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EUROPEAN CHEMICALS AGENCY

New Approach Methodologies in Regulatory Science

Proceedings of a scientific workshop

Helsinki, 19–20 April 2016

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Table of contents

TABLE OF CONTENTS **3**
LIST OF ABBREVIATIONS **4**
EXECUTIVE SUMMARY **6**
1 INTRODUCTION TO THE WORKSHOP **8**
2 THEME 1: DEFINITIVE HAZARD ASSESSMENT, PARTICULARLY READ-ACROSS **9**
2.1 Introduction to Theme 1 9
2.2 Case study 1: PFAA 10
2.2.1 Plenary presentations 10
2.2.2 Discussions: key topics and conclusions drawn 10
2.3 Case Study 2: β-unsaturated alcohols 13
2.3.1 Plenary presentations 13
2.3.2 Discussions: key topics and conclusions drawn 13
2.4 Case Study 3: metabolomics analysis of phenoxy herbicides 18
2.4.1 Plenary presentations 18
2.4.2 Discussions: key topics and conclusions drawn 18
2.5 General comments on the use of NAMs in definitive hazard assessment and read-across in particular 22
2.6 Summary 23
3 THEME 2: SCREENING AND PRIORITY SETTING **26**
3.1 Introduction to Theme 2 26
3.2 Plenary presentations 26
3.3 Discussions: key topics and conclusions drawn 31
3.4 Summary 34
4 THEME 3: PROSPECTS FOR REGULATORY SCIENCE **36**
4.1 Introduction to Theme 3 36
4.2 Plenary presentations 36
4.3 Discussions: key topics and conclusions drawn 41
4.4 Summary 45
5 OVERALL OUTCOMES AND SUGGESTIONS **47**
Appendix 1 List of presentation titles 50
Appendix 2 List of poster titles 51
Appendix 3 List of participants 57
Appendix 4 Speech from the European Partnership for Alternative Approaches (EPAA) presented by Renate Weissenhorn 63

List of abbreviations

ADME	Absorption, distribution, metabolism, and excretion
ADH	Alcohol dehydrogenase
AOP	Adverse outcome pathway
BER	Bioactivity exposure ratio
CEFIC	European Chemical Industry Council
CERAPP	Collaborative estrogen receptor activity prediction project
CLH	Harmonised classification and labelling
CLP	Classification, Labelling and Packaging Regulation
2,4-DP	2-(2,4-dichlorophenoxy)propionic acid
EC10	Effective concentration at 10 % inhibition or the concentration that will have an effect of 10 % on the measured endpoint
ECHA	European Chemicals Agency
ECVAM	European Centre for the Validation of Alternative Methods
EDC	Endocrine disrupting chemical
EDSP	Endocrine screening program
EFSA	European Food Safety Authority
EPAA	European partnership for alternative approaches
EU IMI	European Union innovative medicines initiative
GSH	Glutathione
HTTK	High-throughput toxicokinetics
IATA	Integrated assessment and testing approaches
ICPS	International classification for patient safety
IMAP	Inventory multi-tiered assessment and prioritisation
IPCS	International programme on chemical safety
IUCLID	International uniform chemical information database
LRSS	Long range science strategy
MAQC	Microarray quality control
MCPA	2-methyl-(4-chlorophenoxy) acetic acid
MCPP	(RS)-2-(4-Chloro-2-methylphenoxy)propanoic acid
MIE	Molecular initiating event
MoA	Mode of action
MOE	Margin of exposure
NADH	Nicotinamide adenine dinucleotide
NAMs	New approach methodologies
NICNAS	National industrial chemicals notification and assessment scheme
NOAEL	No observed adverse effect level
NOEL	No observed effect level
OECD	Organisation for Economic Cooperation and Development
OHT	OECD harmonised template
PACT	Public activities coordination tool
PFAA	Perfluorinated alkyl acid
PFOA	Perfluorinated octanoic acid
PPAR	Peroxisome proliferator-activated receptor
QSAR	Quantitative structure–activity relationship
RAAF	Read-across assessment framework
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
SAR	Structure–activity relationship
SOP	Standard operating procedure
SVHC	Substance of very high concern
TTC	Threshold of toxicological concern
US EPA	United States Environmental Protection Agency
US FDA	United States Food and Drug Administration

WHO	World Health Organisation
WoE	Weight-of-evidence

Executive Summary

ECHA's Topical Scientific Workshop (19-20 April 2016) addressed the use of data and information from new approach methodologies (NAMs) to support regulatory decisions for the use of chemical substances. The workshop brought together over 200 stakeholders in person and a further 100 online.

An international audience considered three themes representing the use of NAMs for read-across (Theme 1), for screening and prioritisation (Theme 2) and for future prospects (Theme 3). The main deliberations and conclusions of the workshop are summarised in this document.

NAMs were taken in a broad context to include *in silico* approaches, *in chemico* and *in vitro* assays, as well as the inclusion of information from the exposure of chemicals in the context of hazard assessment. They also include a variety of new testing tools, such as "high-throughput screening" and "high-content methods" e.g. genomics, proteomics, metabolomics; as well as some "conventional" methods that aim to improve understanding of toxic effects, either through improving toxicokinetic or toxicodynamic knowledge for substances.

Three read-across case studies were presented, including an evaluation of the read-across, and the contributions of NAMs to reduce uncertainty, using ECHA's Read-across assessment framework (RAAF).[1]

NAMs were found to support read-across, especially by providing information on toxicodynamics, which increased confidence in mechanistic hypotheses and justification. However, the NAM approaches considered in this workshop were found to be less useful to provide evidence on toxicokinetics to support a read-across argument.

NAMs were also shown to be applied in a variety of scenarios for screening and prioritisation with examples from various regions. The future prospects for the use of NAMs were outlined through presentations on current and anticipated practice.

The workshop recognised the usefulness of the NAMs for a number of regulatory uses.

A number of key suggestions to further apply NAMs in a regulatory context were made. There is a need for standardisation of NAMs as well as a better understanding of their relevance through thorough analysis of their performance and definition of their applicability.

In addition, reporting templates for NAMs are required to encourage their use. The OECD harmonised template (OHT 201) for reporting information on intermediate effects is a potential starting point for developing the templates for recording NAMs. NAMs were demonstrated to provide pertinent information relating to mechanisms of action i.e. toxicodynamics; however, fewer examples of their use for toxicokinetics were available. One of the barriers to the use of NAMs was the lack of transparency in terms of limited

[1] http://echa.europa.eu/en/support/registration/how-to-avoid-unnecessary-testing-on-animals/grouping-of-substances-and-read-across

documentation and/or access to the underlying data and algorithms.

The regulatory use of NAMs is anticipated to increase in a variety of applications and to address a number of regulatory challenges, including supporting read-across, prioritisation and screening; however, they should be applied with a full understanding of their potential advantages (e.g. rapid screening, improved mechanistic understanding) and limitations. To increase uptake and acceptance amongst all stakeholders, case studies and capacity building are required.

The main outcomes and conclusions of the workshop were:

- Data from NAMs were shown to support read-across as well as providing useful and usable information for screening and prioritisation.
- NAMs could be used imaginatively and flexibly, their use may drive changes in regulatory hazard assessment practices in the future.
- There is a need to better define NAMs and provide an overview on where and how NAMs could be applied in the future. A taxonomy of methods for NAMs would be a great benefit.
- There is a need for standardisation of NAM approaches so that they may be made transferable and transparent.
- A standardised template for reporting NAMs is required; existing templates such as OHT 201 could be used as a starting point for developing such templates.
- The intrinsic quality and coverage of NAM data have to be defined and addressed.
- It was shown that NAMs currently provide useful information on toxicodynamics, but little insight into toxicokinetics.
- It was demonstrated that NAMs can support exposure assessment by providing, for example, information on production and use volume.
- There is a need to understand and characterise uncertainty from NAM data and how these will affect WoE.
- There is a need for further case studies to demonstrate the practical application of NAMs.
- Training and education in the use and meaning of NAMs are needed across all stakeholder groups.

1. Introduction to the workshop

The European Chemicals Agency (ECHA), Helsinki, Finland held a *Topical Scientific Workshop on New Approach Methodologies* on 19-20 April 2016, which was attended by over 200 participants and more than 100 attendees online.

Jukka Malm, Deputy Executive Director of ECHA, opened the workshop and welcomed all participants. He outlined the purpose of the workshop stating that ECHA is a regulatory agency. ECHA prepares science-based decisions and opinions and gives scientific and technical advice to companies to help them to comply with their legal obligations under REACH and CLP. ECHA has developed strategic objectives to steer their work. One of these objectives is to be a hub to promote good regulatory science. The topical scientific workshops (of which this is one of a series) are one element in ECHA's functioning as a hub. The workshops provide a platform for academia and regulators to come together and engage in a dialogue to address important challenges in regulatory science.

Malm defined the context of the new approach methodology workshop as being on ECHA's role in alternatives to animal testing and the 3Rs. He emphasised that more knowledge on NAMs is required. European legislation is based on the principle that industry has to develop hazard data on the substances they produce to ensure safe use. To avoid unnecessary testing on animals the companies have a legal obligation under REACH to try to use alternative methods and approaches before relying on animal testing.

This area of science is moving on with new prediction techniques being developed and new alternative data becoming available. The focus is very much on understanding toxicology rather than only observing it. This development brings further opportunities and challenges, especially for combining different kinds of evidence from novel techniques. It would, however, be unrealistic to assume that, for higher-tier endpoints, the new toxicological techniques would soon, or even in the medium term, fully replace testing on animals. Hence, the animal testing and the NAMs will be complementary, and not necessarily competing approaches. As such, it is advisable to see how the design and conduct of animal testing could be improved so that it would better inform the development of read-across or weight-of-evidence (WoE) approaches for analogue chemicals, for example through improved information on toxicokinetics or other mechanistic data.

The regulatory setting and advances in science provided a very pragmatic setting for the *Topical Scientific Workshop on New Approach Methodologies*, specifically:

- to understand the practical problem formulation of regulatory bodies in interpreting data from new approach methodologies, and;
- to understand what the latest developments of science can offer.

The ambition is that the *Topical Scientific Workshop on New Approach Methodologies* will help both the regulatory safety assessment and the science advance in parallel for mutual benefit.

Christian Desaintes summarised the status of current European-level projects, which have supported the development of NAMs (e.g. the SEURAT-1 Cluster) and have the potential to develop approaches in the future (e.g. EU-ToxRisk).

The Workshop was also addressed by Renate Weissenhorn from the European Partnership for Alternative Approaches (EPAA). Her speech is provided in Appendix 4.

2. Theme 1: Definitive hazard assessment, particularly read-across

2.1 Introduction to Theme 1

Theme 1 focused on the use of NAMs to assist in the development of read-across arguments to fill data gaps, especially as they pertain to REACH. To address this issue, three case studies were presented and assessed through the read-across assessment framework.

The goals of the case studies were two-fold. The first goal was to demonstrate how different types of NAMs and other non-animal evidence support reasoning for read-across. In particular, how NAMs can reduce toxicodynamic and mechanistic uncertainties and increase the WoE support of a read-across prediction. The second goal was to aid in identifying any barriers and limitations in using NAMs for data gap filling in hazard assessment. The first two case studies were provided by the SEURAT-1 project (co-funded by the European Commission and Cosmetics Europe) and the third case study was provided by industry (BASF).

