Meeting Report

Regulatory Acceptance of Read-Across

doi:10.14573/altex.1805081

A satellite meeting on "Regulatory Acceptance of Read-Across" was hosted by the Johns Hopkins Center for Alternatives to Animal Testing (CAAT) at the 56th Annual Meeting of the Society of Toxicology in Baltimore, MD, USA in March 2017. This report summarizes the presentations and discussions at this meeting, by speakers from both academia and regulatory agencies, in which the current state of regulatory acceptance of read-across in the EU, US, and Japan was addressed, and the challenges and opportunities for read-across in regulatory toxicology were described.

Read-across allows for the screening, classification, prioritization, and hazard assessment of chemicals based on the toxicological data of similar chemicals and is the most common alternative to animal testing. As both available datasets and improved read-across techniques allow greater confidence in this testing method, it is becoming increasingly important to ensure its straightforward regulatory acceptance.

The satellite meeting featured invited speakers from multiple agencies offering their practical perspectives on the applications of read-across methods in regulatory toxicology decision-making. Based on the meeting presentations and discussions, the current state of read-across acceptance in regulatory toxicology is addressed in this report, along with the challenges and opportunities for read-across use in the decision-making process.

Thomas Hartung – Meeting introduction and the read-across activities of the Johns Hopkins Center for Alternatives to Animal Testing (CAAT)

Thomas Hartung, the director of the Center for Alternatives to Animal Testing (CAAT) at the Johns Hopkins Bloomberg School of Public Health, began the presentations by providing an overview of the motivation for regulatory acceptance of read-across, describing the potential applications of read-across in regulatory toxicology, and outlining the challenges and opportunities associated with regulatory acceptance (Patlewicz et al., 2014, 2017; Hartung, 2016).

Hartung explained that out of the 80 million substances synthesized, less than 10% have undergone toxicity testing (Hartung, 2017a). Considering this along with metabolites, mixtures, and other natural products, for which we do not have sufficient information, testing such a large set of chemicals using current methods would exhaust resources, but read-across could offer a practical solution to this problem.

Hartung then introduced the laws that are relevant to regulatory acceptance of read-across, namely, the Toxic Substance Control Act (TSCA) in the US, which was recently reauthorized as the Lautenberg chemical safety act (LCSA) in June 2016. TSCA regulates the manufacture of chemicals in commerce, but due to the magnitude of new chemicals, Hartung noted, most of the substances in the US come to market without any type of toxicological testing. With the new authorization, the manufacture of a new chemical can only begin if the Environmental Protection Agency (EPA) affirms that the chemical is unlikely to present an unreasonable risk [5(a)(1)(B), 5(g)]. The problem, Hartung said, is obtaining toxicological data for the 1,000 new chemicals synthesized each year, and this problem is not limited to the US, as regulations around the world, such as EU REACH, Korea-REACH, Taiwan-REACH, and the Canadian Environmental Protection Act (CEPA) of 1999, also present challenges due to the magnitude of existing chemicals that require safety assessment. Hartung stated that such legislation was written with the development of new tools in mind and read-across is one of the new approaches that will allow chemical screening and data-gap-filling at larger scales more efficiently.

In addition to the efficiency read-across provides to toxicological assessment, Hartung explained that read-across is a key opportunity for green toxicology, which requires early testing of many more substances that make it to the market (Maertens et al., 2014; Crawford et al., 2017; Maertens and Hartung, 2018). Read-across is especially relevant to two principles of green toxicology, "benign by design" and "reduced toxicity", because read-across can be used as an informative tool to guide the design of new substances.

Hartung also outlined the potential uses of read-across in regulatory toxicology, which include screening, prioritization, and risk assessment of new and existing chemicals for REACH and other global legislation, new product registration for industry, adverse outcome pathway (AOP) (Leist et al., 2017) and integrated approaches to testing and assessment (IATA) frameworks (Tollefsen et al., 2014; Hartung et al., 2013), meeting the dramatic testing requirements of nanomaterials, the development of new pharmaceuticals, and testing of illicit drugs.

