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The challenge of the application of 'omics technologies in chemicals risk assessment: Background and outlook

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ABSTRACT

This survey by the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) highlights that 'omics technologies are generally not yet applied to meet standard information requirements during regulatory hazard assessment. While they are used within weight-of-evidence approaches to investigate substances' modes-of-action, consistent approaches for the generation, processing and interpretation of 'omics data are not applied. To date, no 'omics technology has been standardised or validated. Best practices for performing 'omics studies for regulatory purposes (e.g., microarrays for transcriptome profiling) remain to be established. Therefore, three frameworks for (i) establishing a Good-Laboratory Practice-like context for collecting, storing and curating 'omics data; (ii) 'omics data processing; and (iii) quantitative WoE approaches to interpret 'omics data have been developed, that are presented in this journal supplement. Application of the frameworks will enable between-study comparison of results, which will facilitate the regulatory applicability of 'omics data. The frameworks do not constitute prescriptive protocols precluding any other data analysis method, but provide a baseline for analysis that can be applied to all data allowing ready cross-comparison. Data analysis that does not follow the frameworks can be justified and the resulting data can be compared with the Framework-based common analysis output.

Abbreviations: AOP, Adverse outcome pathway; C&L, Classification and Labelling; CATTPTRA, Committee on Applications of Toxicogenomic Technologies to Predictive Toxicology and Risk Assessment; CEBS, Chemical Effects in Biological Systems database; CEFIC, European Chemical Industry Council; CLP, Classification, labelling, packaging of substances; DEG, Differentially expressed gene; DETECTIVE (project), Detection of endpoints and biomarkers of repeated dose toxicity using *in vitro* systems; DMEL, Derived minimum effect level; DNEL, Derived no effect level; EAGMST, Extended Advisory Group on Molecular Screening and Toxicogenomics; ECETOC, European Centre for the Ecotoxicology and Toxicology of Chemicals; ECHA, European Chemicals Agency; EURL ECVAM, European Union Reference Laboratory for Alternatives to Animal Testing; GHS, Globally harmonised system of classification and labelling of substances; GLP, Good laboratory practice; IATA, integrated approach for testing and assessment; IPCS, International Programme on Chemical Safety; ISATAB, Investigation/Study/Assay tab-delimited format; ITS, Integrated testing strategy; LOAEL / LOAEC, Lowest-observed adverse effect level / concentration; LRI, Long-range Research Initiative; MAD, Mutual acceptance of data; MAQC Consortium, MicroArray Quality Control Consortium; MGED Society, Microarray Gene Expression Society; MIAME, Minimum Information About Microarray Experiments; MIE, Molecular initiating event; MoA, Mode-of-action; NOAEL/C, No-observed adverse effect level / concentration; NRC, National Research Council; OECD, Organisation for the Economic Cooperation and Development; PHE, Public Health England; PoD, Point-of-departure; qRT-PCR, Quantitative real-time polymerase chain reactions; REACH, Registration, Evaluation, Authorisation, Restriction of Chemicals; TG, Test guideline; TG-GATES, Toxicogenomics Project-Genomics Assisted Toxicity Evaluation System; TRF, Transcriptomics reporting framework; WHO, World Health Organisation; WoE, Weight-of-evidence.

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1. Introduction

This survey summarizes legal, regulatory, scientific and technical challenges that have to be met to facilitate the regulatory use of 'omics technologies. Thereby, it serves as background information to the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) workshop report *Applying 'omics technologies in chemicals risk assessment* (Buesen et al., 2017); *the Framework for the quality assurance of 'omics technologies considering GLP requirements* (Kauffmann et al., 2017); *the generic Transcriptomics Reporting Framework (TRF) for 'Omics Data Processing and Analysis* (Gant et al., 2017); *and the Framework for the quantitative weight-of-evidence analysis of 'omics data for regulatory purposes* (Bridges et al., 2017) that are all combined in this journal Supplement.

The following parts of the introduction provide definitions for different 'omics technologies, generally describe how 'omics could be used for regulatory purposes, and discuss how the regulatory use of 'omics differs from their use within research projects that are unrelated to regulatory purposes. The introduction is followed by 4 Sections:

Section 2 presents the outcome of a written inquiry that was undertaken with chemical companies producing for the global market. These key players were invited to share their views on the regulatory use of 'omics technologies, to identify reasons why 'omics are not commonly used, or to share their experience in using them, as applicable.

Section 3 supplements the responses received during the written inquiry. Taking the example of *Regulation (EC) No 1907/2006 on the Registration, Evaluation, Authorisation, and Restriction of Chemicals* (REACH; EP and Council of the EU, 2006), it discusses the legal and regulatory framework that is relevant for the regulatory use of 'omics technologies.

Section 4 explores specific scientific and technical aspects that may stand in the way to the use of 'omics data for human health hazard assessment of substances. This appraisal is focused on microarray technologies used for transcriptome profiling. Other 'omics technologies (e.g. quantitative real-time polymerase chain reactions (qRT-PCR), RNA-sequencing), proteomics and metabolomics are not considered. Also, the use of 'omics technologies for ecotoxicological assessments is excluded.

Section 5 discusses the outcome of the survey and aims at designing a roadmap to facilitate the regulatory acceptance and use of 'omics technologies.

1.1. What are 'omics?

The term 'omics as used in this survey refers to the study of systemic genome responses to substances in cellular systems or whole organisms. For convenience, the major different 'omics technologies currently available can be viewed as follows (OECD, 2005, 2009; CATTPTRA–NRC, 2007):

- **Genomics**: The study of the structure and function of the genome (toxicogenomics in the context of toxicology);
- **Transcriptomics**: The study of genomic-scale changes in RNA expression (e.g. messenger RNA and noncoding RNA (Aigner et al., 2016));
- **Proteomics**: The study of cell- and tissue-wide protein expression;
- **Metabolomics**: The study of cell- and tissue-wide metabolite profiling;
- **Epigenomics**: The study of reversible heritable changes in gene function that occur without a change in the sequence of nuclear DNA (e.g. DNA methylation and histone modifications).

'Omics data first started appearing in the late 1980s with the development of spectroscopy techniques, such as nuclear magnetic resonance. However, the term 'omics really came into use in the late 1990s with the invention of microarrays for transcriptome profiling. It was then applied to the further development of mass spectrometry and nuclear magnetic resonance that made possible proteomics and metabolomics in addition to transcriptomics. High throughput transcriptome sequencing has also been used in 'omics studies (Gant et al., 2009; Rouquié et al., 2015; Xu et al., 2016).

