OPINION

Read-across for Hazard Assessment: The Ugly Duckling is Growing Up

Wera Teubner and Robert Landsiedel

Increasing use of read-across in integrated approaches for the testing and assessment of chemical hazards will ensure that it eventually matures into a beautiful swan

In the hazard assessment of chemicals, read-across describes a technique used to predict physicochemical, ecotoxicological and toxicological endpoints. If it is performed on several substances at a time, it is called 'category formation'. Read-across is based on the experience that similar chemicals exhibit similar properties — with the crucial issue of knowing which properties determine similarity for a given endpoint. In this aspect, it is a relative of the quantitative/qualitative structure–activity relationship (QSAR), and was sometimes simply termed 'expert judgement'. The idea of the read-across concept being an 'ugly duckling' has mostly arisen from the difficulty in verifying the plausibility of its findings without actually performing the experimental studies. The Read-Across Assessment Framework (RAAF),[1] published in May 2015, states that "Under REACH, any read-across approach must be based on structural similarity between the source and target substances". However, the limited verification of read-across, and especially the limitations of the use of the read-across approach only to structural similarities, reflect a state of infancy that needs to be nurtured toward maturity in order to reap its maximum benefits.

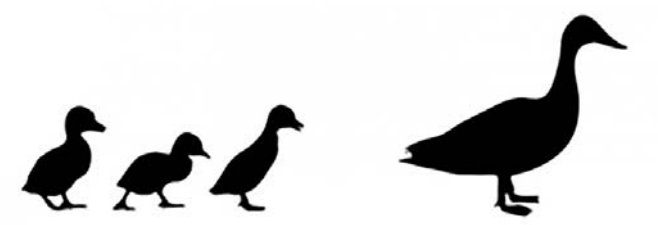
When, in 1959, William Russell and Rex Burch published *The Principles of Humane Experimental Technique*,[2] calling for the *replacement*, *refinement* and *reduction* of animal testing, a major focus was the quality of animal testing and the criticism that poor planning and experimental techniques resulted in animal studies of limited value, and consequently in more testing than should have been needed. With the introduction of Good Laboratory Practice and of Organisation for Economic Co-operation and Development (OECD) test guidelines (TGs) and animal welfare policies, the quality of animal data has become much less of a problem, and *refinement* has considerably improved. The improvement of cell culture, tissue culture and molecular biology technology kindled the hope for *replacement*. Meanwhile, stand-alone *in vitro* methods (e.g. for skin and eye irritation) or batteries of tests (e.g. for skin sensitisation) can address local toxicity. Likewise, methods to address specific early effects or mechanisms, such as genotoxicity or oestrogenic activity, are available.

A major challenge today is the prediction of complex toxicological effects such as systemic and developmental toxicity. Large research programmes, e.g. ToxCast or SEURAT, aim to meet this challenge.[3,4] Any new approach to complex toxicological effects combines various methods (*in silico*, *in vitro* and *in vivo*) in a testing battery or strategy.[5,6] These approaches use mechanistic information, and are constructed according to (putative) adverse-outcome pathways (AOPs).[7] Such information is, of course, also useful in supporting the read-across of apical toxic effects of different chemicals. Read-across can actually become a successful part of many integrated approaches for testing and assessment (IATAs).

Traditionally, chemicals are considered candidates for read-across, if they share structural similarity or are metabolically or spontaneously transformed to common products. It is assumed that structural similarity will result in a common mode-of-action. When assessing wanted pharmacological activities or unwanted toxicological hazards in research and development, applying read-across is already possible when the substance in question still only exists on paper. High-quality predictions are valuable for success in product development. At some point, the predicted effects are determined experimentally for promising candidates, and it is at this point that the consequences of poor read-across hit back. Again, identifying the correct similarity between read-across source and target chemicals is crucial.

The 'ugly duckling' characteristics of read-across (Figure 1) originate from areas in which it is used as a quick (and cheap) means to generate hazard information, either to fulfil regulatory data requirements, or to identify and list substances allegedly of very high concern (no reference given here, since this

Figure 1: The ugly duckling



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PiLAS is not a pillory). It also may originate from the idea that any information is better than no information in situations where there is no budget, or when animal testing is simply out of the question. Global efforts to identify and substitute hazardous chemicals can only succeed, if so-called 'regrettable substitutions' can be avoided. Neither overestimation nor underestimation of hazards by read-across is helpful in this context. Actually, it takes a wide range of thorough considerations to perform a robust and meaningful read-across — and these need to be documented. To toxicologists with long experience in their respective chemical space, similarity may seem so obvious that their read-across justifications are rather frustrating to comprehend.