Norbert Fedtke (ECHA) described the critical aspects in the assessment of read-across adaptations and the role of supporting evidence. He reminded the workshop that the grouping of substances and read-across is one of the most commonly used alternative approaches for filling data gaps in registrations submitted under REACH.

In dossier evaluation, ECHA has to evaluate whether the provisions in the REACH Regulation are met for this adaptation of standard tests. In response to this challenge, ECHA developed and published the read-across assessment framework in May 2015 whereby read-across approaches are assessed through the use of different scenarios and their respective assessment elements and assessment options. The assessment elements investigate the scientific aspects of the read-across hypothesis in a structured way. Supporting evidence is required to substantiate the scientific hypothesis, which establishes the basis for predicting properties and is therefore crucial in the read-across arguments.

All types of supporting evidence are considered when conducting an assessment according to the RAAF which, in the context of the workshop, includes NAMs. A considerable effort in developing new approaches and methodologies for investigating properties of chemicals has been made over the past years. Therefore, it was timely that the workshop explored such techniques in terms of their potential and limitations to investigate their future use in read-across approaches.

In the context of REACH, it must be emphasised that the NAMs are not adaptations to the standard information requirement (see the introduction to the workshop). As such, NAMs are not in themselves subject to acceptance or rejection in the evaluation of the adaptation. With regard to the discussion in this document, data from NAMs can be viewed as support to the read-across hypothesis. Reference to acceptance of NAMs below means the potential for applying NAMs in an acceptable manner.

It should be noted that in the proceedings, the panel and break-out group discussion is aggregated to facilitate the reading by case study in Theme 1.

2.2 Case study 1: PFAA

2.2.1 Plenary presentations

The perfluorinated alkyl acid (PFAA) read-across case study was presented by Terry Schultz (University of Tennessee) and the RAAF assessment of the read-across was presented by Sharon Stuard (Procter and Gamble).

PFAAs are chemically very similar (e.g. highly fluorinated chemicals consisting of a straight-chain hydrocarbon backbone and a single terminal carboxylate moiety). From a toxicokinetics standpoint, PFAAs are absorbed by the gut, bind to albumin and other proteins and are not metabolised in the liver. Their persistence (i.e. low clearance) is markedly influenced by resorption in the kidneys, which is species-, sex- and analogue-dependent.

Toxicodynamically, PFAAs are direct-acting toxicants (i.e. where metabolism is not a factor) with similar modes of action (MoAs), most likely a combination of PPARα/ PPARγ interactions, leading to rat oral repeated-dose hepatotoxicity through perturbations to fatty acid uptake, lipogenesis, fatty acid oxidation, and centrilobular hepatocellular hypertrophy correlated with higher liver weights.

The molecular mechanism of PFAA-induced liver toxicity is not completely characterised. Toxicogenomics studies suggest that PFAAs suppress immunity and induce fatty acid transport and metabolism, as well as inflammation. While PFAAs have been shown to be involved in several mechanistic-relevant events, the length of the fluorocarbon-backbone has not been shown to have an impact on the mechanism of action.

The case study proposed that the NAM data supports the premise that the molecular mechanism of action inducing repeated-dose liver toxicity of PFAAs is PPAR-linked. It further proposed that while the NAM data considered have no impact on the toxicokinetic uncertainty, they reduce the uncertainty and strengthen the WoE associated with the toxicodynamic similarity as such reducing the uncertainty associated with mechanistic relevance, thereby significantly enhancing the justification of the read-across hypothesis.

2.2.2 Discussions: key topics and conclusions drawn

The applicability of the PFAA case study was considered, with some relevant issues being raised.

Key topic: the category formed and read-across premise

- A participant queried the use of PFOA as the source chemical. The presenter commented that PFOA was the most poorly cleared compound thus, if clearance is a driver for toxicity, it could be considered the worst- case scenario.
- Several participants saw the quality of the "anchor data" as a major issue. Further information on the quality of the *in vivo* data was seen as a pre-requisite; this would require detailed analysis of study reports. A greater understanding of the inherent variability (as part of an assessment of quality) of the NAMs, as well as models and *in vivo* data, is required.
- A participant highlighted differences in the NOEL values i.e. the C6 PFAA has a higher NOEL than analogues. This can be explained by understanding the importance of *in vivo* clearance, particularly the differences between the sexes and significant inter-species differences between rats and humans. The presenter commented that there was insufficient clearance data for this category and that, for this case study, NAMs provided no additional information or confidence on clearance and that this was a key uncertainty.

Conclusions*:*
This was a well-defined category with a small number of substances and a clear premise for read-across. However, uncertainty remained not least in the understanding of the quality of the *in vivo* data but also for toxicokinetics.
This category demonstrates the requirements for high quality ADME data and information, specifically in this case for clearance.
Further information (*in vivo* or *in vitro*) on clearance would help to reduce uncertainty in the read-across.

Key topic: the role and use of new approach methodology data

- Several participants confirmed that this case study showed that NAM data, specifically those from ToxCast in this example, supported the mechanistic hypothesis but provided little evidence or support on toxicokinetics.
- A participant queried the specific mechanism of action with regard to PPAR and whether the α or γ form was activated. This is as yet unknown, although NAMs could provide further evidence to clarify this e.g. from hepatocytes.
- A participant indicated that other types of NAM data could be applied to this specific read-across e.g. comparative protein binding and *in vitro* toxicokinetic data.
- Several participants, including panel members, highlighted the pressing need for the demonstration of the standardisation of NAM data. With regard to NAMs, the need for reliable, reproducible and relevant data was highlighted as well as means to demonstrate this.
- Several participants, including panel members, stated that a reporting format for NAMs is desirable (see also below in Themes 2 and 3).
- A participant highlighted, whilst they undoubtedly contain useful information, there is difficulty in interpreting ToxCast data. The presenter confirmed that expert opinion is often required e.g. for unspecific, high concentration, and burst effects associated with cytotoxicity. In addition, the experimental limitations of the system e.g. solubility (especially for the longer chain lengths in this case study) need to be considered.
- A participant suggested that biological read-across could use toxicological data and information from pharmaceuticals (either those in use or not approved). Such compounds often have detailed mechanistic understanding and supporting data. The use of information for pharmaceuticals could also extend to tapplying data from human exposure. The presenter commented that care would be needed as pharmaceuticals may have a different applicability domain to the category being considered.
- ToxCast data have demonstrated their usefulness in supporting mechanistic plausibility in this read-across. However, expertise in interpreting the ToxCast data is required for more extensive use. A participant stated that ToxCast performance standards were not included in the case study, but they are freely available on the US EPA website. SOPs are available for all ToxCast Assays

undertaken internally to US EPA, for proprietary assays a description of the assay and vendor information is available.

Conclusions:
NAM data have helped to reduce the uncertainty in the toxicodynamics of the read-across argument. The NAM data considered did not reduce uncertainty in the toxicokinetics.
NAM data can support detailed mechanistic interpretation and confirmation of toxicodynamics, although further information would be required in this category.
There is a need to demonstrate the relevance of NAM data to support a read-across.
A consistent reporting format for NAM data is desirable.

Key topic: general consideration of the case study 1 read-across scenario

- A panel member stated that NAM is an umbrella term for many different methods e.g. *in silico*, *in chemico*, *in vitro*, high throughput etc. The role of different NAMs needs to be better defined.
- There was a general shared view that RAAF proved to be a flexible framework to assess the PFAA category and associated read-across. The RAAF enabled the contribution of the NAMs to be assessed.
- There was a general discussion about how to derive confidence levels from multiple data, especially to be able to show that NAM data can reduce uncertainty. This raises the possibility that remaining uncertainty in a read-across can be identified, but strategies will still be required to deal with that uncertainty.
- A participant stated that the barriers and difficulties in using NAMs need to be better defined. In addition, there are likely to be different levels of expected and acceptable confidence in a read-across for different regulatory settings i.e. risk assessment, classification and labelling, prioritisation etc.
- A participant emphasised the need for a better understanding of the WoE and use of mechanistic data to support read-across. Specifically there is a requirement for common means to present the information and how to consider aspects such as biological plausibility. There was also a suggestion for frameworks for read-across to be endpoint-specific.

Conclusions:
NAMs have a broad definition and are applicable to a number of read-across scenarios.
The RAAF proved to be a flexible framework to organise information.

There are needs to develop WoE arguments and frameworks in a more rational manner.

Key topic: further possibilities and R&D needs for NAMs

- No suggestions were made for research needs for Case Study 1.

2.3 Case Study 2: β-unsaturated alcohols

2.3.1 Plenary presentations

Mark Cronin (Liverpool John Moores University) presented the β-unsaturated alcohol read-across case study and Andrea-Nicole Richarz (European Commission's Joint Research Centre) presented the RAAF assessment of the read-across.

Short-chain (i.e. C3 to C6) β-olefinic alcohols have similar chemical properties but are structurally either primary or secondary and either straight-chained or branched, with different positions of the methyl substituent.

These structural differences affect chemical reactivity of metabolites and, thus, repeated-dose toxicity. While all short-chain β-olefinic alcohols are rapidly and nearly completely absorbed from the gut; only the primary and secondary alcohols are capable of being metabolised, primarily in the liver, through alcohol dehydrogenase (ADH).

Oxidative metabolism of primary and secondary β-olefinic alcohols results in the corresponding α,β-unsaturated aldehyde or α,β-unsaturated ketone. These α,β-unsaturated derivatives are the definitive electrophilic toxicants and *in vivo* potency is related to relative thiol reactivity; thus, only β-unsaturated alcohols with a metabolism similar to 2-propen-1-ol and a reactive potency similar to acrolein may be read across from 2-propen-1-ol with reasonable certainty.

NAM data, whilst incomplete, support the premise that the molecular mechanism of action inducing repeated-dose liver toxicity of β-olefinic alcohols is metabolically-linked. It is noted that the NAM data considered have no impact on the toxicokinetic uncertainty, but they reduce the uncertainty and increase the WoE associated with the toxicodynamic similarity. To a lesser extent, they reduce the uncertainty associated with mechanistic relevance and completeness of the read-across.

2.3.2 Discussions: key topics and conclusions drawn

Key topic: NAMs have reduced uncertainty in toxicodynamics and could help confirm mechanism of action

- A participant stated that NAM data provide the mechanistic validation of the read-across hypothesis. In this case study, the hypothesis of reactivity and its prediction is central to the read-across argument; therefore, the NAMs should reflect that to provide support to the read-across argument.
- Several participants considered that there could be a possibility to form a hypothesis and/or break down the mechanism into a two-stage process i.e. metabolism by ADH and then reactivity (possibility for reactivity and quantification). This could be the basis for the use and/or development of NAMs and go further to confirm the toxicokinetics and toxicodynamics. It was noted, however, that there is currently no metabolic step in the *in chemico* assays.