'Omics (and epigenomics) technologies all encompass the collection of large data sets. Analysis of these high volume data requires bioinformatic methods (whereas interpretation of data from 'omics studies often requires input from conventional biology, pathophysiology, and toxicology). Therefore, 'omics technologies have advanced conjointly with the science of bioinformatics that incorporates the established principles of statistical data interpretation specifically for application to 'omics data sets. The advances in bioinformatics that developed in parallel to 'omics technologies allowed more measurements to be stored and processed.

In the last decades, 'omics technologies have been applied extensively in research (Raja et al., 2017). 'Omics technologies have the capability of providing a profound insight into the biochemistry and physiology of the cell and any perturbing effects of xenobiotics. This has led to an enthusiastic adoption by research toxicologists. Hopes were expressed that 'omics technologies would provide the tools to identify an array of biomarkers of adverse effects and modes-of-action (MoAs) of toxicity to improve the prediction of human effects during substance hazard assessment and that they would contribute to the development of alternative methods to animal testing (Storck et al., 2002; OECD, 2005, 2009; CTTAEA-NRC, 2007; Gant, 2007; Gant et al., 2009; Phillips et al., 2009; Goodsaid et al., 2010; Buick et al., 2015; Li et al., 2015; Williams et al., 2015). Despite this, the translation of 'omics into the regulatory domain remains at best cautious (Tralau et al., 2015).

1.2. What are 'omics applications?

In toxicological research, 'omics methodologies have been applied as a means to evaluate if substances induce whole genome alterations that could ultimately lead to or be assessed with the development of adverse effects and to identify the MoAs of potentially toxic substances by reference to established adverse outcome pathways (AOPs; see below).

When testing for substance-induced effects (hazard), 'omics data can be used for class comparisons, predictions or discovery. Taking the example of microarray experiments for transcriptome profiling, class comparisons address the question, which genes best distinguish data classes (e.g. the control group and the test group). Class predictions use the pattern of gene expression induced by the test substance to predict the MoA and its effects (Box 1). In a similar manner, the gene expression pattern can be used for comparison with other data and using unsupervised clustering methods to make new predictions about the MoA of the chemical.

1.3. What are MoAs and AOPs and how do 'omics contribute to their understanding?

MoAs describe the biologically plausible sequence of chemical-

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specific key events, starting with exposure and proceeding through the interaction of the substance or its metabolites with a cell, through functional and anatomical changes leading to an observed effect supported by robust experimental observations and mechanistic data. The World Health Organisation (WHO) International Programme on Chemical Safety (IPCS) Harmonization Project is working on MoAs, and it has provided a generic approach to the principles commonly used for evaluating cancer and non-cancer MoAs (Sonich-Mullin et al., 2001; Boobis et al., 2006, 2008, 2009; Meek et al., 2014).

AOPs relate to a linear sequence of events from the interaction of substances with cellular molecules through to an understanding of the adverse effect at the individual level (for human health) or population level (for ecotoxicological endpoints). The key events in an AOP, beginning with the molecular initiating event (MIE), should be definable and make sense from a physiological and biochemical perspective (Ankley et al., 2010; OECD, 2012a, 2013; Meek et al., 2014). In comparison to MoAs, AOPs are endpoint oriented, not substance-specific, and therefore do not include metabolism considerations (ECETOC, 2017). AOPs are the central element of a toxicological knowledge framework being built to support substance hazard and risk assessment based on mechanistic reasoning, e.g. under the Organisation for the Economic Cooperation and Development (OECD) AOP programme (OECD, 2013).

Box 1
Categorisation of microarray experiments for transcriptome profiling (adapted from: CATTPTRA–NRC, 2007)

Class comparison: *Which genes best distinguish the two classes in the data?* Compare gene expression profiles of different phenotypic groups (such as treated and control groups) to discover genes and gene expression patterns that best distinguish the groups.

Class prediction: *Can a particular pattern of gene expression be combined with a mathematical rule to predict the effects of a new compound?* Class prediction experiments attempt to predict biologic effects based on the gene expression profile associated with exposure to a compound. The goal is not merely to separate the samples but to create rules (or algorithms) that predict phenotypic outcomes for new compounds based solely on gene expression profiling data. Each algorithm uses an original set of samples (or training set) to develop a rule that uses the gene expression data (trimmed to a previously identified set of informative genes) for a new compound. Thereby, this new compound is placed into the context of the original sample set, and its hazard class is identified.

Class discovery: *Are there unexpected, but biologically interesting, patterns that exist in the data?* In animals exposed to a range of compounds, analysis of gene expression profiles with unsupervised clustering methods can be used to discover groups of genes that may be involved in cellular responses and suggest hypotheses about the MoA of the compounds.

Mechanistic studies: Moving from class prediction to mechanistic understanding often relies on additional work to translate toxicogenomics-based hypotheses to validated findings. Bioinformatic tools play a key role in developing such hypotheses by integrating information that can facilitate interpretation.

The application of 'omics methodologies to the study of substance-specific MoAs rests on the premise that any chemical injury is mediated by, or reflected in, changes at the RNA, protein, or metabolite level. Under defined conditions of cellular location, time, and biological context, these changes can provide meaningful information about biological responses to a toxicological insult (CATTPTRA–NRC, 2007). Elucidating earlier steps of a MoA or AOP may enhance the earlier detection of apical effects (OECD, 2013; Rouquié et al., 2015). For this to be possible though, it is essential to link the MIE to phenotypic alterations (in whole organisms).

Changes at the molecular level do not necessarily result in the development of adverse effects. They may also reflect inherent biological variability or compensatory, reversible changes that will not result in apical effects. The discrimination of these effects requires biological understanding and interpretation. This caveat aside though, the identification of compensatory changes is useful for hazard assessment. Even though they may not be related to toxicity in the short-term (and may even reflect long-term protection of the organism), they may in some cases eventually give rise to adverse effects (OECD, 2005).

1.4. How could 'omics potentially be used for regulatory submissions, e.g., under the REACH regulation?

The regulatory use of any test method is closely linked to the specific legal framework that is relevant for the substance under investigation. Taking the example of the REACH Regulation (EP and Council of the EU, 2006), this survey summarizes how 'omics-based data could potentially be used for regulatory submissions. As presented in further detail in Section 2 - *Legal and regulatory prerequisites to use 'omics in REACH dossiers*, the REACH Regulation lists production volume-specific standard information requirements that cover specific physico-chemical, toxicological and ecotoxicological endpoints. These data are evaluated during hazard and risk assessment, and they are analysed to determine risk management options accordingly. Generally, the application and integration of 'omics technologies may be useful in different layers of regulatory hazard identification and assessment (Box 2) contributing to:

(i) Classification and labelling (C&L) of substances, for example as part of a tiered testing strategy.
(ii) Weight-of-evidence (WoE) approaches to elucidate the MoA of the substance under investigation.
(iii) Substantiation of chemical similarity for read-across (ECHA, 2015; van Ravenzwaay et al., 2016).
(iv) Determination of points-of-departure (PoDs) for hazard assessment.
(v) Demonstration of species-specific effects and human health relevance (or absence thereof).