There are still challenges faced by adopting read-across in a regulatory setting, such as standardization, Hartung noted, as Good Read-Across Practice is only just emerging (Ball et al., 2016). Also, because read-across is based on the local similarity of a chemical to its neighbors, the process is driven by available data and typically results in a one-to-one or one-to-two chemical correlation. In addition, one of the main drawbacks of read-across is that it is not validated against animal testing as many other alternative methods have been. On the other hand,

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quantitative structure-activity relationships (QSARs) (Cronin et al., 2003) can be validated with enormous consequences for acceptability and applicability. Automated read-across, i.e., a chemical similarity-based QSAR, could be a solution here (Hartung, 2016; Shah et al., 2016). However, current read-across is not a traditional QSAR; although read-across is informed by chemical structure, it is strictly based on local similarity of a chemical with similar chemicals.

Hartung then reported on the read-across activities of CAAT, which began in 2013 with the white paper, *Food for thought: Read-across approaches – Misconceptions, promises and challenges ahead* (Patlewicz et al., 2014). A workshop was held in Baltimore, MD in October 2015, to discuss the development of a guidance document for Good Read-Across Practice (Ball et al., 2016). This guidance document was accompanied by another, which proposed that biological data from *in vitro* or short term *in vivo* animal studies could strengthen the validity of read-across approaches (Zhu et al., 2016). Following this, additional forums for stakeholders were held in Brussels and Washington, DC in February of 2016 (Maertens et al., 2016).

Hartung also highlighted a few changes in regulatory toxicology, which will provide the ideal environment for expanding the capabilities of read-across, including that EFSA began publishing their 10,000 risk assessments in December 2016, the EPA began to electronically release data on 2,000 pesticides in 2017, and the data for 40,000-60,000 chemicals, which would have originally been proprietary, will be made publicly available by ECHA in 2018. These are only a few of the many data sources that could be beneficial for read-across, and these developments demonstrate a willingness to support these types of assessments.

Hartung also reported on the activities involving REACH-across (UL), which he explained is primarily the work of Thomas Luechtefeld, a PhD student at CAAT, who downloaded the publicly accessible ECHA database and then converted the data into a standardized form using natural language processing. These reports were used to produce a searchable database which was mined for a few important endpoints to illustrate its potential (Luechtefeld et al., 2016a,b,c,d). The final tool that was developed using this data, REACH*Across*™[1], is an automated read-across platform that utilizes machine learning to determine chemical similarity within a curated dataset of 70+ million chemical structures, 300,000 endpoint labels, 250,000 high-interest compounds, and 20,000 unique compounds with endpoint information, which represents approximately 30 trillion pairs of chemicals. As of March 2017, the tool includes all endpoints for REACH 2018 with an accuracy similar to that of animal tests. An internal validation has been conducted for an unprecedented number of chemicals (Luechtefeld et al., 2018).

Hartung concluded his talk with a timeline of the main achievements of regulatory acceptance of read-across and stated that the next goal for regulatory acceptance of read-across is validation so that in the future there will be broad use of automated, validated read-across for the toxicological assessment of chemicals.

Takashi Yamada – Recent experiences in the development of read-across for chemical safety assessment at the National Institute of Health Sciences (NIHS) in Japan

Takashi Yamada of the Division of Risk Assessment, National Institute of Health Sciences (NIHS) in Japan started with a summary of the NIHS and International Council of Chemical Associations' Long-Range Research Initiative (ICCA-LRI) Workshop held on Awaji Island, Japan in June 2016, for which a workshop report, *Meeting the Global Challenge of Applying New Scientific Methods to Improve Environmental and Human Health Risk Assessment* is available[2]. One session of the workshop was completely devoted to the challenges of read-across and how to build confidence in read-across for use in decision-making. The conclusions of this session, Yamada explained, were that read-across is a valuable tool for chemical safety assessment, but that there are major barriers to its use in regulatory toxicology due to the lack of validation and standardized methods, and because regulators do not have experience with read-across. At this session it was proposed that to overcome these barriers, more case studies involving read-across need to be performed, guidance should be developed, and training should be available to those who could benefit from using read-across. It was also decided that future research should address the uncertainty and variability in chemical assessments conducted using read-across.

