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21st Century Approaches for Evaluating Exposures, Biological Activity, and Risks of Complex Substances: Workshop highlights

Ursula G. Sauer[a,*], Robert A. Barter[b], Richard A. Becker[c], Emilio Benfenati[d], Elisabet Berggren[e], Bruno Hubesch[f,g], Heli M. Hollnagel[h], Kunifumi Inawaka[i], Athena M. Keene[j], Philipp Mayer[k], Kathleen Plotzke[l], Robert Skoglund[m], Océane Albert[f]

[a] Scientific Consultancy – Animal Welfare, Neubiberg, Germany
[b] ExxonMobil Biomedical Sciences, Inc., Annandale, NJ, USA
[c] American Chemistry Council, Washington DC, USA
[d] Istituto di Ricerche Farmacologiche Mario Negri IRCCS, Milan, Italy
[e] European Commission, Joint Research Centre, Ispra, Italy
[f] European Chemical Industry Council (Cefic), Brussels, Belgium
[g] Hubesch Consult BVBA, Sint-Pieters-Leeuw, Belgium
[h] Dow Europe GmbH, Horgen, Switzerland
[i] Japan Chemical Industry Association, Tokyo, Japan
[j] Afton Chemical Corporation, Richmond, VA, USA
[k] Technical University of Denmark, Kongens Lyngby, Denmark
[l] Dow, Midland, MI, USA
[m] Covestro LLC, Pittsburgh, PA, USA

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ABSTRACT

The June 2019 workshop *21st Century Approaches for Evaluating Exposures, Biological Activity, and Risks of Complex Substances,* co-organised by the International Council of Chemical Association's Long-Range Research Initiative and the European Commission's Joint Research Centre, is summarised. Focus was the need for improved approaches to evaluate the safety of complex substances. Approximately 10% and 20% of substances registered under the EU chemicals legislation are 'multi-constituent substances' and 'substances of unknown or variable compositions, complex reaction products and biological substances' (UVCBs), respectively, and UVCBs comprise approximately 25% of the U.S. Toxic Substances Control Act Inventory. Workshop participants were asked to consider how the full promise of new approach methodologies (NAMs) could be brought to bear to evaluate complex substances. Sessions focused on using NAMs for screening, biological profiling, and in complex risk evaluations; improving read-across approaches employing new data streams; and methods to evaluate exposure and dosimetry. The workshop concluded with facilitated discussions to explore actionable steps forward. Given the diversity of complex substances, no single 'correct' approach was seen as workable. The path forward should focus on 'learning by doing' by developing and openly sharing NAM-based fit-for-purpose case examples for evaluating biological activity, exposures and risks of complex substances.

1. Introduction

Complex substances include multi-constituent substances (MCSs) and substances of unknown or variable compositions, complex reaction products and biological substances (UVCBs) (ECHA, 2012a, b; USEPA, 2015). While MCSs are well-defined substances that possess more than one main constituent, UVCBs are comprised of a complex mixture of individual components that could not practically be formed by deliberate physical mixing. UVCBs may range in complexity from predictable alkyl chain variations (e.g. surfactants) to less predictable multi-constituent biologicals (e.g. fragrance oils), and the number of their components can range from only few to many thousand. Due to their complexity, UVCBs cannot be represented by unique structures or molecular formulas, but are described by a broader indication of the nature of their components. Each of the individual components of MCSs and UVCBs may possess different physicochemical and fate properties

* Corresponding author. Scientific Consultancy – Animal Welfare, Hallstattfeld 16, D-85579, Neubiberg, Germany.
E-mail address: ursula.sauer@sauerug.de (U.G. Sauer).

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Abbreviations

AOP	Adverse outcome pathway
Cefic	European Chemical Industry Council
ECHA	European Chemicals Agency
GenRA	Generalized Read-Across
IATA	Integrated approach for testing and assessment
ICCA	International Council of Chemical Associations
IVIVE	*In vitro – in vivo* extrapolation
JRC	Joint Research Centre
LRI	Long-range Research Initiative
MCS	Multi-constituent substance
MoA	Mode-of-action
NAM	New approach methodology
OLSA	Orthogonal Linear Separation Analysis
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
USEPA	United States Environmental Protection Agency
UVCBs	Substances of unknown or variable compositions, complex reaction products and biological substances

(Clark et al., 2013).