1.5. Are 'omics already being used under the REACH regulation?

In accordance with Article 75(1) of the REACH Regulation, the European Chemicals Agency (ECHA) is in charge of *managing and in some cases carrying out the technical, scientific and administrative aspects of this Regulation and to ensure consistency at Community level in relation to these aspects* (EP and Council of the EU, 2006). ECHA helps companies to comply with the legislation, advances the safe use of chemicals, provides information on chemicals and addresses chemicals of concern. With respect to the application of 'omics technologies for the hazard and risk assessment of substances, ECHA stated in its progress report 2015 (published in 2016), that *experience has shown that different advanced techniques such as new approach methodologies* [that include 'omics

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Box 2
Hazard classification and derivation of derived no effect levels and derived minimum effect levels (DNELs / DMELs) under REACH

The classification of a substance serves to properly identify and communicate any hazardous properties. The provisions for the harmonised classification of substances are laid down in *Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures* (CLP; EP and Council of the EU, 2008) that has implemented most of the *United Nation's Globally Harmonised System of Classification and Labelling of Chemicals* (GHS; United Nations, 2015). For each toxicological endpoint, specific hazard categories are defined.

The derivation of levels of substance exposure above which humans should not be exposed implies determining the DNEL for effects that are elicited by a threshold MoA. For substances that elicit effects by a non-threshold MoA, DMELs shall be established. Rules for the derivation of DNELs or DMELs, as applicable, are laid down in *the ECHA Guidance on information requirements and chemical safety assessment: Chapter R.8 Characterisation of dose [concentration]-response for human health* (ECHA, 2012).

Typically, DNELs are derived from a PoD. PoDs may be, e.g., benchmark doses (the dose causing a predetermined change in response), no-observed adverse effect levels/concentrations (NOAELs/Cs) or lowest-observed adverse effect levels/concentrations (LOAELs/Cs). Specific assessment factors are applied to the PoD to account for, e.g., the uncertainty arising from the variability in the experimental data and from intra- and inter-species variation, the nature and severity of effects, and the sensitivity of a given human (sub-)population (ECHA, 2012).

methodologies] *are not used in many registration dossiers … This lack of use may be an indication that industry does not consider these NAMs to be sufficiently developed* (ECHA, 2016).

This statement from the ECHA highlights that, even after several decades of research, 'omics methodologies are not generally used to fulfil information requirements under the REACH Regulation. The ECHA progress report does not however provide any explanations for why 'omics (or other new) methodologies are not yet considered 'sufficiently developed'. Therefore, it has to be questioned why 'omics science has failed to be translated from research to regulatory use. Which challenges have to be overcome to make the use of 'omics data as acceptable for regulatory submissions (e.g. under REACH) as it is in support of research projects? In addressing these questions, it has to be determined how the use of 'omics for research purposes differs from their application for regulatory purposes.

1.6. How does the use of 'omics for research purposes differ from regulatory use?

The regulatory applicability of 'omics technologies implies standardisation and validation efforts (i.e. formal validation for regulatory purposes) that are not met by criteria applied for 'omics studies that are conducted for research purposes. Further, new test methods (or updates of existing test methods to include, e.g., 'omics technologies) may only be used for regulatory purposes if they have been accepted for regulatory use, and the procedure of regulatory acceptance can be complex and lengthy (Sauer et al., 2016). To further elucidate why 'omics technologies have failed to make a complete translation from research to regulatory use, it is necessary to compare and contrast the ways in which they are applied in research and regulatory settings. Though both settings involve the generation of new data, the way in which that data is used is different, and it is this differential use that indicates the spectrum of challenges to the translation from research to regulatory use.

In the research paradigm, the generation of a hypothesis, followed by statistical testing that results in the rejection or acceptance of the hypothesis lead to novel findings, that in turn provide the incentive for the generation of further hypotheses. Research findings are published in open source literature upon successful completion of a peer review process. Ideally, these findings are then re-tested by others and complemented by data from other sources and derived using other methods. This adds a layer of robustness to the applicability of new methodologies while allowing for individual approaches to the derivation and interpretation of data. Further, and importantly, research findings are not used to justify the application of products to which human exposure (either of workers or the public) or environmental exposure can occur. As a result, research work inherently has more room for trial and error.

By contrast, regulatory requirements are legally binding, and the specific studies that have to be conducted to meet regulatory requirements are laid down in, e.g., substance-specific legislation. Regulatory studies have to be reproducible and interpretable as their results will be used to support, e.g., the introduction of a new product on the market, to which workers or the public can be exposed. For these reasons, procedures used to generate data for regulatory purposes need to be standardised and validated to show their reproducibility and reliability (robustness and predictability for the desired endpoint), and they should be applied in a standardised and controlled manner. Therefore, the current challenge for 'omics data is to overcome the validation barrier. However, as long as there is no consensus within the scientific community on the need to determine and apply best practices in the generation, storing, curating, processing and interpretation of 'omics data, the possibilities of taking 'omics methodologies forward to validation or even regulatory acceptance and use are slight.

In addition, generally, a regulatory test method is designed to assess one specific toxicological endpoint (e.g. genotoxicity). 'Omics methodologies, though, are global by their nature, and provide data that can potentially be relevant for a large number of endpoints. It has to be determined whether a given 'omics-based method shall be applied either for the specific evaluation of one particular endpoint or for an overarching purpose where the final regulatory use of the data depends on the specific outcome of their interpretation. The latter application appears especially attractive because it allows using 'omics methods more widely in the replacement or supplementation of other methods. Notwithstanding, since the current approach to validate new methodologies involves comparing the data collected with the new method against some form of 'gold standard', such an overarching use may provide special challenges to method validation.