Yamada then provided an overview of regulatory acceptance of read-across in a few key Asian countries. In Japan, read-across is an acceptable tool to assess biodegradation and bioaccumulation of industrial chemicals and is being discussed for potential uses in assessing other chemical endpoints. In China and Korea, read-across is generally not considered an acceptable tool to perform chemical safety assessments.

Yamada also provided an overview of the Organisation for Economic Co-operation and Development (OECD) integrated approaches to testing and assessment (IATA) case studies project, which involves a team from Australia, Canada, Denmark, Japan, The Netherlands, Sweden, and the United States, along with the European Commission, the European Chemicals Agency (ECHA), the Business and Industry Advisory Committee (BIAC) to the OECD, and the International Council for Animal Protection in OECD Programmes (ICAPO). These case studies provide a forum to increase experience with the use of IATA for regulatory purposes and should ultimately allow the development of guidance for new tools that can be used in regulatory toxicology. Yamada briefly reported on four case studies that were conducted in 2015, *in vitro* mutagenicity of 3,3' dimethoxybenzidine (DMOB)-based direct dyes (Canada and US), repeated dose toxicity of substituted diphenylamines (SDPA) (Canada), hepatotoxicity of allyl ester category (Japan), and bioaccumulation potential of biodegradation products of 4,4'-bis(chloromethyl)-1,1'-biphenyl (Japan). He stated that all the case studies illustrate a pragmatic use of chemical group-

[1] https://www.ulreachacross.com

[2] https://lri.americanchemistry.com/LRI/LRI-Workshops/

ing methods, while addressing some challenging topics such as the use of adverse outcome pathway (AOP) information. The results of these first four case studies from the IATA case studies project were published along with a reporting template and a considerations document highlighting what was learned in the first review cycle (OECD, 2017).

Yamada concluded that read-across is conceptually simple but practically difficult because it takes a significant amount of time to gather the information needed to begin a read-across assessment. He also stated that transparency, reproducibility, data quality, and certainty are key for regulatory acceptance of read-across in Japan. Although there is currently very little practical use of read-across in regulation in Japan, there is interest, and these case studies are a first attempt to increase regulatory acceptance.

Timothy Adams – Safety evaluation of mixtures: Novel application of read-across, the threshold of toxicological concern (TTC) approach, and the use of chemical groups for safety evaluation of mixtures at the Office of Food Additive Safety (OFAS) of the US Food and Drug Administration (FDA)

Timothy Adams of the Office of Food Additive Safety (OFAS) at the Center for Food Safety & Applied Nutrition (CFSAN) of the US Food and Drug Administration (FDA) began the next talk on a novel application of read-across, the threshold of toxicological concern (TTC) approach (for review see Hartung, 2017b), and the use of chemical groups for safety evaluation of mixtures at the FDA.

Adams first provided a brief historical timeline of the use of read-across at the FDA. The FDA first began to organize flavoring ingredients into categories in scientific literature reviews conducted from 1974 to 1979 in which substances were sorted by structure and functional groups, metabolism, and acute and sub-chronic target organ toxicity. In 1978, the structure-based Cramer decision tree, which relates chemical structure to toxic potential, was developed and validated against chemicals with toxicity data, such as pesticides, drugs, food additives, and industrial chemicals (Cramer et al., 1978). In 1995, the FDA updated the 1970s data and submitted it to the FAO/WHO joint expert committee on food additives (JECFA). Since 1996, 69 chemical categories (CCs) have been submitted to JECFA and these were prioritized by chemical category as well as with the Cramer decision tree. From 2011 to 2016, an expanded decision tree (EDT) was developed with 125 new chemical categories, chemical structure, competing metabolic pathways, mode of action (MoA) data, and factors affecting relevance of MoA to human health, such as peroxisome proliferation, alpha-2-u-globulin, and forestomach tumors. Adams noted that the read-across performed by the FDA considers the EDT class, CC, and the MoA of a chemical. Adams then provided an example for four hydrocarbons: myrcene, limonene, camphene, and pinene, which were determined to belong to a single chemical category due to shared structural features, metabolic fates, pharmacokinetic patterns, and target endpoints, and are all characterized by a relatively narrow range of NOELs. Thus, Adams concluded, the data from one of these chemicals can be used in read-across to extrapolate information about another in this group.

