Workshop report

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

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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 homogeneouseous 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.
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 im-
ported 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 jus-
tification for read-across, exposure-based waiving, weight of
evidence, substance identification, etc., requiring many up-
dates of the submitted dossiers and helping increase the qual-
ity 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 au-
tomatic query capability and data are inserted as unstructured
text rather than numbers, making automatic assessment very
complex. The ECHA interface web page allows the consulta-
tion 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 for-
mat open to many useful applications. Beyond the possibility
of performing statistical evaluation of a number of param-
eters, including the assessment of *in vivo* studies when repeat-
ed for the same substance and the prevalence of a particu-
lar property in the chemical universe, it also could be used to ap-
ply 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 outli-
ers. 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 of-
fered 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 Clarient 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, en-
hanced 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 pub-
lic 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 sub-
stances, such as drugs or plant protection products, including
company data on products that were abandoned before reach-
ing 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 consid-
ered 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 bio-
logical mechanism. ECHA, as representative of the world of
regulators, is leading the process, which represents a great
opportunity for disseminating the idea by providing guide-
lines, 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 re-
quests, 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 Regula-
tion 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 sci-
centific 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 biologi-
cal 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

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