- The hypothesis regarding the mechanism being approached on two levels, metabolism and reactivity, was expanded upon. They could then be split and approached separately, thus providing more confidence in the read-across arguments. For instance, the case study used high quality GSH reactivity data, but the relationship with *in vivo* activity is not known. In addition, little is known about the quantitative aspects of the metabolic component, which increases uncertainty. It could be possible to model metabolism (see the next comment). However, there is a requirement for better knowledge on ADME properties. It was further noted that the proposed mechanism relies on the intra-cellular depletion of GSH. The relative interspecies difference in GSH are also unknown.
- A participant noted that, in reality, for this case study there may be a network of interlinked responses and it is difficult to identify one single mechanism and effect. For instance, the fibrosis adverse outcome pathway (AOP) needs careful consideration e.g. the role of NADH needs to be clarified where mitochondrial effects and NAD+ can affect lipid metabolism.
- Omics was considered to be a useful tool to help determine mechanisms of action. Such technologies could help to determine whether one MoA becomes irrelevant with a group of molecules. The data will also be useful and could help define when the MoA is overriding.
- It was noted that *in vivo* omics data could provide a bridge between *in vitro* NAMs and the *in vivo* data. This may be through confirmation of the activation of toxicological pathways. However, omics technologies are expensive and the outputs rely on the time points at which measurements are taken and concentrations used (see Case Study 3). It was stated that a new EU Innovative Medicines Initiative (IMI) project on quantitative systems toxicology, as well as EU-ToxRisk, will investigate the bridge between *in vitro* and *in vivo* data by considering diseased human tissue to see if pathways are the same as *in vitro*.

Conclusions:
NAM data have reduced uncertainty in the toxicodynamics of the read-across argument by providing evidence of the mechanism of action.
More information may be desirable to reduce uncertainty in the confirmation of mechanism action, this may come from splitting the mechanism into a two-step (metabolism and reactivity) process, the judicious use of molecular modelling and the application of omics may be of value.

Key topic: more in vivo and NAM datadata required to make read-across quantitative

- There was an appreciation that it was not possible to make a quantitative assessment of toxicity from the data provided. A participant suggested that data for GSH and stress reporters should be compared quantitatively - this was not undertaken, or possible, in this case study.
- The non-quantitative nature of the read-across was reinforced by the conclusion that read-across is possible for chemically similar compounds or those with a common molecular initiating event (MIE). This in itself does not allow safety to be assessed and this approach (as applied in the case study) increases the difficulty for quantification. Currently, it is not known how to deal with level of belief/ confidence/uncertainty etc. that may be required for quantitative read-across.

- It was also stated that in the case study, there were insufficient *in vivo* data and a lack of toxicokinetics information for quantification to take place. A solution could be quantitative dose response data for *in vitro* and *in chemico* NAMs. However, the relationship between the *in vitro* dose response and *in vivo* activity is still required. One suggestion was that EC10 values, derived from the experimental dose-response data, could be used to compare from *in vitro* to *in vivo* – this would have increased confidence.

Conclusions:
Quantification of the read-across prediction is desirable but was not achieved in this case study.
To enable quantification, more information on toxicokinetics, as well as the relationship between *in vitro* and *in vivo* is required.

Key topic: more work required on NAMs to reduce uncertainty in toxicokinetics

- There was general agreement that in this case study NAMs have not provided information on toxicokinetics and there were queries on how to deal with toxicokinetics.
- One solution was suggested, namely to test the metabolic profile of the compounds *in vitro* with human hepatocytes. Standard techniques can be used and testing laboratories are able to undertake this work. This could provide a metabolite profile and rates of reaction for relevant compounds. It was noted that HepRG is similar to human cells with regard to ADH, so this could be considered. In addition, it may be possible to use a cell, e.g. a fibroblast that does not have ADH to determine whether ADH is important for metabolism. However, there are a least 15 different ADH enzymes and a further suggestion was that it may be possible to perform a knockout method.
- It was confirmed that in this case study it is clear what the metabolic route is, but there will be case studies where this is less certain. Understanding rates of metabolism and clearance could help in these cases and for this read-across scenario (production of a common metabolite).

Conclusions:
Toxicokinetics were not addressed by NAMs in this case study.
Strategies for applying NAMs include obtaining a metabolic profile, assessing the relevance or otherwise of ADH and obtaining clearance data.

Key topic: identification of other uncertainties

- Read-across is considered to be an adaptation to standard data. As such, it assumes that good data, e.g. a 90-day oral repeated-dose test in this case study, are required to make a prediction. In this case study, the quality of the *in vivo* data was poorly, if at all addressed, which added to the uncertainty. To improve the quality of read-across, there is a need to see the study reports and assess them in more detail.

- It was observed that related to the issue of data quality, there is a need to review the selection of the NO(A)ELs for the read-across in more detail with a clearer definition. The quality of all the data (*in vivo* and NAMs) was largely unknown and unquantifiable leading to uncertainty.
- With regard to the chemical identity, it was noted that stereochemistry was poorly defined. This is an uncertainty as information is required on the stereoisomers tested as this could affect reproducibility of test results, and possibly metabolism and reactivity. It was stated that impurity profiles were not known for the chemicals tested and should be provided.
- With regard to the read-across, the comparison against the most toxic molecule e.g. the smallest (acrolein) as an approach for read-across was raised. Whilst this is precautionary, it increases uncertainty.
- An uncertainty is making sure that NAM data are relevant for the *in vivo* scenario. More effort is required on how to anchor NAMs to *in vivo* data.

Conclusions:
There are a number of uncertainties in the read-across case study, some of which were identified through the RAAF.
Uncertainties included lack of knowledge on data quality and relevance, and proper chemical identification.

Key topic: general consideration of the Case Study 2 read-across scenario

- It was stated that within the regulatory context, adaptation of the regulation (including the use of read-across and NAMs) can be sufficient to replace the results of an animal test. However, current experience in read-across for data gap filling under REACH shows that NAM data alone cannot support a case. There is always a need for a standard guideline data, preferably of high reliability and following GLP. Therefore, regulatory acceptance will depend on conditions of the read-across and how they have been applied. The use and measurement of fit for purpose NAMs is a challenge scientifically and from an educational/expertise/training aspect.
- A participant noted that other creative lines of evidence could be included to reduce uncertainty in a read-across argument. Whilst not relevant to this endpoint, the compounds in this case study are skin sensitisers, which could confirm the Michael addition reaction. Whilst this approach was generally agreed upon, caution in using such data was suggested as their direct relevance would need to be carefully justified.
- It was noted by several participants that *in vivo* biomarkers may be or are required to identify apical endpoints. With the ban on animal testing in some sectors, zebra fish embryo are considered to be a non-animal test, but is a standardised assay that could provide relevant NAM information regarding effects. Linked to this, it was observed that some sets of information on zebra fish becoming available for large datasets of compounds. In addition, more data on *C. elegans* and fruit flies will soon become available. Big data will start to assist in data gap filling and provide a means to supply NAMs and elicit patterns (see Theme 3).

- A participant observed that a suitable coverage of species can be achieved with knowledge of evolutionary aspects of pathways, specifically those relevant to the adverse outcome. If one species is known to be a modern descendent of another, then the pathways are likely to be shared, allowing for an extrapolation of effects using knowledge of pathways. Shared pathways, including those with humans, could be investigated.
- It was noted that toxicology has not kept up with human (personalised) medicine. We do not yet know how much data is required and there could be many learnings from medicine.

Conclusions:

A number of means of using other NAM data, or obtaining further information, were presented including biomarkers, surrogate species, evolutionary pathway analysis and knowledge from techniques used in personalised medicine.

Key topic: further possibilities and R&D needs for NAMs

- It was discussed that (especially for higher tier endpoints) NAMs are not currently a standalone piece of information for a category and need to be linked to *in vivo* data. Further, it was suggested that a package of *in vivo* data with NAMs would be very powerful and could confirm read-across arguments. As such, NAMs are not a disconnected entity, but must be linked to *in vivo* data. Thus, a well-defined category, anchored to high quality *in vivo* data and NAMs would confirm the hypothesis of category membership and hence facilitate read-across.
- There was discussion of if and how minimal *in vivo* data could help accelerate the choice of NAM techniques. However, it was noted that certain sectors are not able to perform further *in vivo* testing. In addition, the Horizon 2020 project EU-ToxRisk cannot perform new *in vivo* experiments. As was the case with SEURAT-1, it is completely focused on safety assessment based on alternative methods to animal testing. However, EU-ToxRisk will be able to compare different models varying in complexity from cell lines up to spheroid models to assess the relevance of the approaches and determine uncertainty for read-across. To assist such progress it was noted that there should be knowledge of the AOP and/or MoA to drive the development, use and acceptability of NAMs.
- There was general agreement that there is a clear need for training and education in the area of read-across and use of NAMs, as well as capacity building both in personnel and facilities.
- There was discussion about a number of other barriers and limitations to the use of NAMs and read-across. The discussion focused in part on the case study. This included that better knowledge on toxicokinetics is required; the need to make and record which NAMs are fit for purpose to predict a given toxicity; the acceptance that NAMs require expertise to measure and interpret; and the cost of NAMs should be lower than *in vivo* testing to stimulate use.
- There was a strong feeling that more case studies are required to illustrate the use of NAMs as applied to read-across. In particular, the challenge was given to industry and regulators. One possibility is to submit a REACH dossier as an educational process using NAMs. The EU-ToxRisk project may take a lead in collaborating with ECHA and other interested contributors. A concept of creating "safety bays", where industry and regulators could work together for the advancement of science, was proposed.

Conclusions:
There are a number of clearly defined R&D needs to increase the acceptability of NAMs for read-across.
Many such R&D needs could be addressed, in part at least, by well-designed case studies centred on a REACH submission.

2.4 Case Study 3: metabolomics analysis of phenoxy herbicides

2.4.1 Plenary presentations

Bennard van Ravenzwaay presented the read-across case study using metabolomics analysis with phenoxy herbicides, as well as the RAAF assessment of this case study.

This case study demonstrates how new technologies, such as metabolomics, can, from a biological perspective, help to establish a read-across case for pesticides (e.g. phenoxy herbicides). Specifically, (RS)-2-(4-Chloro-2-methylphenoxy)propanoic acid (MCPP) was selected as the target chemical and 2-methyl-(4-chlorophenoxy) acetic acid (MCPA) and 2-(2,4-dichlorophenoxy)propionic acid (2,4-DP) were possible source chemicals.

The 28-day metabolome evaluations of the two source chemicals indicate the liver and the kidney as the target organs. The metabolome evaluation of the target chemical provides the same information. The overall comparison of the metabolome data indicate that 2,4-DP is a better source chemical than MCPA.

The read-across of the 90-day repeated-dose data for 2,4-DP predicted that MCPP would show decreased food consumption and body weight gain at 2 500 ppm. The target organs are the liver (weight increase and clinical-pathology changes), as well as the kidney (weight increase and clinical-pathology changes). A moderate reduction of red-blood cell parameters would also be expected at this dose level.

Qualitatively, these predictions are very similar to the results of the actual 90-day study in rats performed with MCPA (i.e. reduced food consumption and body weight gain, target organs: liver and kidney – weight increases with concomitant clinical-pathology changes, reduced red blood cells values).

Quantitatively, the predicted NOAEL (150 ppm) is in the range of that of the actual study (NOEL 75 ppm, NOAEL below 500 ppm). The case study concluded the 90-day rat toxicity study of MCPA could have been waived and substituted by the 90-day results of 2,4-DP.