2. Written inquiry on the regulatory use of 'omics

In the summer of 2016, a written inquiry was conducted with 11 ECETOC member chemical companies producing products for the global market. The recipients were invited to share their experience in using 'omics data in regulatory dossiers and regulatory submissions or to specify reasons for not using 'omics data for regulatory purposes. Specifically, the following questions were asked:

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- Has your company already included 'omics data in any regulatory dossiers or submissions?
- If so, can you share details on the specific type of 'omics data used; the technology applied to generate and store data and to analyse and interpret data; the toxicological endpoint addressed; how the 'omics data contributed to hazard categorisation, the determination of no-observed adverse effect levels/concentrations (NOAELs/Cs), etc.; and the acceptance of such data by the responsible authority or authorities.
- If you have not yet included 'omics data in regulatory dossiers, would you be in a position to share with us further details on the reasons why you refrained from doing so and specific prerequisites that you believe should be met to foster applicability of 'omics technologies for chemicals risk assessment.

By mid-September 2016, experts from 5 chemical companies had responded. Table 1 provides an overview of the responses received (for the complete (anonymised) responses, cf. www.ECETOC.org).

The individual companies' experiences were diverging largely reflecting the respective companies' product portfolios. Generally, 'omics technologies are not used to meet REACH standard information requirements. The respondents identified a lack of standardisation and validation of these technologies as major reasons for not using 'omics technologies in REACH dossiers. 'Omics are, however, used in-house to support decision-making and to identify MoAs. In the sector of crop protection products, they have also been used in regulatory submissions.

3. Legal and regulatory prerequisites to use 'omics in REACH dossiers

Since 'omics technologies are not yet generally being used in REACH dossiers, this section further elucidates which legal and regulatory prerequisites 'omics technologies have to meet in order to become applicable under REACH.

3.1. REACH standard information requirements

The REACH Regulation prescribes that all substances produced or imported in annual quantities of 1 tonne or more have to be registered. In the REACH Regulation, the cumulative Annexes VII, VIII, IX, and X list standard information requirements covering specific physico-chemical, toxicological and ecotoxicological endpoints that should be fulfilled when submitting registration dossiers for substances produced or imported in quantities of 1, 10, 100, or 1000 tonnes or more, respectively. Taken together, the standard information requirements in Annexes VII-X list the following human health endpoints: Skin irritation or skin corrosion, eye irritation, skin sensitisation, mutagenicity, acute toxicity, repeated-dose toxicity, reproductive toxicity, and carcinogenicity.

For a new method (or methodology) to become applicable to meet the standard information requirements under the REACH Regulation, it should provide information that can be used for hazard classification, and/or it should allow determination of a PoD (cf. Box 2) for one of the toxicological endpoints that are mentioned in the REACH Regulation.

Further, and importantly, the method should be adopted for use under the REACH Regulation. Generally, test methods may be used to fulfil the REACH standard information requirements if they are listed in the Annex of *Regulation (EC) No 440/2008 laying down test methods pursuant to REACH* (Test Methods Regulation; Council of the EU, 2008) and the corresponding REACH Annexes have been amended accordingly. As a rule, the Test Methods Regulation lists test methods that have been adopted as OECD Test Guidelines (TG). Accordingly, all activities related to new test methods taking place on the level of the OECD are directly relevant for the REACH system.

3.2. Consideration of 'omics in OECD programmes

To date, there is no OECD TG covering one of the human health endpoints mentioned in the REACH standard information requirements in which any type of 'omics technology constitutes the primary endpoint detection method. However, 'omics-based parameters might also be introduced as additional, new parameter(s)

Table 1
Summary of responses from the written inquiry: Chemical companies' experiences with the regulatory use of 'omics technologies.

Respondent	Regulatory use of 'omics?	If yes: For which purpose? If no: Why not?	Regulatory acceptance of 'omics data?	Technical comments
A	Yes, transcriptomics.	MoA categorisation for hepatocarcinogenesis; classification based on data similarity by the hierarchical clustering method.	Under consideration.	Procedure conducted in accordance with Affymetrix or Agilent protocols.
B	No.	Results not sufficiently reliable to ensure worker protection; 'omics data do not satisfy REACH provisions.		'Omics technologies must deliver reliable endpoint-relevant results, which can be used for the derivation of Derived No Effect Levels or Predicted No Effect Concentrations and for C&L.
C	Yes, but not in REACH dossiers.	'Omics were applied to support occupational exposure limits.		Data generation is not controlled; the outcome is too experimental condition-specific to be meaningful; it is difficult to correlate 'omics data to actual adverse effects and to determine human relevance.
D	Yes, Next Generation Sequencing, transcriptomics.	For internal decision making and as supporting data for all crop protection products. A few situations where full 'omics data were used for regulatory submission – to elucidate very complex MoAs.	Differently: (1) To help support a MoA and determine relevance, or lack thereof, to humans; (2) To derive NOAEL; (3) One case: 2-year cancer bioassay waived based upon MoA and gene expression data.	
E	Yes, but not in REACH dossiers	Pesticide registrations. For important commodity chemicals, research studies using 'omics approaches have been applied for product stewardship and for establishing MoAs.		

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into an already existing OECD TG. Also in this regard, none of the currently available OECD TG specifying *in vivo* test methods for any of the human health endpoints covered by the REACH Regulation make any form of reference to the term "omics".

For the assessment of endocrine disrupting properties (that are not discrete standard information requirements under REACH, but that are assessed in the course of repeated-dose and reproductive toxicity studies), 'omics technologies are mentioned in OECD Guidance Document No. 150 on standardised test guidelines for evaluating chemicals for endocrine disruption: *Use of other technologies (for example gene expression analysis or "omics" data) may help in understanding the link between endocrine-related mechanisms and apical effects in a WoE approach* (OECD, 2012b). Clearly, this statement is merely suggestive and does not provide guidance on how to perform 'omics studies or how to use the outcomes of such studies for hazard and risk assessment. Further and importantly, other than OECD TGs, OECD guidance documents are not covered by the OECD Council Decision on the Mutual Acceptance of Data (MAD; available at: http://www.oecd.org/env/ehs/mutualacceptanceofdatamad.htm). Therefore, consideration of 'omics technologies in an OECD guidance document does not have the same standing as their (theoretical) inclusion in OECD TGs.

To foster the development and use of 'omics technologies, in 2012, the OECD launched its AOP programme. This AOP programme is conducted under the umbrella of the *Extended Advisory Group on Molecular Screening and Toxicogenomics* (EAGMST; http://www.oecd.org/chemicalsafety/testing/adverse-outcome-pathways-molecular-screening-and-toxicogenomics.htm) that is co-chaired by the European Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) and the US Environmental Protection Agency (Zuang et al., 2015). The activities of the OECD AOP programme are coordinated with the WHO/IPCS Harmonization Project that has developed a framework on MoA/species concordance analysis (Sonich-Mullin et al., 2001; Boobis et al., 2006, 2008, 2009; Meek et al., 2014).