Adams then provided an overview of the key additions to the EDT, which include new EDT questions for elements and functional groups present in the majority of food substances, including food contact substances, pesticide residues, and other contaminants, a revision of most existing CDT questions, improved delineation of EDT classes, a sufficient database of NOELs to support each EDT class, and a two-tailed 5th percentile NOEL analysis for each structural class.

Adams stated that a current effort is a new strategy for safety evaluation of non-functional constituents (NFCs). The strategy involves a process of identifying the constituents in a mixture, assigning the constituents to a specific chemical group, determining exposure to a chemical group based on intake of the NFC, collecting safety data for representative members of each chemical group and comparing biochemical fate and toxicity data for each, and prioritizing the chemical groups based on percent content and EDT class. Adams noted that only constituents with intake that exceeds the threshold of regulation (1.5 µg/day) need to be identified and only if the constituents meet the practical analytical limit of 0.01% NFC with intake of naturals containing class IV, V, or VI chemical groups or 0.1% NFC with intake of class I, II, or III chemical groups. Adams then provided examples of how this analysis was conducted for peppermint oil, corn mint oil, and nutmeg oil. Adams said that the goals of the safety evaluation of NFCs are that they are comprehensive with no significant risk associated with the intake of NFC mixture left unevaluated, that they can be applied to the global market for NFC use in food, and that they become a practical and economical method to evaluate safety in use of mixtures without testing all NFCs or their constituents individually or testing on animals.

Derek Knight – Regulatory acceptance of read-across at the European Chemicals Agency (ECHA)

Derek J. Knight, Senior Scientific Officer of the European Chemicals Agency (ECHA), began the next talk on regulatory acceptance of read-across in Europe for chemicals legislation. Knight first introduced the EU regulatory framework for chemicals, which is comprised of the Classification, Labelling and Packaging (CLP) regulation, the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation, and Product Legislation, which is separate from REACH and CLP but overlaps with both. Knight then explained that through REACH and CLP, industry is responsible for gathering information and informing risk management through pre-registration of chemicals, data sharing, registration, and self-classification. In the evaluation period for REACH, ECHA and member states competent authorities (MSCAs) preform evaluations of the information provided by industry and can request further infor-

mation, if needed, to authorize or restrict a chemical and harmonize classification and labeling. Following this, the European Commission, with support of ECHA and MSCAs, then applies community-wide risk management measures.

Knight explained that the information requirement under REACH has three purposes: classification and labeling, hazard characterization, and risk characterization. In parallel, ECHA and other regulators also conduct screening of the information provided by industry to identify substances of potential risk. Knight then stated that REACH standard information Annexes are tiered per annum tonnage bands (1-10, 10-100, 100-1000 and > 1000) the industry plans to supply and that Annex XI allows adaptation to fulfil information requirements by prediction but this must be adequate for risk assessment and classification (for review see Hartung, 2010). Knight also emphasized that ECHA provides guidance and advisory documents, training, and the OECD QSAR toolbox to encourage registrants to meet their obligation to use alternative approaches so that new animal testing is only a last resort.

Knight then provided an overview of the use of read-across under REACH to predict chemical properties and said that read-across has been a relatively popular alternative approach submitted by registrants to meet REACH requirements. He noted, however, that because similar chemical structures are the basis for read-across, scientific justification with supporting evidence and test data is needed to support read-across findings and explain why a minor difference in structure is also minor for differences in biological effects.