2.4.2 Discussions: key topics and conclusions drawn

Key topic: interpretation and added-value of metabolomics data as NAMs to support a read-across hypothesis

- There was a common view that the metabolomics case study demonstrated that, in the context of NAMs, the information from the analysis confirmed the probability of liver and kidney toxicity through activation of/binding to PPARα.
- In this case study, the NAMs were the basis of the mechanistic understanding, thus providing considerable added value and showing how metabolomics can

increase confidence in a read-across hypothesis. In this manner, the read-across case study relied completely on the NAMs.

- It was generally agreed that this kind of metabolomics analysis and NAMs can support a read-across argument. However, this must be considered case-by-case. The overall concept is that if a source and the target chemicals have a similar metabolic profile, then this could confirm similarity and would provide a strong basis for regulatory acceptance, negating the requirement for animal testing.
- There was an appreciation that for this case study there was strong understanding of the patterns of metabolites and their interpretation, in part due to the anchorage to the full dataset. However, uncertainty remained about how this information could be applied in a more general context.
- The presenter stated that there are different metabolomics patterns for different toxicities; some are quite specific, such as agonist of PPARα. Having such mechanistic IDs for specific patterns allows for the use of the patterns as fingerprints to compare the profiles for different substances without the specific need to translate or interpret the effects.
- It was noted that the current (arbitrary) statistical threshold of 90 % of metabolites needed to be changed (up- or down-regulated) could also add to the variability of interpretation of metabolomics and hence increase uncertainty.
- There was concern regarding the over-sensitivity of some enzymes and the need to identify those enzymes and metabolic responses that are meaningful. This and the relevance of other effects that may or may not be important to hazard identification was a source of uncertainty.
- The presenter addressed concern that adverse effects were also observed to the testes and adrenal glands. These increased uncertainty as these effects were not consistent with the mechanistic hypothesis. However, it was stated that these effects were an artefact of the test relating to reduced food intake and being non-adverse respectively.
- It was concluded that metabolomics analysis is suitable to provide information on general effects only.
- There was also discussion on the species relevance of NAMs. The current case study provided information relevant to rat, but not to humans. The importance of species, strain and experimental conditions must be considered when interpreting metabolomics information.

Conclusions:
NAM data from metabolomics have provided a robust and interpretable basis for the mechanism of action which has supported the hypothesis of category membership and facilitates read-across.
There are still uncertainties in the use of NAM data from metabolomics in addition to a lack of knowledge and expertise in their meaning.

Key topic: better use of animal studies

- There was a general discussion regarding the temporal aspects of gaining NAM data from metabolomics. This broadened into a general discussion of the added value of a 28-day study compared to a 90-day study. The presenter stated that

for the three chemicals in this case study, the metabolomics responses peaked between day 14 and 28 and that at 90 days there were lesser effects. The lesser effects at 90 days could be because the animals return to a form of homeostasis.

- There was general discussion about the value of the 90-day repeated dose assay and whether it added any greater information than the 28-day (or even 14-day) assays. Currently, there is evidence that there is no added value in continuing a 28-day study to 90 days for some chemicals e.g. low toxicity. However, no strong conclusions could be determined from this area.
- A participant stated that if studies could be combined e.g. repeated dose toxicity testing and metabolomics there could be a saving in animal usage. For instance, on the basis of the information provided by the case study, the current 90-day study test guidelines could, possibly, be replaced by a 28-day study with additional metabolomics analysis. A note of caution was sounded from one participant in using short-term data e.g. 28-day as a replacement for 90 as they may not take account of bioaccumulation.
- A participant stated the metabolomics required the use of animals to obtain the information. This raised issues over the use of animals and whether *in vitro* tests could provide the same information. The presenter stated that obtaining comparable information *in vitro* was currently a challenge. However, some progress is being made in this area but it should be remembered that it would need a stabilised system, to be undertaken organ-by-organ and may need more than 30 cultures of cell lines.
- A participant suggested that comparisons could be made with the human genome project and linking to information from that source.

Conclusions:
There is a possibility, for some compounds at least, that a combination of a 14-28-day repeated dose test, combined with metabolomics analysis, could replace the 90-day test, depending on the purpose and the information requirements.
The use of *in vitro* omics analysis to replace *in vivo* testing requires further assessment.

Key topic: general consideration of the Case Study 3 read-across scenario

- There was a general appreciation of the need to identify stereoisomers as well as to have access to the impurity profiles.
- A participant commented that the use of Tanimoto scores, e.g. from chemical fingerprints or other descriptors, for similarity assessment is limited and should be used with caution. Another participant suggested that there is no single best measure of similarity, but the use of a number of approaches would be appropriate. A further participant stated there were a number of methods to determine molecular similarity and structural alerts (e.g. in the OECD QSAR Toolbox) were a good starting point. Similarity scores such as Tanimoto may be better used for grouping/sub-categorisation, termed fine-grain similarity, once a group of compounds has been formed from, for instance, a mechanistic profiler or a series of analogues. It was additionally noted that Tanimoto scores of similarity should not be viewed as a linear scale.

Conclusions:
There is a need to define chemical structures adequately for read-across assessments.
A better understanding of the use of the Tanimoto coefficient to define chemical similarity is required.

Key topic: further possibilities and R&D needs for NAMs

- There was general discussion that the use of NAMs from metabolomics analysis required considerable investment and expertise in:
  - analytical chemistry,
  - statistical processing power, and
  - the availability of a database of existing information.
- The limiting factor is likely to be the availability of a database of high quality toxicological information on which to anchor the mechanistic interpretation and relevance of the findings. However, there is a considerable cost and capacity implication in all of these issues. Linked to the need of the database was the need for the appreciation of the chemical space it represents.
- Several participants noted that linked to the need for a database is the requirement for biomarkers and understanding the patterns of biomarkers. The EU IMI Safe-T project, which focused on developing new safety biomarkers to help significantly improve patient safety and reduce safety related attrition in drug development, was mentioned as a potential source of biomarkers, however, with an emphasis on pharmaceuticals.
- It was agreed that greater transparency is required in the use of metabolomics as NAM data. Specifically access to the data and the algorithms used in the assessment, as well as relevant information on validation results would be required by scientists from regulatory agencies so that they are able to judge the validity (or otherwise) of these methods.
- A participant stated that the current documentation and guidance may need to be updated for future application of metabolomics data as NAMs. This could be extended to an understanding of the plausibility of metabolomics data and realistic expectations e.g. for transferability.
- It was also stated that there is a need for standardisation of metabolomics methods and their interpretation to form reliable NAMs.
- There is a need to validate further metabolomics approaches to provide NAMs, with clear criteria being required for validation.
- A participant emphasised how metabolomics could fit into the overall bottom-up/top-down approach to understanding toxicology. Toxicological relevance (even to humans) could be extended by including, or placing in the format of, a mode-of-action ontology. The ability to perform screening (top-down) could be combined with detailed metabolomics (bottom-up) analysis within categories.
- Further research is required to investigate how NAMs, based on metabolomics and other assays, could be used to confirm no or low toxicity.

Conclusions:
There are a number of R&D needs including a database to support metabolomics, standardisation, validation and reporting formats.

2.5 General comments on the use of NAMs in definitive hazard assessment and read-across in particular

A number of comments were made which are generic to the issue of NAMs being used to support a read-across hypothesis. These issues are generic and applicable to the use of NAMs in general and not necessarily a specific read-across case study.

Overview

- A panel member concluded that NAMs have a very positive future in terms of supporting read-across. However, it was stressed that no one single approach will fit all categories. Further research is required to determine which NAMs are appropriate e.g. for negative and positive read-across predictions. There was general agreement that it is not a question of "one size fits all" for the use of NAMs in read-across.

Possible application of NAMs

- With regard to the acceptance of read-across, especially when supported by NAMs, it was agreed that differing levels of uncertainty will be acceptable in different contexts e.g. prioritisation, classification and labelling, risk assessment.
- The RAAF introduces a spectrum of read-across elements for checking chemical structure, similarity, biological effects/mechanisms of action, and toxicokinetics, each supported by the appropriate evidence. The presence of a spectrum was acknowledged within the workshop, while the exact process for applying the information was seen as less well developed.
- It is clear that similarity is context- and case- dependent and in most, if not all, cases both chemical and biological data (including those from NAMs) would be required to some extent. The need for chemical and biological similarity was also referred to in terms of the use of the read-across scenario.
- The current use of read-across according to Annex XI to the REACH Regulation there is a requirement for chemical similarity which is a pre-condition for the consideration of any other type of similarity, e.g. biological read-across.
- There was agreement that NAM data must be mechanistically based. They can be derived from a range of processes including *in silico* modelling and *in vitro* measurements. Ideally some, or all NAMs, would relate or include evidence from the MIE or key events as defined by the AOP.
- The ability to make better risk assessments from read-across by adding in NAM information was highlighted. The possibility was also raised that including NAMs may allow the chemical coverage of categories to be increased i.e. disparate structural categories could be joined together on the basis of biological similarity – such a process is commonly applied in the development of pharmaceuticals. However, it is acknowledged that these compounds are designed with a specific and targeted activity, which can be used as a basis for a complex grouping of non-structurally similar compounds. This will require a process of cyclical and iterative development of categories linking biologically similar molecules.

Summary of future R&D needs and further steps

- The need to better understand uncertainty and the quality of data was seen to be very important. This could be achieved through further case studies. There was general agreement that uncertainty in the case studies was increased due to the unknown quality of the *in vivo* data.
- There is a great need for capacity building and better appreciation of what NAM data are. Frameworks to apply read-across and especially NAMs are required, these could, for instance, build on the RAAF. These could encourage registrants (for REACH) to structure and assess the scientific arguments.
- In terms of applying NAMs, more effort and thought is required to validate all the approaches. This could require new approaches to validation (see Themes 2/3 in this report) and also financial commitment.
- There was agreement that the use of NAMs must be case-by-case. More work is required to understand what is the minimum number of assays and datasets. A participant commented that common standards are required for the use of NAMs to support read-across. This could include statements on the reliability of the tests.
- The need for case studies was emphasised which could be provided by industry. It was also noted that the OECD has a project where member countries and other stakeholders submit and review case studies related to the use of IATA which could provide further information. Indeed, SEURAT-1 read-across and *ab initio* case studies are to be presented to the OECD in 2016.
- The development of NAMs by industry was discussed by a participant. The drivers for industry uptake will include cost, the time to register and the level of certainty that NAMs will provide. Currently, many NAM techniques are no cheaper than the *in vivo* tests with no certainty of acceptance from regulators as a direct replacement of standard information requirements. An opinion exists that it is "safer" to use traditional testing regimes, rather than NAMs, to increase the likelihood of acceptance.

2.6 Summary

A number of key conclusions were drawn from Theme 1 of the workshop. These and other shared views between the participants are summarised in Table 1.

