverse Outcome Pathways (AOP) or Pathways of Toxicity (PoT). The AOP is a mechanistically-based approach that may explain the similarity in the biological behavior of two or more substances with the complement of pharmacokinetics considerations that should consider metabolism, distribution in the organism and kinetics of the excretion. Information on substance metabolism, in particular, is central to supporting similarity between two or more substances for read-across purposes as metabolism may determine a biological difference between two substances that look similar from a chemical point of view. However, this information is not often available or the biological data often lacks standardization, preventing good automatic comparison.

The definition of the principle and the format for justifying and presenting the data in read-across is not enough and users need suitable tools to exploit the read-across opportunity on a strong scientific basis, including clear applicability domain, robust statistical evaluation plus transparent and objective outcome. The availability of large quantities of data and test results acquired for chemicals whose structure and physical chemical properties are well defined is a fundamental basis for a robust statistical evaluation and feed the read-across approach.

In this sense, the public access of the REACH registration dossiers on the ECHA website represents a tremendous opportunity with 14,000 registration dossiers that contain relevant chemicals assessment data (http://www.echa.europa.

eu/information-on-chemicals/registered-substances). This is the largest ever reservoir of information, even though re-elaboration requires careful assessment, as the data in the registration dossiers are under the sole responsibility of the registrants with no formal control of the regulators. The first REACH deadline regarding substances manufactured or imported in quantities above 1000 t/y was in 2010. At that time, registrants had no experience on this new regulation and in some cases the dossiers were presented in a very superficial format. After 6 years, there is much more awareness of the importance of the REACH program and ECHA, together with the Member State Committee (MSC), has also started the re-evaluation of many dossiers, asking for detailed justification of the approach used in the dossiers, including scientific justification for read-across, exposure-based waiving, weight of evidence, substance identification, etc., requiring many updates of the submitted dossiers and helping increase the quality of the new ones. The big advantage of the ECHA database is that it is complete and includes data on chemical structures, physical-chemical properties and biological behavior, with no restrictions on a particular use or characteristic, as is the case for many other databases. However, the database has no automatic query capability and data are inserted as unstructured text rather than numbers, making automatic assessment very complex. The ECHA interface web page allows the consultation of only one substance at a time following manual query, and information about similar substances is hidden.

CAAT at Johns Hopkins University has developed a system to transform the ECHA database into a machine-readable format open to many useful applications. Beyond the possibility of performing statistical evaluation of a number of parameters, including the assessment of *in vivo* studies when repeated for the same substance and the prevalence of a particular property in the chemical universe, it also could be used to apply the read-across principle to new chemicals. The automatic search engine may be used to discriminate good data from bad data by combining the results with other parameters that are available for the substance by either considering the quality of the experimental study or through the identification of outliers. This tool, called ToxTrack, already has been successfully applied for a general study of oral and eye irritation and skin sensitization (Luechtefeld et al., 2016b-d) demonstrating its incredible potential. Currently, formal approval from ECHA for use of the data is pending.

Another possibility to harness the ECHA database is offered by the AMBIT tool, an open software product designed to support companies by facilitating high quality chemical safety prediction. The development of this tool was supported by Cefic- "Long Range Research Initiative" (LRI) programme in collaboration with Clariant and IdeaConsult. Thanks to the opportunity offered by ECHA to refer to the non-confidential REACH dataset, the "predictive toxicity model" in AMBIT can apply the principles of read-across and categorization by combining the possibility of directly querying in the ECHA database or into own data after securely offline uploading in the software. AMBIT is freely available (http://cefic-lri.org/lri_toolbox/ambit/) and it comprises a database of more than 450,000 chemical structures and functional modules, enhanced search functionality, with several data export formats, including the REACH IUCLID format. The tool is designed to enable the secure import of external databases from several sources.

Further improvement for a broader applicability of any tool requires clear rules on how to access the database of the REACH registration dossiers, which are not completely public and missing many parts of the submitted dossiers that are considered confidential. Moreover, data accessibility should be enlarged to other sectors related to specific classes of substances, such as drugs or plant protection products, including company data on products that were abandoned before reaching the market.

Complementary to the ECHA database, another important open source reservoir of data is the archive of the ToxCast data, as published by EPA (http://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data), which contains results from several thousands of *in vitro* tested chemicals, measuring hundreds of endpoints each. This can be considered the ideal complement to the classic approach of read-across-based chemical similarity (Zhu et al., 2016). In fact, the ECHA RAAF document explicitly refers to the possibility of using *in vitro* data to support the similarity between two or more substances as the *in vitro* data may elucidate a specific mechanism or demonstrate the shared AOP. New *in vitro* data can be easily generated following a specific strategy (Rovida et al., 2015) and the ToxCast set of assays may represent a valid possibility.