Knight introduced ECHA's read-across assessment framework (RAAF)[3], which is an internal tool for ECHA to evaluate read-across justifications made by registrants. Registrants can also use the RAAF to understand the aspects of read-across that ECHA considers to be crucial to their evaluation. The outcome of a RAAF assessment is the identification of the strengths and weaknesses of an approach and characterization of the degree of confidence of the assessor in the read-across. Considering the RAAF, Knight listed reasons for rejections of read-across in ECHA decisions, such as a lack of supporting information (source data unavailable, unsubstantiated hypothesis, systemic exposure profile unknown), scientific plausibility (metabolism data in conflict with hypothesis, toxicity profiles contradictory to claimed similarity, extrapolation vs. interpolation), or no explanation of substance identity (composition of source and target substance) (Ball et al., 2016).

Knight then reported on ECHA's topical scientific workshop of new approach methodologies (NAM) in regulatory science held in April 2016, which explored the regulatory applications of a better understanding of the biology behind how chemicals cause adverse effects to human health and new tools and techniques that produce a huge amount of data available from omics and high-throughput screening methods. Knight noted that this workshop drew inspiration from the EU research program SEURAT-1 and the US Tox21 initiative. Knight also stated that evidence from NAMs was helpful to support read-across cases of toxicodynamic aspects but there is often a major knowledge gap regarding toxicokinetics.

Knight also explained that ECHA recently published an extension of the original RAAF to address environmental properties, with a greater focus on ecotoxicological and environmental fate properties of chemicals[3]. Knight said that the next effort is to extend this further to assess read-across for multi-constituent substances and unknown or variable composition, complex reaction products and biological materials (UVCB substances)[4]. Knight noted that ECHA has recently published considerations on specific challenges related to assessing read-across of complex-composition substances.

Nicole Kleinstreuer – Improving read-across using biological data and quality assessment at the Interagency Center for the Evaluation of Alternative Toxicological Methods of the National Toxicology Program

Nicole Kleinstreuer, the deputy director of the Interagency Center for the Evaluation of Alternative Toxicological Methods of the National Toxicology Program (NICEATM), began the next talk on improving read-across using biological data and quality assessment by stating that it is important from a scientific standpoint to ultimately achieve regulatory acceptance in the US. The main goal of this effort, Kleinstreuer stated, is to incorporate newer biological data streams, such as high-throughput screening data, through the Tox21 consortium into read-across assessments.

Kleinstreuer first introduced "hybrid" read-across, which incorporates biological data from *in vitro* assays into read-across assessment. This is beneficial because often knowledge of chemical descriptors is not enough to understand the effect of a chemical on human health, and for this reason, biological descriptors, from cell-based and cell-free *in vitro* assays as well as small model organism assays, can be used to further characterize chemicals and inform risk assessment.

Kleinstreuer then provided an overview of the distance-based pairwise similarity metrics used in read-across and how these can be informed by biological data. She explained that the similarity metrics that should be used are dependent on what is being used to quantify and characterize chemical fingerprints. Kleinstreuer provided the example of the Tanimoto index, which is the most common metric used to identify pairwise similarity for read-across. She explained that the formula for this similarity function does not include a variable for the features that two chemicals mutually lack. Thus, if the goal of a read-across assessment was to identify whether a group of chemicals would not bind to a specific receptor, it would be necessary to use a different similarity function. This example, she noted, demonstrates that biological data can inform the choice of an appropriate similarity metric.

[3] https://echa.europa.eu/documents/10162/13628/raaf_en.pdf

[4] https://echa.europa.eu/documents/10162/13630/raaf_uvcb_report_en.pdf/3f79684d-07a5-e439-16c3-d2c8da96a316

Next, Kleinstreuer introduced the Integrative Chemical-Biological Read-Across (CBRA), which is an open source tool available for read-across that was developed by the Fourches Laboratory at North Carolina Chapel Hill. This program determines similarity via the Tanimoto coefficient and can infer the activity of a compound from both chemical and biological analogues to generate a plot that represents a chemical's neighborhood in both the chemical and biological space. Kleinstreuer also discussed chemical dataset curation work of the Fourches Laboratory, which, she noted, is very important for read-across. Kleinstreuer stated that duplicate analysis, experimental variability, source exclusion, activity cliffs, model-ability, consensus QSAR, and identity errors are all important considerations when curating consistent and reliable data for use in read-across.