Table 1. Key conclusions drawn from Theme 1 of the workshop

Topic	Workshop shared views
Added value from NAMs	Data from NAMs have the potential to add value to a read-across hypothesis by reducing uncertainties in e.g. mechanistic plausibility.
Case-by-case use of NAMs	There is no "one size fits all" with NAMs to support read-across. They will need to be used selectively and appropriately to the read-across scenario.
Possibility for application	The applicability of NAMs and when they are of sufficient quality is not yet fully known.
Toxicodynamics	When used appropriately, NAMs currently have the capability to support the toxicodynamic hypothesis underpinning a read-across argument and hence reduce uncertainties.

Toxicokinetics	In the case studies, NAMs, e.g. metabolism simulators or PBTK models, were not used to reduce the uncertainty regarding toxicokinetic aspects. Potential reasons for this could be lack of availability of such NAMs and/or particularly high barriers precluding their application. This is a clear area for further development.
Mechanistic basis	NAMs (for toxicodynamics) are more useful when there is a clear mechanistic basis and/or link to an AOP.
Chemo-bio similarity	There is a need to consider both chemical similarity and (wherever possible) biological similarity in the development of a category. Subsequently, it has to be determined whether belonging to this category allows for the prediction of certain toxicological effects or the absence thereof.
In vitro to *in vivo* relationships	A better understanding of how *in vitro* NAMs relate to *in vivo* response is required. This is for all levels of NAMs e.g. from cellular responses, to cells and organoids.
Metabolomics	NAM data from metabolomics have the possibility to confirm mechanistic plausibility. Some clear R&D needs were identified including better understanding and the need for a high quality database.
Research needs for NAMs	A number of clear research needs for NAMs were identified ranging from the better use of biomarkers, development of *in vitro* methods to replace reliance on *in vivo* testing (i.e. for pathway analysis), imaginative use of current testing species and understanding of species-specific pathways.
Data quality	Data quality was poorly addressed in the case studies due to a lack of access to original study reports (for both *in vivo* and NAM data) and a lack of criteria to assess data quality.
Data accessibility	Greater accessibility to (*in vivo* and NAM) data is required to support read-across.
NAM standardisation	There is a need to standardise NAM approaches and their output.
NAM validation	Further validation (in terms of their acceptance for purpose) of many NAMs is desirable.
NAM reporting	A standardised reporting format for NAMs is required which could, for instance, be developed from OECD OHT 201.
Case studies	Further case studies using NAMs, including a possible REACH submission dossier, are encouraged. More specifically, case studies integrating NAMs pertinent to toxicokinetic aspects would be welcome.
Better understanding	There is a clear requirement for better understanding of NAMs and the role they may play in reducing uncertainty in a read-across scenario.
Training and education	There is a need for training and education in the area of NAMs and their application for read-across.

Capacity building	There is a need to build capacity, in terms of facilities, to support the use of NAMs.
Outreach	There are possibilities to learn from areas such as personalised medicine. Current projects such as EU-ToxRisk in the EU and Tox21 in the US may provide vital input into the use of NAMs to support read-across.
RAAF	The RAAF was found to be a flexible framework to assess a read-across prediction. It shows how uncertainties can be reduced by adding NAM data.

3. Theme 2: screening and priority setting

3.1 Introduction to Theme 2

Theme 2 focused on the role of NAMs in supporting screening and priority setting of chemicals in the regulatory context. It focused on the existing use of "Screening Technologies – In Silico and In Vitro" (as outlined in Section 2.2 of the Workshop Background Document available from: http://echa.europa.eu/documents/10162/22049802/tsws_background_document_en.pdf).
The presentations and discussion brought in experience and knowledge from four regions, with differing means of applying NAMs and providing WoE to make regulatory decisions, appropriate to local legislation. Common threads and elements of good practice have been drawn together in this section.

3.2 Plenary presentations

Four plenary presentations were made in this session representing four geographical regions. The presentations focused on regulatory frameworks and how NAMs could be used and were given by the following presenters:

- Kerry Nugent from the National Industrial Chemicals Notification and Assessment Scheme (NICNAS), Australia described "*The NICNAS Inventory Multi-tiered Assessment and Prioritisation (IMAP) Program*".
- Richard Judson from the United States Environmental Protection Agency outlined "*The Application of Computational and High-Throughput in vitro Screening for Prioritization*".
- Christine Norman from Health Canada gave a presentation "*Integrating New Approach Methodologies under Canada's Chemicals Management Plan*".
- Panagiotis Karamertzanis from ECHA explained "*A common screening approach for REACH and CLP processes*".

Table 2 summarises the main points made with regard to the use of NAMs, without necessarily focusing on the regulatory framework itself.

Table 2. Key points regarding the use of NAMs and good practice from the plenary presentations on screening and priority setting.

i) Region ii) Regulatory framework iii) Type of approach	**Key elements of regulatory programme**	**Use of NAMs**	**Good practice**
i i) Australia ii) NICNAS Inventory Multi-tiered Assessment and Prioritisation (IMAP) Program iii) Three-tier prioritisation scheme	Three tiers: • Identification of chemicals which, using exposure data, do not require further consideration. • Identification of relevant data, and preparation of a brief report to characterise the likely risks for chemical identified from exposure data. • Examination of whether appropriate risk management measures already exist, and whether the available data are sufficient to justify relevant risk management measures. Tier III comprises assessment of any critical questions identified in the Tier II examination of the available data.	• Exposure information may be considered as a contributor to NAMs. • Exposure can be considered as a function of use and volume – this gives an exposure score which placed a chemical into an exposure "band". • Tier I uses (Q)SAR for some endpoints in at least some cases. • Tier II/III use of expert judgement including read-across/grouping, (Q)SAR, bioelution, other hazard and mechanistic data i.e. anything that can contribute to weight of evidence.	• Prioritisation by consideration of a matrix of exposure and hazard. • Hazard "bands" i.e. assessments were based on GHS classification scheme. These were "data agnostic" i.e. any NAMs could be applied. • Clear linkage to exposure scenarios throughout. • QSAR is used to determine mechanistic relevance e.g. to support category formation. • A flexible scheme which can accommodate further new types of data. • Future work may consider an extension of the TTC concept to risk management recommendations.
i) USA ii) Endocrine disruptor screening program iii) Computational and *in vitro* prioritisation of potential endocrine	• Multiple high throughput *in vitro* assays aimed at replacing uterotrophic assay. • All available assays for e.g. oestrogen receptor binding, were considered. • A mathematical model was used to integrate the outputs from the	• Multiple *in vitro* assays • Different high throughput screening / content technologies applied • Different points of pathways considered • Multiple QSARs used, with results combined.	• The NAM approaches are considered valid by the US EPA to replace guideline studies. • Well characterised *in vitro* assays and a thorough understanding of the data. • Appreciation of limitations of *in vitro* assays i.e. that some will be incorrect as well as the uncertainties.

disruptors	assays. • QSARs for oestrogen receptor binding, agonist and antagonist activity – consensus approach (the Collaborative Estrogen Receptor Activity Prediction Project (CERAPP)).		• Consideration of variability and uncertainties in *in vivo* data/ guideline studies (uterotrophic assay) allowing for a better understanding of the guideline data and identifying chemicals with a high quality response. • Strength in numbers – many assays and the possibility to form a consensus. • NAMs founded on pathways. • Anchorage to *in vivo* data. • Establishing the effect of data quality on use of NAMs and modelling. • Use of consensus QSAR approaches, with the consensus providing a better prediction than a single approach. • All data are made public: transparency of all data through the EDSP21 Dashboard. • Validation of NAMs not based on OECD criteria, but the ability to show good correspondence between *in vitro* and *in vivo* responses, as well as good documentation and a public review.
i) Canada ii) Chemicals Management Plan (CMP) iii) Three-phase risk assessment initiative	• Focus of risk assessment programme is based on past priority setting exercise of industrial chemicals (Canada's Domestic Substances List Categorisation). • Priority setting moved 4 300 chemicals to risk assessment to be assessed in three phases by 2020; many with limited datasets. • Margin of exposure (MOE) approach routinely applied as traditional risk	• CMP Phase 2 used grouping, read-across and QSAR approaches. • The proposed BER approach provides a method for applying *in vitro* data e.g. from ToxCast in risk assessment. • BER and MOE similarly apply real-life exposure scenarios. • High-throughput toxicokinetic data is required to convert *in vitro* bioactivity to oral equivalent dose	• Collaborative activity with the US EPA. • Case study on substituted phenols applied ToxCast data to assess BER and compare to traditional MOE; systematic approaches to identify valid analogues and integrate NAMs into IATA-based hazard characterisation was also illustrated. • Case study provided valuable information – combines data rich and data poor substances; many are high

	assessment method. • Bioactivity exposure ratio (BER_) is being explored for priority setting and risk assessment. • A "risk assessment toolbox" developed under the CMP proposes to apply NAMs for risk assessments of different levels of complexity e.g. Type 2 screening approaches, Type 3 grouping and read-across approaches. • Exploration to date suggests that the BER provides a valuable metric linking activity of a substance (following conversion to an oral equivalent dose using pharmacokinetics and *in vitro* to *in vivo* extrapolation) to the estimated human exposure of that substance; the magnitude of the BER may be a useful indicator of the potential for concern arising from the detection of positive responses, which can be integrated into decision making.	for use in screening and risk assessment. • NAMs may also be used to support IATA based hazard classification. • A custom similarity metric including substituent position and chemical identity was used to identify analogues and to establish closest neighbours. • Data matrix of QSAR predictions and *in vitro* data have been collected for oestrogen receptor pathways; data matrix is consistent with the approach proposed by the OECD IATA case studies project.	volume chemicals • Use of *in vitro* data and real world exposure values to calculate BER to compare to MOE calculated using *in vivo* toxicity data and same exposure values • Shows the utility of ToxCast/ExpoCast approaches. • Recognises the need to interpret how to use BERs in relation to MOEs; i.e. the need to benchmark against "traditional" information. • BER shown to be health protective; further proof-of-concept for the use of HTS data in regulatory applications is ongoing. • Case study demonstrated use of high quality QSAR-ready dataset i.e. curated structures. • Similarity metric for analogues is more sophisticated than a traditional Tanimoto index alone. • Data collection, including NAMs, for parent chemical as well as analogues was completed to support data-gap filling and read-across for risk assessment.
i) European Union ii) REACH and CLP iii) Harmonised classification and labelling (CLH); identification of SVHCs (possibly leading to	• Common screening identifies and prioritises substances most important for health or the environment. • Member States heavily involved in substance evaluation of shortlisted substances.	• IUCLID database contains data including NAMs (for screening). • Sources of information include registration data, C&L notification data, external datasets and predictive methods. • Predictive methods include structural alerts, QSAR models and	• Use of IUCLID to capture NAMs. • Use of many and varied sources of information to produce hazard results, exposure considerations. • Algorithm for predictions encourages use of NAM data. • All information for common screening placed in an internal (ECHA) database.

authorisation); restriction Screening of a shortlist of substances		known metabolism paths and predictions. • A comprehensive set of algorithms is run to make an assessment from all data. • Chemical structures vital to link to other information and for grouping. • Use of priority lists, text searching, QSAR, ToxCast, categorise into modes of action, data in registration dossiers. • Many external experimental datasets used – these include NAMs. • Exposure is considered, as well as hazard, to prioritise chemicals for short listing. • Grouping is used – structural similarity and read-across – to search areas of chemical space.	Information is summarised in screening definition documents. • Structures generated for "substances" within IUCLID to identify individual components – 200 000 non-UVCB structures used in screening. • Searches many data sources in a flexible manner to obtain data and NAMs. • Encourages industry to provide tonnage per use and other information to support exposure assessment; this information can also be captured in IUCLID. • Consider groups of compounds rather than single compounds – means that information can be optimised with local areas of chemical space. • Use dendrograms and other techniques to visualise groupings, demonstrate boundaries of chemicals. • Documentation of processes on ECHA website for transparency. • Infocard for each chemical on website to summarise information used and decisions.