During the workshop, there was the general agreement that read-across has come to a crossroads, moving from pure chemistry to one of the means to help understand the biological mechanism. ECHA, as representative of the world of regulators, is leading the process, which represents a great opportunity for disseminating the idea by providing guidelines, organizing meetings and evaluating new proposals from registrants. The ECHA RAAF document represents the first official document prepared by regulators and it sets the basis for enlarging the read-across strategy within other legal requests, even with the limitation that outside the EU there is no formal acceptability yet, and in some countries, rejection is explicit. Even within the EU, other legislation, like Regulation CE 1107/2009 on plant protection products, may benefit from this opportunity and also may provide very useful data on metabolism and pharmacokinetics, which are probably the key to future improvement of the read-across strategy.

The enthusiasm should not fade in light of the strict scientific procedure that must be rigorously applied, starting from the limit of the applicability domain, which excludes most of the UVCB (unknown, of variable composition, or of biological origin) substances. It is also important to keep in mind that read-across is just an opportunity and not the panacea. It may help the risk assessment process, but with the support of other sources of information. Moreover, read-across is based on the elaboration of existing data whose reliability and accuracy are

often not confirmed. This is the reason why the process for building Good Read-across Practice has just started and is far from being well-defined (Ball et al., 2016). Another important issue that was identified during the workshops is the actual scientific limit in the measure of the uncertainty, which is inherently linked to any conclusion. A noteworthy aspect is also the reliability of conclusions for non-toxicity that in case of wrong prediction may lead to severe consequence for human health or environment safety.

Regarding further opportunities, the next step is moving beyond the EU. For example, Korea, Taiwan, Turkey and other countries are already working on specific programs for the implementation of the read-across principle. In this sense, the contribution from OECD should be highly relevant. Hopefully, the opening to new markets may contribute to the accessibility of larger databases.

References

Ball, N., Cronin, M. T. D., Shen, J. et al. (2016). Toward good read-across practice (GRAP) guidance. *ALTEX 33*, 149-166. http://dx.doi.org/10.14573/altex.1601251

ECHA (2014). The Use of Alternatives to Testing on Animals for the REACH Regulation Second report under Article 117(3) of the REACH Regulation. ECHA-14-A-07-EN. http://echa.europa.eu/documents/10162/13639/alternatives_test_animals_2014_en.pdf

ECHA (2015). Read-Across Assessment Framework (RAAF). ECHA-15-R-07-EN http://echa.europa.eu/documents/10162/13628/raaf_en.pdf

Hartung, T. (2016). Making big sense from big data in toxicology by read-across. *ALTEX 33*, 83-93. http://dx.doi.org/10.14573/altex.1603091

Luechtefeld, T., Maertens, A., Russo, D. P. et al. (2016a). Global analysis of publicly available safety data for 9,801 substances registered under REACH from 2008-2014. *ALTEX 33*, 95-109. http://dx.doi.org/10.14573/altex.1510052

Luechtefeld, T., Maertens, A., Russo, D. P. et al. (2016b). Analysis of public oral toxicity data from REACH registrations 2008-2014. *ALTEX 33*, 111-122. http://dx.doi.org/10.14573/altex.1510054

Luechtefeld, T., Maertens, A., Russo, D. P. et al. (2016c). Analysis of Draize eye irritation testing and its prediction by mining publicly available 2008-2014 REACH data. *ALTEX 33*, 123-134. http://dx.doi.org/10.14573/altex.1510053

Luechtefeld, T., Maertens, A., Russo, D. P. et al. (2016d). Analysis of publically available skin sensitization data from REACH registrations 2008-2014. *ALTEX 33*, 135-148. http://dx.doi.org/10.14573/altex.1510055

Rovida, C., Alépée, N., Api, A. M. et al. (2015). Integrated Testing Strategies (ITS) for safety assessment. *ALTEX 32*, 25-40. http://www.altex.ch/resources/altex_2015_1_025_040_Rovida_sva1.pdf

Zhu, H., Bouhifd, M., Kleinstreuer, N. et al. (2016). Supporting read-across using biological data. *ALTEX 33*, 167-182. http://doi.org/10.14573/altex.1601252.

Correspondence to

Costanza Rovida
CAAT-Europe
University of Konstanz
Box 600
78457 Konstanz, Germany
Phone: +39 340 4008118
e-mail: costanza.rovida@uni-konstanz.de