Kleinstreuer also discussed a different type of similarity metric, machine learning algorithms, such as unsupervised random forest or self-organizing maps, that determine chemical similarity. She explained that the benefit of using such algorithms to determine chemical similarity is that they can consider the interdependency of the features and variables used to characterize chemicals and define chemical similarity within that broader context.

Kleinstreuer then gave a brief overview of the Tox21/ToxCast Federal Partnership and explained that the data generated with this high-throughput screening (8,000 chemicals in 60 assays in Tox21 and 3,000 chemicals in 800 assays in ToxCast) is a valuable resource to inform read-across. She also presented a case study on the use of biological data in read-across assessment, in which an unsupervised random forest was used to determine chemical similarity based on chemical structure as well as biological descriptors generated with the ToxCast dataset to develop chemical proximity matrices (Zhu et al., 2016). Kleinstreuer also gave a brief overview of a case study in which a read-across assessment informed by data from 16 ToxCast/Tox21 estrogen receptor (ER) pathway assays was performed using an unsupervised random forest similarity metric and the results were compared to a database of *in vivo* rodent uterotrophic bioactivity (Browne et al., 2015). When the read-across informed by the *in vitro* ER pathway dataset was compared to an uncurated *in vivo* dataset, the performance was relatively low, with a sensitivity and specificity of 70%, but when the *in vivo* dataset was curated to include only relevant endpoints, the read-across assessment had high sensitivity (95%) and specificity (98%). In a second case study, an unsupervised self-organizing map similarity metric was used to perform a read-across informed by a dataset of ToxCast chemicals screened in a primary human cell assay system called the biological multiplex activity profile (BioMAP) system. The read-across was used to identify clusters of chemicals that were enriched with chemicals, for which data already exists.

Kleinstreuer concluded that read-across can be significantly enhanced with data beyond chemical structure such as physicochemical properties and *in vitro* bioactivity information and that curation of this data informed by knowledge of biological pathways is key when correlating structural features and *in vitro* activity patterns with apical *in vivo* toxicity endpoints. She stated that the ToxCast and Tox21 data provides a wealth of biological information for thousands of chemicals and hundreds of molecular and cellular targets. Kleinstreuer also emphasized that there are many similarity metrics ranging from simple formulae to complex machine-learning algorithms that can be optimized to improve the predictive accuracy and applicability of read-across to increase its relevance and promote regulatory acceptance.

George Kass – Read-across for chemicals in food by the European Food Safety Authority (EFSA)

George Kass of the European Food Safety Authority (EFSA) presented on the topic of read-across for chemicals in food by EFSA. Kass first provided an overview of the activity of EFSA and explained that read-across has been used for risk assessment in the areas of plant protection, chemical contaminants, and food additives and packaging. He noted that read-across has been used most extensively in the analysis of flavoring substances, which include 2,637 substances in the EU that the consumer is exposed to in relatively low amounts. Kass explained that EFSA has two main functions regarding flavoring substances: evaluating currently marketed flavoring substances and assessing applications for the authorization of new flavorings. There are 34 chemical groups for flavoring substances, and these are determined based on structural similarities as well as common metabolic and biological behaviors.

Kass provided an example of a specific flavoring group, FGE.19, which is populated by the α,β-unsaturated carbonyls and their precursors and is predicted to be genotoxic by read-across. He explained that once such a prediction by read-across is made, the available genotoxicity and carcinogenicity data for the compound is examined, but if the evidence is negative or insufficient, the compound is evaluated based on a special procedure. This procedure consists of assigning the substances with common structural elements to compound classes (Cramer et al., 1978), comparison of intake amounts to the threshold of toxicological concern (TTC) for each class, and assessment of potential metabolites. Traditionally there were two outcomes of this procedure: 1) if the metabolite was determined innocuous and the intake is below the TTC, it was determined that there was no safety concern at the estimated level of intake, or 2) if the metabolite was considered potentially harmful and the intake was above the TTC, a toxicological assessment was conducted to determine the NOAEL and adequate margin of safety.