Approximately 10% and 20% of the substances registered under Regulation (EC) 1907/2006 on the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH; EP and Council, 2006) have been declared as MCSs and UVCBs, respectively (ECHA, 2017a). Similarly, approximately 25% of the substances listed on the United States Environmental Protection Agency (USEPA) Toxic Substances Control Act Inventory are flagged as UVCBs (USEPA, 2015, 2019). The inherent properties of UVCBs and MCSs present technical and scientific challenges when evaluating their biological activity, exposures and risks (Clark et al., 2013).

New approach methodologies (NAMs) include e.g. *in vitro* and *in chemico* methods, *in silico* tools and 'omics technologies (National Academies of Sciences, Engineering, and Medicine, 2017; National Research Council, 2007). USEPA defines NAMs as a "… broadly descriptive reference to any technology, methodology, approach (…), or combination thereof that can be used to provide information on chemical hazard and risk assessment that avoids the use of intact animals" (USEPA, 2018). Generally speaking, NAMs include methods to compute or infer chemical structure, physicochemical properties, which in turn inform evaluations of dosimetry, exposure, and environmental fate/persistence, methods to evaluate biological activity, including hazard inference methods, and innovative exposure assessment tools. The effective use of NAMs should include consideration of e.g. dosimetry and *in vitro - in vivo* extrapolation (IVIVE). NAMs can be incorporated into Integrated Approaches to Testing and Assessment (IATAs) that can be built using the Adverse Outcome Pathway (AOP) framework (USEPA, 2018). Many NAMs are now sufficiently far advanced to be applied for the risk assessment of chemicals and chemical products for priority setting, regulatory decision-making and product stewardship (Berggren et al., 2015, 2017). Nevertheless, the specific challenges related to UVCBs and MCSs still impair the use of NAMs for the exposure, hazard and risk assessment of complex substances.

Against this background, the International Council of Chemical Associations (ICCA) Long-Range Research Initiative (LRI) convened an international workshop *21st Century Approaches for Evaluating Exposures, Biological Activity, and Risks of Complex Substances* that took place on 19 and 20 June 2019 in Stresa, Italy. This workshop, that was co-organised with the European Commission, Joint Research Centre (JRC), brought together approx. 50 experts from academia, government, authorities, industry, and non-governmental organisations from Canada, Europe, Japan and the USA.

A brief summary of the workshop is presented below, and the full report is available online at https://lri.americanchemistry.com/ICCA-LRI-2019-WS-Full-Report-20191218.pdf.

2. Setting the stage: opportunities to use new technologies - perspectives from users and agencies

There is widespread recognition in the regulatory science communities, because of the number and variability of complex substances, it is not feasible, nor desirable from animal welfare, costs and efficiency perspectives, to employ traditional laboratory animal toxicity tests to characterise hazard profiles of complex substances. Therefore, it is not a question of whether NAMs will be applied, but instead how they will be applied and how confidence in the assessments will be established. A series of presentations served to frame the workshop, articulate the challenges, to stimulate participants to think and engage creatively to envision the opportunities of these new technologies, and to consider actions needed to surmount the barriers that must be overcome to actualise the use of NAMs for product stewardship and regulatory decision-making. In reality, combined exposure is normal and is always occurring, whether it is by contact with complex substances or by co-exposures to mono-constituents. The challenges, experiences and barriers in the use of NAMs to evaluate mono-constituent substances also apply to complex substances. While component-based approaches are be useful, they may be the exception rather than the rule for complex substances where information on identity and toxicology of components may be lacking. Hence, whole-mixture testing may be necessary for complex substances. A major challenge when assessing complex substances is the determination of the relevant chemical space, i.e. all possible (relevant) molecules and multi-dimensional conceptual spaces representing the structural diversity of these molecules (Awale et al., 2017) and understanding of relevant exposures. Problem formulation takes on a particular importance in the context of combined exposure to multiple chemicals because the demarcation of the problem is generally more complex than for single substances, and consideration should be given to exposure- and risk-based decision frameworks in the design of assessments. The opportunities in developing a new safety evaluation assessment paradigm based on exposure-driven assessments using NAMs, and the implications of failing to do so, need to be fully appreciated and communicated widely. Experiences with applying NAMs, incorporated into IATAs, need to be identified, and transparently discussed. Reservations in decision-making based on such approaches need to be identified and addressed e.g. by training for new skills and by implementing necessary adaptations of processes and tools.