3.3 Discussions: key topics and conclusions drawn

The following is a summary of the discussions and findings from the plenary presentations with regard to the use of NAMs for prioritisation and screening.

Key topic: relevance and understanding of NAM data for screening and prioritisation – the importance of data quality

- With regard to how NAMs can be used, a panel member stated that they must be used to address specific endpoints. The utility of NAMs in the broadest sense will be the same as using *in vitro* data.
- NAMs may (currently) be best used to screen for/prioritise/identify chemicals of high concern e.g. associated with a particular endpoint for adverse effect. There is, however, a need to determine the relevance of NAMs. As such, it is important to pay attention to the meaning of the data, especially for relatively data poor chemicals. NAMs may support mechanistically based read-across. NAMs may also be used to deprioritise.
- A panellist raised the issue that *in vitro* data must be treated with caution. In certain circumstances, false positive *in vitro* data occur e.g. in ToxCast there is a well-established "burst effect" at high concentration. However, when the meaning and relevance of data are known they can be used with reasonable confidence to confirm the safety of foods as opposed the relative hazard of pesticides.
- EDSP analysis of uterotrophic data illustrates reproducibility – or lack of it – of *in vivo* guideline data. The data set of uterotrophic assay results, curated by the US EPA, is of high quality and demonstrates good practice in curating data.

Conclusions:
There is a growing understanding, which is in no way complete, as to how NAMs may be used in global schemes for prioritisation and screening. Data and information from NAMs are accepted in various regulatory schemes around the world.
Understanding of data, which includes their meaning, relevance, and the reliability of the information, is crucial for their successful application.

Key topic: role of exposure in screening and prioritisation schemes

- A presenter stated that exposure can be estimated by use and volume.
- A panellist confirmed the concept of using exposure in volume bands in the REACH legislation and that models for exposure have been used internationally.
- A panellist confirmed that, from the European perspective, there is promise in considering exposure information, but it is difficult to obtain this information and complex to apply. One scenario suggested the use of exposure in priority setting. Another panellist suggested the use of exposure in screening alone.
- In Canada, the aim of the bioactivity exposure approach is not necessarily to be predictive, but to be health protective for data poor substances e.g. to learn from historical margins of exposures to calibrate exposure from bioactivity exposure ratios – especially when an endpoint is not known. A variety of NAMs could be used e.g. *in vitro* combined with (Q)SARs and software such as OASIS-TIMES to assess metabolic activation.

- A participant queried the role of exposure in schemes, in some schemes exposure is put before hazard assessment. There is clearly disparity in global schemes with differences in the manner in which exposure is expressed and described, as well as being applied. A panellist confirmed the differences in different global scheme. The panellist described the Australian system where use and exposure is different, for instance for cosmetics (e.g. frequent dermal application) and industrial chemicals (e.g. very limited exposure in manufacture). There are also complications to use of exposure e.g. an ingredient may be used both in cosmetics and pesticides, thus with different use levels and exposure scenarios. The use of exposure-based banding in novel notification schemes was suggested.
- It was noted that the threshold of toxicological concern (TTC) is a form of exposure consideration, with different final exposure scenarios. TTC provides a qualitative assessment of the potential for risk of chemicals with low levels of exposure.
- Other findings on exposure are reported in the section on *"achievable short-term goals".*

Conclusions:
In the absence of "traditional" exposure data e.g. measurements of blood concentrations etc. information on exposure from other sources such as use and tonnage can be extremely useful for screening and prioritisation.
Information on exposure may be obtained from various sources, e.g. an understanding of use and/or tonnage, or from *in vitro* assessments combined with HTTK such as ExpoCast.

Key topic: short- to mid-term goals with the use of NAMs in screening and prioritisation schemes

- There was discussion of the achievable short-term goals for the use of NAMs. A panellist said the easiest NAMs to use with confidence would be those that are closest to the MIE for a mechanism of action. Taken as an example, NAMs can identify if a chemical could bind with the oestrogen receptor, but it would be more difficult to prove birth defects from NAMs. With regard to the EDSP, evidence of the activation of pathways may be an indication that chemicals could cause teratogenic effects, but evidence of downstream effects will be more difficult to determine.
- The panellist confirmed that the clearer the linkage of the MIE to the adversity, the easier it is to use the information for regulatory purposes, thus emphasising the need for robust AOPs. NAMs measure key events (within an AOP) with an emphasis on the first key event, or MIE. A panellist also stated that the potential severity of effect must be considered.
- A panellist said efforts could be placed into better characterising uncertainty. It is important to understand the limitations of the NAM data, especially for environmental effects. The role of WoE and uncertainty analysis is very important.
- A number of achievable short-term goals with regard to exposure were identified by a panel member. The panellist stated that exposure is important for decision making. An upcoming initiative within Cosmetic Europe's long range science

strategy (LRSS) will target exposure, particularly to identify realistic and usable exposure data.

- In the Canadian scheme, NAMs support consistency across a category and may be used for read-across. They support groupings and the validity of chemical membership of a grouping – for endpoints of concern on the basis of exposure scenarios. A panellist queried whether in this scheme and use it would be possible to make a decision on a single chemical rather than a category. The panellist stated that a small number of chemicals under regulatory consideration were of low concern, and the NAMs confirmed this. More experience would be required to move NAMs from screening and prioritisation to making decisions on single chemicals, which may be of greater concern.

Conclusions:

In addition to the current use of NAMs for screening and prioritisation, there are achievable short-term goals in areas such as better understanding the role of exposure, characterisation of uncertainty, definition of the meaning of NAMs with regard to the MIE and/or other key events and supporting grouping.

Key topic: how can NAMs be applied for no- and low-toxicity chemicals?

- A panellist emphasised the challenge of confirming the no/low toxicity of chemicals. It was agreed that NAMs can be used for identifying hazard, but there is less experience and knowledge for no/low toxicity chemicals.
- A panellist responded that this topic could be related to exposure and the understanding of uncertainties with NAM data. It is possible to calculate exposure estimates and, in combination with data from cell lines (e.g. ToxCast) and toxicokinetics, confirmation of no/low toxicity relative to the estimated levels of exposure could be made. There is a need for, as a minimum, *in vitro* pharmacokinetic models to support this effort. Ultimately, a knowledge that exposure (in particular) is significantly less than the point of departure for any pathway would result in a classification of acceptable risk. Exposure models e.g. the US EPA ExpoCast are improving. When they are improved, they will become more acceptable.
- A panellist confirmed that more research is required in this particular area, particularly to set aside certain chemicals. Indeed, one of the challenges for the developers of NAMs is to set criteria for compounds of lower concern.
- A word of caution was sounded by a participant who stated that for NAMs such as ToxCast, a negative result from an assay cannot necessarily be relied upon. However, a positive value from ToxCast could be used to provide a NOEL. There is a greater need to understand the data and degree of certainty in the negative values; whilst having no traditional data also should be viewed in terms of the lack of certainty this brings.
- A participant noted that for the EDC assays, ToxCast data have been used successfully to de-prioritise known weakly active and/or safe compounds. A compound such as genistein, which is a weak oestrogen agonist, could be used to provide boundaries to help understand and/or use the information from NAMs. Benchmarks could be sought for similar types of activities to provide a rationale to de-prioritise chemicals.

Conclusions:
There is potential for NAMs to screen for chemicals with no/low toxicity, for instance by including hazard (e.g. ToxCast) and exposure (e.g. ExpoCast) data.
More work on how to apply NAMs to chemicals with no/low toxicity is required.

Key topic: other comments on how NAMs can be applied for screening and prioritisation

- A participant raised the issue of whether we need NAMs to be predictive or to be protective. The current paradigm is somewhat dated and could be reconsidered in terms of mapping protection goals (for risk management of chemicals) onto information requirements. The original protection goals were based on information that is decades out of date. Other participants confirmed the importance of protection goals as well as ensuring the correct questions are asked. As an overall consequence of this, one outcome may be that to exploit the possibilities and potential of NAMs to their fullest it may be necessary to change the regulatory context and framework i.e. adapt risk management frameworks to make best use of NAMs.
- A participant suggested that new hazard classifications could be established that were not linked to the traditional animal methods, but from NAMs which may be more relevant e.g. bioactivity spectrum.
- Other comments were directed towards use of technologies such as metabolomics in screening and prioritisation. Whilst this may provide useful patterns of information, more work is required to understand practical issues such as reproducibility. A comparison was made with medical diagnostics, about whether all links are required to be known – in principle NAMs may be acceptable when all the linkages are not known, but NAMs cannot be accepted where the validity and reproducibility of the technique is not known.
- EDSP illustrates strength of using *in vitro* data (i.e. oestrogen receptor binding) to create QSARs. In addition, the development of multiple consensus QSARs (CERAPP) showed promise. Caution was recommended when using the same data for interpretation and to develop QSARs, which again are used for interpretation.

Conclusions:
The use of NAMs to make decisions regarding screening and prioritisation could be considered further in light of the information they provide as compared to the traditional paradigm of animal testing.

3.4 Summary

A number of key conclusions were drawn from Theme 2 of the workshop. These and other shared views between the participants are summarised in Table 3.

Table 3. A summary of the workshop shared views on the use of NAMs for screening and prioritisation.

Topic	Workshop shared views
Variety of NAMs used for screening and prioritisation	A number of different types of NAMs, including (Q)SAR, read-across, *in vitro*, HTTK and exposure information are used to satisfy global regulations for screening and prioritisation.
Differences and similarities in global regulation for screening and prioritisation	Each regulatory framework uses NAMs differently. However, there are commonalities e.g. in the use of QSARs, read-across for hazard identification, as well consideration of exposure data.
Opportunities for sharing of knowledge, common good practice and data	There are distinct possibilities to share good practice with regard to the use of NAMs for screening and prioritisation. In addition, NAM data on chemicals could be shared e.g. a greater use of the ToxCast data.
Assessment of exposure	NAMs can contribute to an understanding of exposure both through usage considerations or measurements from *in vitro* and integration of HTTK.
Use of exposure data	Information from exposure can be used for screening and prioritisation.
Reliability and quality of NAM data	The reliability of NAMs is variable and should be ascertained, as this will influence the quality of the information that may be derived from them.
Screening and (de-)prioritisation of chemicals with no/ low toxicity	There is a possibility to use NAMs to "screen out" or "deprioritise" chemicals with no/low toxicity. There is a need to better understand how NAMs could facilitate this process.
Case studies required	Case studies of the use of NAMs for screening and prioritisation are useful and more could be considered (e.g. within EU-ToxRisk) to assist with specific issues and for chemicals with no/low toxicity.
Consensus approaches	There is strong evidence that consensus approaches for using NAM data, whether they are from computational and/or experimental approaches, are more reliable than any single NAM method.
Human protection, not prediction of animal tests	NAMs give the opportunity to provide information for human (and environmental) protection directly. This should be viewed as progress and a step-change from the goal of predicting traditional animal tests.
Opportunity to update regulatory paradigms	Currently, regulatory frameworks rely on the findings from traditional animal testing; these are not necessarily relevant to the information NAMs may provide. Means of updating the regulatory context, so that decisions may be made from NAMs, should be sought.