Kass then described a case study evaluation of calcium L-ascorbate, a nutrient source with threonate content of up to 2% (EFSA, 2011). He noted that read-across is not generally used to evaluate food additives and nutrient sources, because full dossiers with a full set of toxicological data for risk assessment is required for individual food additives, but this case was an exception. Initially, no safety concern was identified when calcium L-ascorbate was evaluated in 2007 by EFSA; however, in 2011, the manufacturer proposed an improved production process, which would decrease the threonate content to 1.2%, but create a new by-product, 4-hydroxy-5-methyl-3(2H)-furanone (4-HMF) at up to 0.06% w/w. There was not sufficient data to evaluate

4-HMF, however, so read-across was utilized to evaluate the compound. It was then determined that 4-HMF had been previously evaluated as a flavoring substance by EFSA, was known as "toffee furanone" and belonged within a group of five structurally related substances, the α,β-unsaturated 3(2)-furanones, which through QSAR were predicted to cause genotoxicity. The related compound, 4-hydroxy-2,5-dimethylfuran-3(2H)-one (HDMF), which had been evaluated by JECFA in 2004 in a 2-year carcinogenicity study, was considered as the representative compound for this group, and *in vitro* and *in vivo* testing conducted for this structurally similar chemical, HDMF, provided sufficient information to rule out genotoxic concern for 4-HMF.

Kass also described two case reports recently conducted by EFSA on the use of read-across in the evaluation of food contact materials. The first was 2-hydroxypropyl methacrylate oligomers, for which read-across was used to assess genotoxicity and *in vivo* toxicity. The second evaluation was conducted for ethylene glycol dipalmitate, and read-across was used to assess genotoxicity and *in vivo* oral and prenatal developmental toxicity.

Kass then introduced a recent guidance document released by EFSA on the establishment of the residue definition for dietary risk assessment (EFSA PPR Panel, 2016). To illustrate the relevance of read-across to dietary residue definition, he provided the example of spiroxamine, which is a systemic fungicide used to protect cereals and fruits. When considering a plant protective product, the metabolites formed by humans must be taken into consideration as well as the metabolites formed by the plant. In the case of spiroxamine, a total of 43 metabolites were considered, QSAR predicted positive genotoxicity for 20 metabolites in at least one model and negative genotoxicity for 6 metabolites in all models. All metabolites were then analyzed with read-across, and no new alerts were identified in read-across for 4 metabolites predicted as negative by all QSAR models and for 12 metabolites predicted as positive by QSAR models. Read-across did not eliminate the possibility of genotoxicity for 6 metabolites.

In summary, Kass stated that the use of read-across is limited to certain sectors of EFSA. There is no legislation for the use of read-across in food chemical risk assessment or formalized approach to read-across; however, EFSA has an opportunity to develop guidance documents where the use of read-across is supported in the sectors of flavoring substances and plant protection products and their metabolites. EFSA has ongoing projects to assess the wider applicability of read-across in the food sector.

Conclusion

Speakers at the satellite meeting "Regulatory Acceptance of Read-Across" described many motivations that are driving increased regulatory acceptance of read-across, including that many chemicals need to be tested to comply with current regulations but there is not enough time for traditional toxicity testing and that many new tools are being developed to conduct read-across in accordance with regulatory needs. It was reported that regulatory acceptance has been aided by relevant case studies which illustrate the accuracy of predictions using read-across for chemical safety assessment. It was also suggested that informing read-across assessments with bioactivity information generated in high-throughput *in vitro* assays could be used to increase the relevance of findings. Along with these suggestions, the development of guidance documents, validation of methods, and development of training opportunities were recommended as future goals to build confidence in this new approach and expand the use of read-across for regulatory toxicology.

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Conflict of interest

The authors declare the following competing financial interest(s): T. H. and T. L. consult Underwriters Laboratories (UL) on computational toxicology, especially read-across, and have a share of their respective sales. T. L. cofounded ToxTrack LLC, Baltimore, developing among others automated read-across tools.

Acknowledgements

This work was supported by the EU-ToxRisk project (An Integrated European "Flagship" Program Driving Mechanism-Based Toxicity Testing and Risk Assessment for the 21st Century) funded by the European Commission under the Horizon 2020 program (Grant Agreement No. 681002).

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