To the best of the authors' knowledge, the EAGMST has not to date been engaged in providing guidance on the standardisation and validation of 'omics technologies. (In the written inquiry, chemical companies identified standardisation and validation as key elements to facilitate the regulatory use of 'omics.) For instance, the summary report of the 7th meeting of the EAGMST (OECD, 2014) almost exclusively focuses on work conducted to develop specific AOPs. Under 'other relevant activities', reference is made to two 'omics-related activities of which EAGMST was made aware, but are not activities of EAGMST *per se*. These activities address technical software requirements that are not the focus of the present survey (Box 3).

In December 2015, Public Health England (PHE) submitted a Standard Project Submission Form (as specified in the OECD TG Programme) to the OECD secretariat describing *appropriate application of univariate analysis methods to high throughput data, particularly 'omic data*. The underlying analysis concepts had been developed as part of ECETOC Expert Team activities in providing guidance on analysis of microarray data from the European Chemical Industry Council (CEFIC) Long-range Research Initiative (LRI) Project EMSG56 *Combined Low-dose Exposures to Anti-androgenic Substances*.

At its 23–24 June 2016 meeting, the EAGMST confirmed its support of the work described in the Standard Project Submission Form and proposed scoping a series of initiatives to facilitate the use and application of 'omics technologies in regulatory decision making. It is planned to closely link this work of the EAGMST with the ECETOC work on *the Framework for the quality assurance of 'omics technologies considering GLP requirements* (Kauffmann et al., 2017); *the generic Transcriptomics Reporting Framework (TRF) for 'Omics Data Processing and Analysis* (Gant et al., 2017); *and the Framework for the quantitative weight-of-evidence analysis of 'omics data for regulatory purposes* (Bridges et al., 2017) that are all combined in this journal Supplement.

3.3. REACH provisions on WoE approaches

Test methods that have not yet been adopted as OECD TG or taken up into the Test Methods Regulation may nevertheless be used to meet REACH information requirements, i.e. in WoE approaches. In WoE approaches, an overall appraisal of all data that are available at a given point in time are used, e.g. in the form of integrated approaches for testing and assessment (IATAs), to come to a conclusion that a specific substance may or may not have a hazardous property. Importantly, WoE approaches may also be used to improve the understanding of the specific mechanisms of toxicity by which a substance elicits toxicity.

In the area of chemical hazard and risk assessment the phrase 'weight of evidence' is used increasingly, but without a clear understanding or definition for this term. Therefore, different documents may imply different procedures when referring to WoE. The

Box 3
'Omics-related activities mentioned in the EAGMST report (OECD, 2014)

Under 'other relevant activities' of the EAGMST meeting report (OECD, 2014), reference is made to two 'omics-related activities: *Jun Kanno (Japan) presented the utility of percellome toxicogenomics in repeated dose Sick House Syndrome-level inhalation toxicity. [...] Steve Edwards (US) presented activities of the Toxicogenomics Interoperatability Interest Group at Research Data Alliance project. The group focus is on alleviating the barriers of data availability and interoperability. The exchange [of] TG-GATES and DrugMatrix datasets between diXa and CEBS using ISATAB was chosen as a case study for illustrating the problem and potential solution.*; Abbreviations in this quote:

DrugMatrix® is a large molecular toxicology reference database located at the US Department of Health and Human Services National Toxicology Program.

diXa stands for a data infrastructure for chemical safety assessment that was developed in the course of an EU 7th Research Framework Programme-funded project (Hendrickx et al., 2015).

CEBS stands for the Chemical Effects in Biological Systems database of the US National Institute of Environmental Health Sciences (http://www.niehs.nih.gov/research/resources/databases/cebs/).

ISATAB stands for the Investigation/Study/Assay (ISA) tab-delimited (TAB) format, a general purpose framework with which to collect and communicate complex metadata (i.e. sample characteristics, technologies used, type of measurements made) from 'omics-based' experiments employing a combination of technologies (http://www.dcc.ac.uk/resources/metadata-standards/isa-tab).

TG-GATES (Toxicogenomics Project-Genomics Assisted Toxicity Evaluation System) is a large-scale toxicogenomics database developed by the Japanese Toxicogenomics Project consortium (Igarashi et al., 2015).

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Framework for the quantitative weight-of-evidence analysis of 'omics data for regulatory purposes (Bridges et al., 2017), presented in this journal Supplement, intends to provide guidance on how to define and standardise WoE approaches.

Section 1.2 of Annex XI of the REACH Regulation lays down the provisions for applying WoE approaches: *There may be sufficient WoE from several independent sources of information leading to the assumption/conclusion that a substance has or has not a particular dangerous property, while the information from each single source alone is regarded insufficient to* support *this notion. There may be sufficient WoE from the use of newly developed test methods … Where sufficient WoE for the presence or absence of a particular dangerous property is available, further testing on vertebrate animals for that property shall be omitted.*

The ECHA expects that 'omics technologies will mostly be applied for the grouping of substances and in integrated testing strategies (ITSs) and IATAs: *The 'omics' technologies are expected to provide a tool for optimising the grouping, and new high-throughput assays could indicate further similarities than those indicated by chemical structure. […] Basic elements of an ITS/IATA are, for example, physicochemical data, in vitro data, human data, animal data, computational methods, "omics" data, mechanisms/modes of action (MoA) and biokinetic models* (ECHA, 2014).

While Annex XI of the REACH Regulation, in principle, allows using methods that have not been adopted into the Test Methods Regulation to fulfil the REACH information requirements, data submitted within WoE approaches do not have the same standing as data that were collected using test methods that are listed in the Test Methods Regulation. Generally, it is the task of the dossier submitter to ensure data sufficiency, and regulatory authorities will check if the dossier fulfils the regulatory requirements. In the case of accepted and established test methods this can be done routinely. By contrast, they are required to assess the sufficiency of WoE approaches in the course of compliance checks of registrations with respect to adaptations to the standard testing regime, and they may request further testing, if considered necessary (Sauer et al., 2016). The *Framework for the quantitative weight-of-evidence analysis of 'omics data for regulatory purposes* (Bridges et al., 2017), presented in this journal Supplement, aims at objectifying estimations of the 'sufficiency' of WoE approaches.