3. Screening, biological profiling, and more complex risk evaluations

Determining the potential biological effects of complex substances can be difficult. The physicochemical properties of the constituents of complex substances influence the fate, transport and concentrations of each constituent at the sites of contact, and at the sites of biological interactions, in the environment and in test systems. Strategies to achieve defined, constant exposures in test systems include the avoidance or minimisation of losses by non-depletion testing using gas-tight glass vials instead of open wells or flasks, or passive dosing systems to yield steady-state freely dissolved concentrations during the test (Smith et al., 2010). A consistent evaluation of effective exposures in *in vitro* systems is key to improving a broader utility of the *in vitro* data.

High-throughput and high-content screening methods hold considerable promise for rapid, cost-effective profiling of biological activities of chemicals. While considerable progress has been made in applying these methods to individual chemicals, limitations such as solubility and volatility may impact use for profiling complex

substances. Virtually all substances, including extracts of fruits and vegetables, can produce bioactivity perturbations in *in vitro* assays, similar to those produced by known chemical toxicants (Wetmore et al., 2019). 'Omics technologies generate large complex data sets that can be used to probe wide swaths of biological response space. However, making sense of such data can be challenging. Both targeted and non-targeted approaches to 'omics data analyses have strengths and limitations, and evolving methods such as Orthogonal Linear Separation Analysis (OLSA; Mizuno et al., 2019) may prove useful in analysing datasets from complex substances. Complex substances can, and do, interact, and composite dose responses are likely caused by superimposed effects of their different constituents. NAM-based testing approaches need to focus on environmentally relevant exposures/concentrations and need to take these factors into consideration.

4. Improved methods for using read-across approaches on new data streams

In silico methods have proven to be practical for read-across inference determinations for a variety of substances (Toropov et al., 2018). Supervised clustering and unsupervised clustering methods can be used for characterising common properties of constituents of complex substances as well as uncommon properties of individual family members. While exposure to complex substances as a whole as well as to mixtures is the 'normal' real-world scenario, mixture assessment can be a complex task requiring sophisticated *in silico* tools. Challenges with respect to tool development relate to data availability, the identification of deviations from concentration addition, and the availability of information on differences of effects.

Efforts to systematise read-across are advancing (ECHA, 2016, 2017b), however they remain highly challenging for complex substances and mixtures. The Cat-App project (www.concawe.eu/cat-app) was set up to address these challenges by aiming to determine biological response and toxicogenomic profiles to facilitate chemical-biological grouping and read across. In addition, the Cat-App project is contributing to a tiered testing strategy for human health risk assessment of petroleum substances which aims at avoiding unnecessary animal testing under REACH. Ongoing research work also aims at introducing dose-response information into USEPA's Generalized Read-Across (GenRA) method to refine the scope of predictions beyond binary outcomes, transitioning from qualitative to quantitative predictions of effect levels or points of departure (Helman et al., 2019).

5. Exposure methods to evaluate fate, transport, and dosimetry

Prevailing difficulties and uncertainties in mixture assessment relate to incomplete information on their composition; the determination of external versus internal concentrations; temporal aspects of exposure to mixtures; emerging chemicals (that can be addressed by non-targeted analysis and modelling), and, finally, non-detects (that can be addressed by worst/best case assumptions). Overall, improved data sharing, the complementary use of monitoring data and modelling, establishing links between external and internal exposure and human biomonitoring (just as monitoring in wildlife) will serve to enhance mixture assessments.

21st century exposure science requires NAMs, including higher-throughput monitoring techniques. While the employment of targeted environmental exposure analysis for complex substances remains challenging, advances are being made in the use of non-targeted approaches. Experiments using non-targeted analysis tools for top-down exposomics (based upon biomonitoring) or bottom-up exposomics (based upon environmental sampling (Rappaport, 2011)) are shedding light on novel chemicals that might be relevant for exposure assessments. Advanced data processing tools for the assessment of complex mixtures are being developed, such as the web platform Mixture Touch that allows non-targeted data to be analysed and interpreted by non-expert users (http://www.mixture-platform.net/Mixture_Touch_open/). Integrating fugacity-based fate and transport models into the platform has potential to improve exposure and risk evaluations of complex substances in the environment. The selection of relevant constituents and fractions of the UVCBs can provide a sound basis as a starting point for the evaluation of fate and transport and predictions of degradation in key environmental compartments can be integrated into ecological risk evaluations.