4. Theme 3: prospects for regulatory science

4.1 Introduction to Theme 3

Theme 3 was a forward-looking assessment of how NAMs could be applied in regulatory science in the short/medium term. It built upon the previous studies by illustrating some of the requirements in a broad context i.e. future potential regulatory application in the EU and the USA as well as specific issues that need to be addressed for the successful use of NAMs in regulatory science.

4.2 Plenary presentations

Four plenary presentations were made. These included illustrations of how NAMs could be applied from ECHA and the US EPA. The four plenary presentations were:

- Russell Thomas, United States Environmental Protection Agency, outlined *"Moving Towards Version 2.0 of Toxicity Testing in the 21st Century and Application to Regulatory Decision-Making".*
- Romualdo Benigni, Istituto Superiore di Sanità, Italy, gave an overview of *"How to Overcome Limitations of New Approach Methodologies in the Context of Regulatory Science".*
- Timothy W Gant, Public Health England, presented *"Analysing Data: Towards a Framework for Transcriptomics and Other Big Data Analysis for Regulatory Application".*
- George Fotakis, ECHA, described *"Using new approach methodologies in regulatory science: tools and methods for integration of evidence".*

Table 4 summarises the key aspects from the plenary presentations, emphasising the role of NAMs in regulatory science.

Table 4. Key points from the Workshop's Theme 3 plenary presentations regarding the prospects for regulatory science.

i) Region ii) Type of approach	Key elements	Use of NAMs	Good practice
i) USA ii) Moving Towards Version 2.0 of Toxicity in the 21st Century	• Version 2.0 has a number of new assays and software following learnings from "ver 1.0" implementation of NAMs. • Solid chemical characterisation in terms of chemical structure. • Incorporation of high-throughput transcriptomics will allow a comprehensive survey of potential biological pathways and MoAs of chemicals. • ToxCast/Tox21 assays used to confirm MIEs and pathway effects from transcriptomic screen. • Addition of organotypic assays and microphysiological systems used to interpret organ- and tissue-level effects of pathway perturbations. • Combination of NAMs into a tiered testing framework. • Inclusion of high throughput toxicokinetics from *in vitro* data and computational modelling provide dose context. • Population exposure is estimated from use and production volume, this can be compared with bioactivities for various product classes to provide risk context.	• Generalised approach to read-across on the basis of chemistry, biology or chemotype. • Biological NAM data bin chemicals according to relative selectivity with many MIEs being targeted. • High-throughput transcriptomics to broadly survey potential biological pathway purturbations and MoAs. • ToxCast/Tox21 assays provide orthogonal confirmation of MoA and pathway effects. • Retrofit *in vitro* assays with metabolic competence. • Organotypic assays used to translate pathway perturbations into organ- and tissue-level effects. • HTTK to provide dose context. • Additional HTTK assays for chemical classes where transporter functions are important e.g. to predict *in vivo* pharmacokinetics.	• Chemical characterisation is required to be robust i.e. high quality structures to build datasets and QSARs. • Generalised read-across approach gives a quantitative estimate of uncertainty. • Use of broad/in depth (NAMs) assays to gain understanding regarding mode of action/AOP – allows for a range of MoA/AOP to be considered. • ToxCast information being evaluated according to gene coverage/toxicological space e.g. high throughput transcriptomics platforms. • Inclusion of metabolic competence, e.g. plate lid with a sphere of S9. • Using an intelligent tiered testing framework to rationally interpret test results allowing for interpretation of NAM output into adverse effects. • HTTK being assessed on a "by class" basis to determine where they are most predictive. • Case studies to demonstrate application of NAMs in ver 2.0 of 21st Century Toxicology to regulatory decision making.
i) Italy	• QSAR and *in vitro* approaches are	• QSAR and *in vitro* methods to	• Errors in a predictive model can inform

ii) Computational and data analysis of information from NAMs	used to provide information to predict toxicity. • Data analysis to assess the reliability of NAM data. • Systematic evaluation of ToxCast data. • Analysis of QSARs to provide mechanistic information.	predict apical endpoints and assess mechanism of action. • Use of NAMs to predict skin sensitisation and identify the rate limiting step. • Correlations sought between ToxCast (ver 1.0) data and *in vivo* responses. ToxCast assays found to be better correlated with specific responses (e.g. endocrine disruption) as opposed to unspecific effects e.g. repeated dose toxicity which are less defined mechanistically.	how to improve models. • Data analysis allows for better understanding of model performance and identification of rate limiting steps (which are often MIEs). • Difficulties established in modelling /finding assays for intermediate key events. • QSARs may/should be evaluated according to their meaning and mechanistic relevance. • Successful predictive models are usually based on the quantification of one, or a small number of, rate limiting steps (often MIEs, but intermediate effects are less useful). • There are educational issues to good practice of data analysis which mean that toxicologists should be involved in the analysis.
i) UK ii) A framework for the analysis of transcriptomics and big data for regulatory applications	• Determination of how to analyse big datasets for regulatory submission. • Work arose from an observation of inconsistencies in the approach of different groups to that analysis of these datasets and the lack of a standard. • A common approach and standard is not so important in research work where approaches to data analysis are justified but is vital in regulatory work where a consistent approach is required. • Exemplar study was of three	• Omics can be used to identify MIEs and AOPs but data may be unreliable if not consistently analysed. • Exemplar data from a two-generation study has been used, a number of time endpoints and effects noted (7.56 million data points). • A primary challenge was identified with manufacturer based processed data that is not consistent between platforms. • The example showed the use of	• Provision of "Robust Reproducible Gene Lists for Regulatory Guidance" to address data quality and availability. • Use of one benchmark method for the analysis of data. • A (July 2015) workshop has developed a framework to analyse big data from omics. • A process of pre-normalisation, logarithmically transformation of the data, outlier identification, normalisation was presented. • Method deals with the low expression level data where small changes in

	chemicals, across two generations with three doses and three timepoints plus control • Purpose is to build on work from the US FDA MAQC consortium that dealt with best practice in data generation and MIAME that dealt with the recording of metadata to develop a widely applicable framework of best practice to go from raw data to gene lists (but not interpretation). • A process from experiment to output to analyse big data has been applied, especially data processing. • No guidance documents at the OECD at the current time to analyse omics data • The work is on-going and various other projects will attempt to evaluate the framework to other datasets.	non-manufacturer specific (generic) data with a process involving a simple normalisation and statistical analysis available to everyone. • The purpose of this work is not to be doctorial but to set a common method that everyone can use to set a 'sea level' against which other methods can be assessed if used but which provides the benchmark in all studies.	experimental accuracy can lead to large changes in the final data and false discoveries. • A process to identify "high value" genes was presented.
i) European Union ii) Tools and methods for integrating data and evidence	• How NAM data can be used as evidence, in a WoE approach for regulatory science. • The tools that may be used. • Use of harmonised templates. • WoE defined with similar principles as WHO/IPCS MoA, HRF, OECD AOPs and IATAs. • A six step structure approach to WoE.	• NAMs may be standardised or non-standardised guidelines. • Use of a WoE approach, with various requirements. • Level of detail in WoE and its integration depends on assessment i.e. for risk assessment it is greater than for prioritisation. • NAMs may be integrated through the AOP approach. • The next IUCLID version will incorporate (WHO/ICPS) templates for MoA analysis etc.	• "Evidence" includes any type of NAMs that contribute to hazard assessment. • Harmonised templates are used to collect data and information – the OHT 201 harmonised template will report NAM-generated data. • Well defined requirements and defined types of data for WoE including well defined uses and terminology. • A six step WoE approach was presented. • A process for weighing of evidence is performed to assess quality of evidence. • Quality of evidence is assessed in terms

			of adequacy, reliability and relevance. ECHA Guidance has been provided. • OECD Guidance is available for non-standard assays, as does OHT 201 and OECD Guidance Document No 211 (Series on Testing and Assessment) are available for reporting and describing NAMs. • A number of frameworks for integrating data exist and can be applied. The use of these frameworks is flexible. Evidence is comprehensively analysed e.g. consistency, plausibility, confidence etc. • Confidence levels are defined (high, medium, low) and can be applied for WoE. Confidence is defined from criteria associated with the quality of individual data as well as their consistency. Guidance is available to assess confidence levels.

4.3 Discussions: key topics and conclusions drawn

Key topic: general consideration of NAMs for future regulatory processes

- There was general agreement that NAMs have been used successfully to support a variety of regulatory processes and that the use of NAMs is desirable and will increase in the future. A panellist stated that we had passed a tipping point with the use of NAMs and how fast we continue should be considered. A panellist stated that it may be necessary to demonstrate that NAMs can be used for different regulatory purposes. There is a general willingness from ECHA to consider NAMs in REACH processes. However, ECHA does not need only to teach but also to be taught.
- A presenter and participants raised the question of whether regulation will keep up with technology as the evolution of testing is at a different pace to regulation. However, it was noted that for REACH the use of NAMs is, in theory, possible. The limiting factor is absence of quality indicators to allow for use – it may be possible to provide guidance documents e.g. from assay developers for the users of such information. It was mentioned that OECD Guidance Document No 211 (Series on Testing and Assessment) provides a means to describe non-guidance test methods. Later efforts could address performance standards.
- A presenter stated that the future will involve multiple technologies (i.e. NAMs) to solve the problem of regulatory decision making.
- A presenter stated that it is important to take account of the differences between human and animal data when developing and using NAMs.
- There are applications of NAMs in different areas e.g. in food safety, contaminants for food safety may also have been considered under REACH.

Conclusions:
NAMs have been used for a variety of regulatory uses and the development of their use is inevitable.

Key topic: big data

- There was a common view that big data from omics can be a valuable tool for regulatory use.
- The presenter stated that there must be a standardised approach to analysing big data. Different types of analysis may change the results, which may be a problem for regulatory application. A participant stressed the importance of understanding the data and their robustness.
- The presenter clarified that the approach presented was not for the interpretation of responses i.e. pathways, but to identify the gene list. The gene list must be correct before interpretation can be attempted. The microarray quality control (MAQC) analysis demonstrated that further analysis was needed, including assessment cut-offs to create robustness within a dataset.
- A participant stated that many genes can be affected and how this can be addressed. The presenter confirmed that some genes will move, other genes will not and it is the identification of these genes that is important for mechanistic interpretation. This will be important in e.g. read-across approaches.

Conclusions:
Big data from omics provide useful information on the genes that may be perturbed ultimately resulting in an adverse effect.
A robust and standardised procedure is required to analyse big data.