The application of read-across and the related category approach received a boost when the European Union (EU) introduced the REACH programme in 2006. The REACH legislation (EC *Regulation 1907/2006*)[8] requires the hazard characterisation of all chemicals marketed in the EU, with actual data requirements dependent on the production and import tonnage and the use conditions. With the estimation that more than 20,000 chemicals would need to be assessed, the legislation needed to include provisions to use animal testing only as a last resort. The obligation of the European Chemicals Agency (ECHA) to report on the status of the implementation and use of non-animal test methods and testing strategies is actually laid down in Article 117(3) of the legislation. As of 1 October 2013, dossiers for 8,729 substances have been submitted to the ECHA. A read-across or category approach was used in up to 75% of analysed dossiers for at least one endpoint.[9]

Considering the huge number of chemicals that were to be registered within the short period of eight years, the REACH legislation introduced a previously mostly-unknown component to chemical legislation. It was proposed that acceptance of registration, if appropriate, would be granted after automated dossier screening. Any scientific review of toxicological data would then be performed at a later stage, and this review would have to be conducted for at least 5% of the registered substances. With this procedure, the opportunity for an upfront discussion on the data requirements and suitability to support a read-across approach is in no way considered. This registration strategy has the advantage of speed and a certainty of meeting submission deadlines, but the disadvantage of uncertainty with regard to follow-up activities, the latter arising from the possibility that the read-across assessment might be judged to be deficient and the decision would then be made that the target substance must be tested.

Unlike standard testing requirements explicitly calling for a given and well-described method (often an animal-study; e.g. OECD TG 412 and TG 413 for systemic toxicity), mechanistic and toxicokinetic studies that may be needed to support a read-across are not part of the mandatory data set. The decision to invest in non-standard mechanistic (and possibly animal) studies, over choosing the straightforward way of routine testing, needs careful consideration. In the absence of a fixed and pre-agreed method for read-across, there is always room for discussions on potential uncertainties and whether the presented read-across case is comprehensive and convincing. Such discussions concern the quality of the experimental data of the analogue substance(s), the lack of adequate justification why similarity is given, or the conclusion that the uncertainty is too high. Ball *et al.*[10] have published their experience with the read-across assessment of an acetate ester, specifically the argumentation that ester hydrolysis is sufficiently fast to follow the line of a common metabolite. Certainly, read-across needs to be rejected, if it was done 'quick and dirty' and as a result does not provide robust data (i.e. the soundness of the resulting hazard information cannot be judged). Schultz *et al.*[11] have recently published a proposal on how to structure and assess the acceptability of a read-across approach. Their strategy of including any helpful information from non-guideline studies to QSAR models is — at least in terms of REACH terminology — turning any read-across into a so-called 'weight-of-evidence' assessment.

Both challenges and improvements to read-across approaches have been triggered by cases where apparently small changes in structures resulted in vast changes of the hazard properties (so called 'activity cliffs'). The most prominent examples originate from differences in the interactions of substances with enzymes and receptors. The substances 2-acetylaminofluorene (2-AAF) and 4-acetylaminofluorene (4-AAF) are structurally very similar. As well as being a bladder carcinogen, 2-AAF is a strong liver enzyme inducer, leading in long-term studies to liver tumours. However, 4-AAF only slightly induces liver enzymes and does not induce the formation of liver tumours.[12] Enantiomers of 1-hydroxyethylpyrene are activated to mutagenic sulphates by different sulphotransferases,[13] and the enantiomers of Carvone smell of caraway or spearmint,[14] to name but two examples. When looking at the two-dimensional description of a chemical only (e.g. SMARTS pattern or Tanimoto score), stereoisomers appear identical, but three-dimensional structure modelling for receptor-binding simulation can differentiate stereoisomers.

Regardless, stereo-isomeric and regio-isomeric differences of molecules appear to be small alterations, as compared to the changes usually bridged by read-across (e.g. homologous series). It is important to know which aspect of similarity between two chemicals is governing their similar hazardous properties.

Structure–hazard relationships are a 'long-shot': In between the structure of a chemical and its apical toxic effect are its material properties (e.g. electrophilicity), system-dependent properties (e.g. ROS generation), molecular interactions (e.g. receptor-binding and DNA-binding) and early cellular responses (e.g. mutagenicity). It is crucial to know when structure information is sufficient, or when additional data, possibly closer to the apical effect, are needed, but this should not undervalue the research efforts undertaken to derive such properties from information on structure, nor does it mean that structure and material property are unrelated. This has been exemplified with skin sensitising chemicals of low molecular weight, where reaction classes identified from the chemical structure may be a more-instructive property to predict the protein-binding than general molecular descriptors.[15-17] The reaction class is considering only the property that is essential to initiate the molecular initiating event of skin sensitisation, i.e. protein binding, whereas general molecular descriptors can 'dilute' this information with molecular features of less relevance.