4. The need to standardise and validate 'omics

4.1. State-of-the-art standardisation and validation of 'omics

As the preceding sections demonstrate, the standardisation and validation of 'omics technologies are fundamental prerequisites to facilitate their regulatory applicability and use. For any test method to be considered for adoption as an OECD TG and hence also for inclusion in the REACH system, its development should be sufficiently advanced that it contains a standardised test protocol, and it should have successfully passed a formal validation study in accordance with standardised criteria (OECD, 2005; Corvi et al., 2006; CATTPTRA–NRC, 2007; cf. Box 4).

Potentially, test methods using 'omics technologies could replace, reduce or refine animal testing, and, the EURL ECVAM has published a framework on the validation of transcriptomics-based *in vitro* methods (Corvi et al., 2016). Scientists in the EU that have developed (*in vivo* or *in vitro*) test methods using 'omics technologies should submit them to the EURL ECVAM. The EURL ECVAM conducts an initial appraisal of the submitted method, with possibly one or more rounds of re-inquiry with the method developer. If the EURL ECVAM considers the new method suitable for validation, the formal pre-validation and validation procedure is initiated. Finally, the EURL ECVAM forwards the outcomes of the validation study to the ECVAM Scientific Advisory Committee for peer review and a formal statement on the scientific validity of the method.

Applying this procedural framework, the EURL ECVAM evaluated a number of test method submissions for transcriptomics-based *in vitro* assays, e.g. the Genomic Allergen Detection test method (Johansson et al., 2011, 2013), a transcriptomics-based *in vitro assay* proposed to discriminate between skin sensitising and non-sensitising chemicals (Zuang et al., 2015). The transcriptomics-based *in vitro* assay SENS-IS has been submitted to the EURL ECVAM, and it is currently under evaluation (Zuang et al., 2016). Further, the EURL ECVAM has been involved in the CarcinoGENOMICS project where it coordinated an inter-laboratory study to investigate the reproducibility of 'omics-based *in vitro* methods for assessing carcinogenic potential (Herwig et al., 2016). Similarly, the EURL ECVAM has been engaged in the DETECTIVE project (Detection of endpoints and biomarkers of repeated dose toxicity using *in vitro* systems). In the course of this project, human liver, kidney and heart cell models were exposed to test substances, and the effects were assessed at the 'omics and the functional levels. The DETECTIVE project also explored whether repeated dose effects on epigenetics and noncoding microRNA expression served to expand the understanding of MoAs (Zuang et al., 2015).

4.2. Scientific and technical aspects to consider in standardising 'omics: the example of microarrays used for transcriptome profiling

The three *Frameworks* (Bridges et al., 2017; Gant et al., 2017; Kauffmann et al., 2017) that are conjoined in this journal

Box 4
Recommendations for the validation of 'omics technologies (CATTPTRA–NRC, 2007)

> The utility of toxicogenomic technologies ultimately depends on how reliable, reproducible, and generalizable the results are from a particular study or individual method of analysis. Moving beyond laboratory assays to more widespread use requires some level of validation, which can be defined as the process of ensuring that a test reliably measures and reports the determined end point(s) and encompasses both technical and platform qualification in addition to biologic qualification. […] Validation must be carried out at various levels.
>
> First, technology platforms must be shown to provide consistent, reliable results, which includes assessment of device stability and determination of analytical sensitivity and assay limits of detection, interference, and precision (reproducibility and repeatability).
>
> Second, the software used to collect and analyse data for an application must provide valid results.
>
> Third, the application, consisting of both hardware and software, must be tested and validated in the context of the biologic system to which it will be applied. Fourth, the application, or a related application based on the original, must be shown to be generalizable to a broader population or to be highly specific for a smaller, target population.
>
> Finally, one must consider how these technologies and applications based on them can be validated for regulatory use.

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Supplement are conceived as a first step towards the standardisation of 'omics studies in a regulatory context. To further explore the rationale for developing these frameworks, this part of Section 3, taking the example of transcriptomics microarray platform technologies, presents specific scientific and technical aspects to consider in the standardisation of 'omics technologies.

In microarray platforms, a solid matrix surface supports thousands of different, surface-bound DNA, which are hybridised against a RNA-containing test sample to measure gene expression. Microarray platform technologies can be used to generate large amounts of data at moderate cost, but their use is limited to the evaluation of those genes that are included in the given microarray (CATTPTRA–NRC, 2007).

Generally, the application of transcriptomics microarray platform technologies (or other 'omics technologies) encompasses five steps:

(i) Data generation, storing and curating;
(ii) Data processing and normalisation;
(iii) Definition and recognition of probe and sample outliers;
(iv) Statistical analysis of the processed data;
(v) Data interpretation.

If relevant for the given platform, the step of data normalisation and processing may be preceded by a further step covering the aggregation of multiple signals per gene or transcript.

Aspects relevant for the standardisation of the first step (data generation, storing and curating) and the fifth step (data interpretation) have been the focus of the work of the MicroArray Quality Control (MAQC) Consortium that is led by the US Food and Drug Administration (MAQC Consortium et al., 2006; Xu et al., 2016). Specifically, the first phase of the MAQC project (MAQC-I) extensively evaluated the technical performance of microarray platforms in identifying differentially expressed genes (DEGs) that could potentially constitute biomarkers for cellular or apical effects.

The MAQC-I found high intra-platform reproducibility across test sites, as well as inter-platform concordance of DEG lists (Fan et al., 2009, 2010; Shi et al., 2005, 2008, 2010). The proficiency of individual laboratories was found to affect the outcomes of microarray platform-based studies (Shi et al., 2005). In examining data for intra-platform consistency, only DEGs that were detected in the majority of replicates were considered. This filter accounted for the different manners in which microarray platforms identified genes below their quality thresholds (MAQC Consortium et al., 2006). A direct comparison of results across platforms was challenging because of inherent differences in protocols, numbers of data points per platform, and data preprocessing methods (MAQC Consortium et al., 2006). This inability to compare across platforms further indicates the need for an analysis framework.

The MAQC-II project focused on the development of accurate and reproducible multivariate gene expression–based prediction models to enable class predictions (cf. Box 2 above) during data interpretation (Shi et al., 2010). DEG lists were found to be more reproducible across laboratories and platforms when fold change-based ranking coupled with a non-stringent p-value threshold was used for gene selection than when selections were based on a p-value-based ranking method (Shi et al., 2005, 2008; Fan et al., 2009). The establishment of calibrated RNA samples and reference datasets were identified as crucial for an objective assessment of the performance of different microarray platforms (Shi et al., 2010; Tong et al., 2006). Box 5 summarizes sources of variability that can affect the performance of multivariate gene expression-based prediction models (Shi et al., 2010).