The original composition of a complex substance can change when it is evaluated in test systems, or released into the environment. Since monitoring typically involves discrete substances, it is challenging and potentially ambiguous to link monitoring data to a specific complex substance. The representative chemical selection tool to assist in the conservative selection of representative chemicals for each subclass within a UVCB developed by Environment and Climate Change Canada ranks intra-class bioavailability, persistence and ecotoxicity of the UVCB constituents, and can be used to inform environmental monitoring efforts and weight-of-evidence ecological risk assessments (Fernandez et al., 2017).

6. Facilitated discussion: challenges and opportunities for implementing NAM-based approaches for assessing complex substances

NAMs can play a unique role in facilitating the exposure, hazard and risk assessment of complex substances and mixtures (for further details, see full workshop report available at https://lri.americanchemistry.com/ICCA-LRI-2019-WS-Full-Report-20191218.pdf). However, prevailing scientific and conceptual challenges need to be solved to enable their full exploitation. These challenges should be addressed in view of the ultimate goal to ensure appropriate risk management for complex substances and mixtures. This will require greater attention to refine and standardise approaches and procedures to: 1) enable fit-for-purpose identification of complex substances and mixtures (and of representative components); 2) ensure defined and constant exposure levels in experiments; 3) account for the potential interaction of compounds in mixtures with large numbers of constituents; and 4) to perform quantitative IVIVE.

The use of NAMs in implementing a tiered risk assessment framework for complex substances and mixtures necessitates research and a paradigm shift in the risk assessment approaches (see for example, Andersen et al., 2019). Specific recommendations for evolving risk assessment practices are presented in Table 1.

Such a paradigm shift in toxicity testing and safety evaluation has also been recommended following an international workshop convened among senior leaders from regulatory agencies including the USEPA, the European Chemicals Agency (ECHA) and Health Canada (Kavlock et al., 2018). Case studies were announced "to explore new ways of describing hazard (i.e., pathway perturbations as a measure of adversity) and new ways of describing risk (i.e., using NAMs to identify protective levels without necessarily being predictive of a specific hazard)" (Kavlock et al., 2018). Similarly, discussions at the ICCA LRI workshop emphasised that case studies should be initiated to put tiered risk assessment frameworks for complex substances and mixtures into practice. The selection of complex substances and mixtures for these case studies can be based upon, for example, exposure potential (related to workplace exposure, consumer exposure; contaminated sites, etc.). Similarly, case studies can be selected by their relevance for topical issues (e.g. circular economy, sustainability of the water cycle) or presumption of environmental or human health effects. To begin with, evaluations should focus on combinations of representative substances, reflecting real-world exposures as far as technically possible, and ultimately aim at investigating the exposome.

Table 1
Recommendations for progressing the use of NAMs in the risk evaluations of complex substances.

1. The necessary paradigm shift in the risk assessment approach for complex substances should place a focus on exposure-driven testing and assessment so that only such data are collected that are relevant for exposure, bioactivity, risk assessment and risk management.
2. The limitations of the traditional methods need to be clearly communicated to help stakeholders from all sectors understand the necessity of NAM-based approaches. Such limitations include high animal use and time expenditure for properly designed studies to assess combined exposures, shortcomings in addressing the spatial and temporal variance of real-world exposures, and the need to determine individual dose-responses for each toxicological endpoint.
3. The integration of NAMs into tiered risk assessment frameworks will allow avoiding unnecessary animal testing. These evaluation frameworks should make use of all types of NAMs, i.e. *in silico* modelling, *in vitro* assays, 'omics, grouping and read-across, exposure prediction modelling, etc.
4. NAMs should both be used to inform bioactivity potential and likely modes-of-action (MoAs) of the test substance.
5. IATAs built using quantitative AOP frameworks should be used, where relevant and applicable, as mechanistic understanding will be key to supporting such a paradigm shift.
6. *In vivo* studies should be restricted to the higher-tiers where only a limited number of substances would be candidates for evaluation (e.g. representative components from within a group or UVCB identified as bioactive in the lower tiers), and the types of *in vivo* studies should be selected to focus on the likely MoAs.

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Declaration of competing interest

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