Evidently, properties and effects closer to the apical toxic effects are more predictive and less uncertain. Lately, the concept of applying 'functionality' rather than (or in addition to) material descriptors was proposed for nanomaterials.[18-20] This can be taken a step further: Rather than using the molecular structure or the 'functionality', read-across can be based on the early biological effects or common modes-of-actions of two (or more) substances. Actually, such a concept is typically represented by the common classification of any chemical with a pH of > 11 as corrosive, but no one would consider calling it functionality-based or mode-of-action-based read-across. The concept of biologically-based activity relationship (QBAR, i.e. referring to QSAR, the structure-based activity relationship) has been discussed and exemplified by van Ravenzwaay *et al.*[12] The example of different toxicities of the structurally-similar isomers, 2-AAF and 4-AAF, was given above. These differences are reflected in different metabolome-patterns induced by these two compounds. Another example are fibrates with structural similarity. Most of these fibrates also show toxicological and pharmacological similarity, based on the metabolome data. Gemfibrozil, however, does have different pharmacological and toxicological effects. The differences in the target organ (e.g. the kidney) for Gemfibrozil and its pharmacological effect (cholesterol lowering) can be identified, based on the metabolome data. This example shows that structurally similar chemicals need not necessarily have the same apical effects, and in this case biological data are needed to prove toxicological similarity.

The call for good science and documentation in hazard assessment, that was made by Russell and Burch,[2] is as relevant now as it was in 1959. Indeed, guidance documents and reporting templates have undergone several refinements,[21-23] strategies have been published,[11,24,25] and recently, the ECHA has published the Read-Across Assessment Framework (RAAF).[1] The latter aims at the quality control and transparency of read-across evaluations. It provides structure, and ensures that all relevant elements are addressed and will lead to a conclusion on whether or not a read-across is scientifically acceptable.

Documentation and justification for a read-across approach, in a form that it is sufficient and immediately understandable for an independent reviewer, is both challenging and time consuming. It is a considerable cost factor, which is easily underestimated in the preparation of registration dossiers. In addition, a letter of access, granting the rights to use the experimental data on read-across substances, must be available. In cases where more than one study are needed, the costs for getting the rights to refer to all read-across studies may match, or even exceed, the cost of a new study. In a favourable situation, the data on the read-across substances are already owned by one of the registrants, or they have been published in sufficient detail in a peer-review journal. In this case, refusal of a read-across assessment upon evaluation is much less costly, as compared to the situation where registrants have paid a competing company for a letter of access to now-useless read-across studies.

Read-across approaches rely on existing experimental data on potential read-across source substances. Both the generation of new data and their dissemination via the ECHA website continue to provide opportunities for read-across. Most importantly, IT tools facilitate the identification of analogues and the easy display of existing data. The most sophisticated tool in this regard is the OECD QSAR toolbox,[26] but already, simpler search tools such as eChemPortal[27] permit a quick search for potential read-across candidates.

Read-across has found its way in other modern chemical legislation, such as the new chemical legislations in Korea (K-REACH) and China. It helps in the hazard assessment of new cosmetic products that are banned from animal testing in the EU. Read-across case studies are discussed at the OECD level,[28] illustrating the current worldwide interest in this approach.

One of the many important points made by Russell and Burch in their 1959 book,[2] is the inappropriateness of blindly taking mammalian studies as the 'gold standard' for human health hazard assessment. It needs to be remembered that this can also be applied to the read-across approach, since most of the experimental data on the similar chemicals are animal data. Read-across assessments predicting the outcome of animal studies may be perfect with regard

Figure 2: The beautiful swans of IATAs

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to fulfilling regulatory requirements, but the ultimate aim remains human health hazard assessment.

Developing sound and well-justified read-across and grouping will be neither quick nor easy (hence it should not be termed 'non-testing'), and it will often require fortification by 'mode-of-action-tailored' experimental data, in order to cover chemicals with similar early interactions, but at first glance not necessarily closely-related structures. Newly generated 'omics' and *in vitro* data addressing early (biological) effects, as well as already-existing REACH dossiers,[29] SEURAT[30] and Toxcast[31] data, offer tools to improve read-across, based on properties closer to the hazard (the apical effect) beyond the traditional concept based only on QSARs. Established AOPs and the identification of molecular initiating events (MIEs) facilitate this use of read-across (and were, on the other hand, often identified from a set of experimental data from structurally-related chemicals). The combination of different experimental data and their relation to apical toxic effects may indeed offer the most powerful tools to advance the Three Rs. Considerations of relevant data in creating a read-across case are also used to build IATAs. Both require a sound scientific case, relevant data to support them, and awareness (and acceptance) of their limitations.

Consensus on what an acceptable read-across looks like, is emerging whilst it is in the process of being used. For this, we have to nourish and nurture the duckling — and we have to recognise when it is no longer an ugly duckling, but has matured and become a beautiful swan (Figure 2).

Author for correspondence:
Dr Robert Landsiedel
BASF SE
Experimental Toxicology and Ecology
Ludwigshafen am Rhein
Germany
E-mail: robert.landsiedel@basf.com

Dr Wera Teubner
BASF Schweiz AG
Product Safety
Basel
Switzerland

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