The results from the MAQC projects provided important information that was taken into account in the development of the *TRF*.

Since the MAQC projects specifically addressed the first and the fifth steps of transcriptomics microarray studies, i.e. data generation, storing and curating as well as data interpretation, this framework focuses on the steps in between, i.e. the processing and normalisation of the raw gene expression profiling data, the recognition of outliers, and the statistical analysis of the processed data to yield a list of DEGs. Clearly, these steps are fundamental to ensuring the relevance, reliability, and transparency of the outcome of the fourth step, i.e. data interpretation, and hence of the overall study results.

Numerous tools and approaches are available and have been applied to process and normalise 'omics data, to recognize outliers, and to statistically analyse the processed data (cf., e.g., Irizarry et al., 2003; Fan et al., 2004; Kanno et al., 2006; Liggett, 2006; Ambroise et al., 2011; Asare et al., 2009; Dalman et al., 2012; Nueda et al., 2012; Welsh et al., 2013; Chawade et al., 2014; Kim et al., 2014; Wei et al., 2014). Frequently, the need to standardise the processing, normalisation and analysis of 'omics data is highlighted in the reviews and reports (Hoffmann et al., 2002; Bammler et al., 2005; OECD, 2005; CATTPTRA–NRC, 2007; Fan and Niu, 2007; Kerr, 2007; Klebanov and Yakovlev, 2007; Cooke et al., 2011; McCall et al., 2011; Schneider and Orchard, 2011; Leung et al., 2012; Micheel et al., 2012; Ghosh and Li, 2014; Roden et al., 2014; cf. Section 1 of the Supplementary Information for further details). However, none provide specific guidance on aspects to consider in conducting these steps of 'omics studies, either for research purposes or in a regulatory context.

Generally, the different tools and approaches that are available to process, normalise and analyse 'omics data are neither right or wrong. However, by applying different tools and approaches, different outcomes (e.g. in terms of specific compositions of DEG lists or the magnitudes of gene expression changes) can be

Box 5

Conclusions from the MAQC-II project regarding the development of multivariate gene expression-based prediction models (Shi et al., 2010)

1. The performance of prediction models is largely endpoint (biology) dependent. Some endpoints are highly predictive based on the nature of the data. For such endpoints, good models can be built, provided that sound modelling procedures are used. Other endpoints are inherently difficult to predict regardless of the model development protocol.
2. There are clear differences in proficiency between data analysis teams, and such differences are correlated with the level of experience of the team.
3. The internal validation performance from well-implemented, unbiased cross-validation shows a high degree of concordance with the external validation performance in a blind study.
4. Many models with similar performance can be developed from a given data set. Similar prediction performance is attainable when using different modelling algorithms and parameters, and simpler data analysis methods often perform as well as more complicated approaches.
5. Applying good modelling practices appeared to be more important than the actual choice of a particular algorithm over the others within the same step in the modelling process.

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generated from the same starting set of 'omics data (Chepelev et al., 2015). The case study presented in the *generic TRF for 'Omics Data Processing and Analysis* (Gant et al., 2017) in this journal Supplement also reveals that the inconsistent application of bioinformatic methods for the generation, processing and interpretation of 'omics data results in different DEG lists. Frequently, it is almost impossible to retrieve retrospectively decisions that were made during 'omics studies (Shi et al., 2010).

These observations underline the need to comprehensively record and report all tools and approaches applied during the processing, normalisation and analysis of 'omics data. Full transparency on all parts of 'omics studies is essential if any meaningful conclusions are to be drawn from the data (Micheel et al., 2012). As no tool or approach is likely to either be fully right or fully wrong, the development of a framework describing how the processing and analysis of 'omics data should be reported appears best suitable to facilitate the standardisation of 'omics studies. This is the rationale underlying the development of the *TRF.* Applying this framework in designing and reporting 'omics studies, all tools and approaches that are available may be considered if fully described and a justification presented (including the application of reference substances to assess the quality of the procedure). Thereby, the framework ensures flexibility in analytical options and enhances the broad applicability of 'omics studies in a regulatory context. The scope of the framework is not to be prescriptive in the analysis method applied, but to lay out one common standard against which all other methods can be compared and that allows cross comparison thus setting a 'base-line' for comparison.

In 2001, the Microarray Gene Expression (MGED) Society initiated the development of a reporting structure for describing microarray experiments, assisting in the identification of technical measures correlated with data interpretability (the *Minimum Information About Microarray Experiments* (MIAME) guidelines; Brazma et al., 2001). The MIAME guidelines recommend reporting, in particular the degree of signal linearity and hybridisation specificity, the normalisation strategy applied, and the use of exogenous and internal controls. The scientific community has endorsed the MIAME guidelines, and most scientific journals require adherence to them for publishing toxicogenomic studies (CATTPTRA–NRC, 2007). Specific aspects to be addressed in reporting the processing of microarray data from the MIAME guidelines (Brazma et al., 2001) are summarised in Box 6.

The reporting structure presented in the MIAME guidelines was conceived for research studies, whereas the three Frameworks presented in this journal Supplement (Bridges et al., 2017; Gant et al., 2017; Kauffmann et al., 2017) specifically address aspects to take into consideration in applying 'omics technologies for regulatory purposes. As discussed above, the prerequisites for conducting (and hence reporting) studies for regulatory purposes fundamentally differ from the prerequisites for conducting (and reporting) studies for research purposes. Accordingly, the three frameworks aim to further the efforts of MIAME in proposing a reporting structure for 'omics-based studies that are intended to be conducted in a regulatory context. Additionally, the *Framework for the quality assurance of 'omics technologies considering Good Laboratory Practice (GLP) requirements* aims at fulfilling a recommendation from the OECD/IPCS workshop on toxicogenomics, i.e. to increase the regulators' confidence in 'omics data by developing a GLP-like best practice for toxicogenomics (OECD, 2005). To the best of the authors' knowledge, the work for this Framework is not paralleled by any other ongoing or finalised similar activity.

While there are fundamental differences between regulatory studies and research studies, considerations that serve to improve the design of the one type of study may also be beneficial for the other. Therefore, recent peer-reviewed publications that addressed substance-induced alterations of RNA expression using microarray transcriptomics (Shi et al., 2010; Thomas et al., 2011, 2012, 2013; Bourdon et al., 2012; Thomson et al., 2013; Jackson et al., 2014; Chepelev et al., 2015; Tilton et al., 2015; Verbist et al., 2015; Lake et al., 2016) were evaluated to record how the processing of 'omics data was reported therein (cf. Section 2 of the Supplementary Information for further details). In all research articles, microarrays were used in the form of commercially available test kits (from Affymetrix®, Agilent Technologies, or Illumina®). Frequently, it was recorded that the corresponding software tools provided by the manufacturers of the respective microarrays were used for data processing and normalisation, as well as for statistical analysis and data interpretation. Consistently, it was reported that data were normalised, but different normalisation methods were applied between different articles. Twice, log transformation of the data was reported. In the other research articles, it was neither reported that log transformation was applied, nor that it was not applied. Twice, the identification of outliers was reported. Different statistical tests were applied for statistical analysis. The specific approaches applied for all steps of data processing, normalisation

Box 6

Considerations relevant for data processing presented in the MIAME guidelines

From: Brazma et al. (2001), cf. also: http://fged.org/projects/miame/

During any given microarray study, three levels of data are relevant: (i) the scanned images (raw data); (ii) the quantitative outputs from the image analysis procedure (microarray quantitation matrices); (iii) the measurements derived after normalisation and consolidation from possible replicates (gene expression data matrices).

For each experimental image, a microarray quantification matrix contains the complete image analysis output generated by the respective software. For a given image, this is a 2D matrix, where array elements (usually genes) constitute one dimension and quantification types are the second dimension. The quantifications used (e.g. mean or median intensity, mean or median background intensity) need to be defined.

In a typical microarray study, reported hybridisation intensities derived from image processing must first be normalised. Normalisation adjusts for a number of technical variations between and within single hybridisations, namely quantity of starting RNA and labelling and detection efficiencies for each sample.

At the time of writing the MIAME guidelines in 2001, there are no widely used standard controls for microarray assays. Microarray data from different sources use different measurement units, whose conversion factors are typically unknown and may even vary depending on expression level.

Parameters relevant to normalisation and control elements mentioned in the MIAME guidelines include (i) the normalisation strategy; (ii) the normalisation and quality control algorithms (III) the identities, type and location of the array elements serving as controls; (iv) the hybridisation extract preparation, detailing how the control samples are included in sample targets prior to hybridisation.

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and analysis were not reported in a fully comprehensive manner in any of the publications. In summary, different research teams apply different 'omics data extraction and analytical approaches, and generally do not report sufficient information to ensure that another research team can reproduce their exact approach.

CATTPTRA–NRC (2007) underlined that the specific software tools used in the processing and analysis of 'omics data can play as significant a role in determining the final outcome of an experiment as the selected microarray platform. *Consequently, considerable attention must be paid to validating the computational approaches. The combination of technology platform data collection and processing algorithms must be appropriately selected and validated for application to the biologic system for each study* (CATTPTRA–NRC, 2007). Further, different standards might be necessary for the tools provided by different manufacturers: *The consensus was that the diversity in platforms, experimental designs, and applications makes it unlikely that a single universal measure of quality will be possible. However, there was confidence that standards based on universal principles could be developed for each platform, for example, one for Affymetrix GeneChips and a separate, but similar, standard for spotted oligonucleotide microarrays* (CATTPTRA–NRC, 2007).

The aim of the *TRF* though is to establish a reporting structure that is applicable to all types of transcriptomics data from all platforms so that a cross-comparison of these data can be easily made. As stated previously, this is not a prescriptive method, but one that allows comparisons between data sets and with other analysis methods.

5. Discussion towards facilitating the regulatory acceptance and use of transcriptomics microarray methodologies

The rapid advancement of 'omics technologies comes with several challenges to facilitate their use for hazard assessment, particularly from the regulatory submission perspective. Sets of 'big data' have to be condensed applying complex technologies and methodologies and using very specific knowledge to obtain information that is relevant for hazard assessment. Knowledge in this area is part of a rapidly evolving scientific field that many scientists working in the regulatory setting are not necessarily familiar with. Accordingly, the lack of regulatory uptake of 'omics technologies is not only linked to a lack of best practices frameworks and quality criteria, but also to a lack of confidence in the analysis and the level of uncertainty with respect to what constitutes enough data (Healy et al., 2016). Scientists and regulators have to first gain experience with the data obtained from a new technology to build confidence in its applicability.

In the present survey, challenges have been identified that should be met to facilitate the use of transcriptomics data for regulatory hazard and risk assessment (McConnell et al., 2014; Bourdon-Lacombe et al., 2015). In regulatory submissions, standard protocols that are fit-for-purpose need to be agreed upon and used. As the present survey reveals, for the time being validated and accepted standard protocols do not exist for 'omics technologies.

Further, regulatory and legal issues remain to be addressed in order to achieve regulatory applicability of 'omics data, and these issues are more complex than the technical issues related to their standardisation and validation.

Irrespective of legal issues, it is necessary to establish robust, transparent processes to ensure that the 'omics study results are not compromised by uncertainty developed during the generation, storing, curating, and processing of the data. This will provide confidence in the regulatory use of such technologies.

The three Frameworks presented in this journal Supplement (Bridges et al., 2017; Gant et al., 2017; Kauffmann et al., 2017) have been conceived to be robust and flexible, and to provide transparency on the different steps that form part of 'omics studies. Application of the frameworks will enhance the quality of 'omics studies. This is expected to increase confidence in the given study results, thereby also facilitating the transition of transcriptomics technologies from research to regulatory use.

Frameworks must be flexible enough to cope with the rapidly evolving technological advancements on the field of 'omics. As new technologies continue to become available, existing data are likely to be reused. Adherence to the Frameworks will facilitate data transferability and reproducibility and the sharing of resources. In the longer-term, adherence to the Frameworks may also contribute to identifying which tool or approach produces a result (e.g. in terms of DEGs) that most accurately reflects the toxicological MoAs and apical endpoints under investigation. Such knowledge will be relevant to establishing best practices for 'omics studies, and it may provide opportunities to use 'omics data, not only to enhance a mechanistic understanding of substance-induced effects, but also to derive the *single number* during regulatory hazard assessment. Finally, the Frameworks can be used as a starting point to train all those involved in the regulatory use of 'omics data in the assessment of the scientific validity of the underlying studies.

Conflict of interest

UGS was hired by ECETOC and CEFIC LRI to assist in the preparation of the manuscript. The other authors were engaged in the course of their normal employment. The authors alone are responsible for the content and writing of the paper.

Disclaimer

The views presented in this article do not necessarily reflect current or future opinion or policy of the U.S. Food and Drug Administration. Any mention of commercial products is for clarification and not intended as an endorsement.

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Appendix A. Supplementary data

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Transparency document

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