Key topic: weight of evidence

- A participant confirmed that data within WoE can be very subjective, better approaches are required to address this subjectivity. The presenter confirmed the need for guidance and expert judgement and expertise – this will normally be those with hands-on experience of measuring the data. Another participant suggested international harmonisation of the understanding of test methods and their applicability domain. A panellist confirmed that it is vital to address the reliability and relevance of NAMs.
- A panellist stated that EFSA's WoE framework includes exposure as well as hazard (toxicokinetics and toxicodynamics are split). This tiered approach combines hazard and exposure to assess risk.
- A participant stated that Canada has used templates developed in the OECD IATA case studies project – important to standardise reporting information for e.g. read-across.

Conclusions:
WoE is a key element in the use of NAMs in future regulatory science.
The use and application of WoE requires further development.

Key topic: challenges for the future prospects of the use of NAMs

- A presenter defined some of the challenges for future regulatory use of NAMs:
  - understanding, defining and addressing the technical limitations of individual assays;
  - the ability to move from an apical effect to defining adversity;
  - translation to human safety and toxicity;
  - how to combine NAM data to address biological complexity;
  - integration of different streams of data in a risk-based, WoE assessment;
  - quantification and incorporation of uncertainty and variability;
  - how to define and ensure an assay is fit for purpose; and
  - ensuring that NAMs are legally defensible.
- A participant asked whether version 2.0 of Tox21 would be extended to mixture testing. This is a challenge and is currently being addressed by cumulative effects of single chemicals using ToxCast assays as well as cumulative effects of exposure and testing of mixtures.

- It was noted that there is a need to continue the development of templates to report NAMs/MoAs to allow for WoE analysis. It was suggested that the continued development of WoE templates to assess the quality of evidence and overall WoE could be useful for defining and assessing NAMs for read-across.
- For implementation, there is a trade-off between prescriptiveness and flexibility. Developers need to work with users to ensure NAMs are fit-for-purpose.
- A participant stated more information is required on how to advise a registrant [to ECHA] regarding how to use NAMs. It was suggested to make a parallel with pharmaceuticals whereby NAMs (particularly toxicokinetics) could be run side-by-side with a traditional study, to inform all of the process and use of the NAMs. As noted by a participant, the parallel studies also require planning and resources.
- A panellist said the NAMs should first be used for data poor regions of space to inform, then more onto higher tier tests
- EFSA have collected data from hundreds of studies and it may be possible to learn about how QSAR/ToxCast methods could be applied.
- Agreement on the quality of NAM data should be agreed, e.g. a systematic review process for NAMs.
- NAMs could speed up "group regulation", i.e. regulation with categories.

Conclusions:
There are numerous challenges to the increased use of NAMs to enable regulatory decisions.
Parallel assessment of traditional (animal-based) and new (NAM-based) cases with accounting for uncertainty appears to be necessary.

Key topic: short-term challenges that can be addressed

- A panellist stated that some short-term barriers e.g. metabolism and expanding chemical space could be overcome. Other achievable challenges to meet include understanding the meaning of a negative prediction.
- A panellist indicated that templates for reporting could be addressed e.g. with reference to the OECD IATA case studies project.
- A better understanding of the policy problem/perspective will help NAM solution providers, e.g. to consider the level of protection, as opposed to direct replacement of tests. More effort is required to understand the use of NAMs for the different contexts.
- Validation frameworks to define the meaning of success along with definable benchmarks. Expectations should go beyond predictive performance to allow for acceptance. Establish different types/frameworks for validation.
- A participant stated that a demonstration of "safety determination" could be a short-term goal. This could include *in vitro* bioactivity, kinetics, and exposure scenario – to determine margin of exposure is sufficient for safety. This is similar to the SEURAT-1 *ab initio* case study, which includes a workflow on how to make a risk assessment with no "standard" toxicological data.
- More case studies could be presented e.g. through EU-ToxRisk to help understanding. Specific case study topics were proposed:

- Case studies could broaden out from read-across to other applications such as screening and/or prioritisation. New case studies could demonstrate how integration of NAMs could be applied;
- Case studies could address metabolism, studying human relevance of NAM data for metabolic pathway;
- Case studies could address the definition and quantification of uncertainty with regard to e.g. read-across and how NAMs may reduce uncertainty;
- Case studies could take more account of the RAAF and the specific assessment elements, especially to stimulate and allow for a high level of interaction with ECHA scientists;
- Development of a case study, which develops a dossier that would be acceptable to ECHA.
- The PACT list could be communicated, have a regulatory debate over a small number of chemicals to open up to NAM data. This could show that key regulatory drivers could be answered;
- Use of OECD platforms would help link NAM data with regulatory platforms. OECD IATA project is considering NAMs, the OECD could provide a very useful platform to discussing and promoting NAMs;
- More thought should be put into case studies to make them more strategic. This will mean linking with all, and correct, stakeholders.

- Provision of an integrated framework to consider model species and evolutionary aspects, as well as similar chemicals.
- New advances should be strategically targeted rather than being broad brush.

Conclusions:
A number of barriers that could be addressed in the short term (up to three years) have been identified.
Most barriers relate to the implementation of NAMs, rather than further development. Many of these could be addressed through strategic and well-designed case studies.

4.4 Summary

A number of key conclusions were drawn from Theme 3 of the workshop. These and other shared views between the participants are summarised in Table 5.

Table 5. Conclusions drawn and shared views from Theme 3 of the workshop.

Topic	Workshop shared views
NAMs are being developed and becoming more useful	There is a clear motivation and willingness from regulatory agencies, including ECHA, to consider the use of NAMs.
Evaluation and anchoring of *in vitro* assays	*In vitro* assays should be evaluated in terms of robustness, reliability and relevance with the findings documented. Where possible, they should be anchored on high quality *in vivo* data and responses, but with the realisation that the current *in vivo* studies have their own false positive and false negative error rates.
Anchoring *in vitro* assays to the MIE	Grossly simplified models of animal toxicity must be built on or capture important features such as the rate limiting step or MIE.
Inclusion of metabolic capability to NAMs	NAMs should be developed with metabolic capability and capacity, this could be an area for further development e.g. ToxCast version 2.0.
HTTK to support extrapolation of *in vitro* doses to *in vivo* scenarios	HTTK can support the implementation of NAMs through assessment of the margin of exposure following calculation of exposure from e.g. *in vitro* assays for TK-related parameters.
Characterisation of chemical structure	For NAMs to be implemented, especially to support techniques such as read-across, the characterisation and definition of chemical structures needs to be clear and unequivocal.
Much variability in analysing big data sets from omics	There is demonstrable variability in the analysis of big data from omics resulting in different conclusions (i.e. gene sets) being obtained.
A framework has been presented for analysing big datasets	Analysis of big data from omics to identify useable and relevant genes for use of NAMs should be performed within a robust framework as presented.
A number of key challenges have been defined	A number of short term, achievable challenges to increase the applicability of NAMs for regulatory application have been identified. Some of these are summarised below and include issues such as transparency, accessibility as well as uncertainty/reliability analyses.
Application of case studies	Clearly defined and strategic case studies are required to demonstrate the use of NAMs, but also to develop them further for regulatory use. A number of key areas for case studies have been identified.

Framework for integration of NAM information for WoE	Various frameworks are available for evaluating NAM data in a WoE scenario. These should be extended and used.
Confidence levels for WoE can be assessed	WoE can be assessed with regard to relative confidence, guidance and templates are available.
Reporting tools for WoE should be improved	There is a need to develop and gain acceptance for reporting tools for WoE.
OHT 201 and OECD Guidance Document No 211 (Series on Testing and Assessment)	These provide a standardised reporting template and guidance that could be used or adapted for NAMs.
Mixtures	Further work is required to address the issues of mixtures with NAMs.

5. Overall outcomes and suggestions

Workshop overview

The workshop provided a forum for a diverse group of stakeholders to meet and provide a view on the role of NAMs in regulatory science. There was a positive outlook to the workshop, tempered by a realisation that more work is required in key areas to make NAMs (more) useful and applicable in regulatory science. The workshop not only illustrated aspects of the state-of-the-art of NAMs, but highlighted areas where progress in NAMs is required.

Existing use of NAMs

NAMs were found to be applicable in a number of areas of regulatory science for the risk assessment of chemicals. Examples of many types of NAMs were presented. Tools such as the RAAF were found to be useful to evaluate the performance of NAMs for read-across.

Data from NAMs were shown to support read-across as well as providing useful and usable information for screening and prioritisation. NAMs are being used actively to provide solutions to address legislation in the EU, USA, Canada and Australia (as well as elsewhere e.g. Japan, South Korea).

The workshop demonstrated a clear appetite and desire to use NAMs, and to extend the use of NAMs for these purposes. It also indicated that NAMs must be applied appropriately, bearing in mind issues such as the regulatory context and applicability domain.

There was an overall shared view in the workshop that **NAMs could be used imaginatively and flexibly**, and that their use may **drive changes in regulatory hazard assessment practices in the future.**

Future use of NAMs: identification and definitions

For many in the workshop, the term "new approach methodologies – NAMs" was novel. To increase understanding, there is a **need to define and scope NAMs.** For instance, the workshop heard a description of NAMs as being: *in silico* approaches, *in chemico* and *in vitro* assays including high-throughput and high-content techniques, omics with a focus on metabolomics, the use of exposure data in terms of volume and use etc.

There is **no current comprehensive overview** of where and how NAMs are being defined and used, and how they could be applied in the future. A taxonomy of methods for NAMs would be a great benefit.

Future use of NAMs: research needs to stimulate uptake

A number of practical issues with the use of NAMs were identified. There is a need for **standardisation** of NAM approaches such that they may be made transferable and transparent.

The **relevance** (for regulatory use as well as predictors of adverse effects) of NAMs is required to improve uptake. In addition, better understanding of the **reliability** of NAMs, in terms of performance and applicability is required.

A key element missing from the use of NAMs is (globally) **agreed standardised**

reporting templates. Whilst a number of potential templates, such as OHT 201 and OECD Guidance Document No 211 were suggested, it is clear that further work will be required to allow for the definition of all details.

Standardisation of frameworks for utilising NAM data is a pre-requisite. In addition, there needs to be consideration of the openness of the results and the accessibility of the methodologies. Further, the development of a taxonomy and the production of an inventory for NAMs are also desirable.

The workshop demonstrated the need for **data quality and coverage to be addressed**. With regard to NAMs this is, in part, a need for standardisation, performance characteristics etc. However, there is a need to better characterise the quality of *in vivo* data (e.g. through access to study reports for read-across, or an overall assessment of toxicological assays). The clear identity of the chemical structures and substances must also be assured.

It was clearly demonstrated that **NAMs provide useful information on toxicodynamics**. This can support a number of regulatory applications, especially read-across. Where possible, there is value in aligning NAMs to AOPs, with the most "interpretable" assays either being indicative of, or closely linked to the MIE.

Metabolomics data were found to be valuable to contribute to mechanistic understanding and support techniques such as read-across. Conversely, currently **NAMs provide little insight into toxicokinetics**, although there are some notable exceptions e.g. US EPA ToxCast and ExpoCast.

Understanding of exposure was demonstrated to be an important area for a number of regulatory applications (e.g. screening and prioritisation). The role of exposure is firmly embedded in many geographic regions; the possibilities of **NAMs to support exposure assessment** were presented. For instance, the use of NAMs to estimate exposure includes information on volume of production/import within a region as well as use scenarios. Further, NAMs can result from, and contribute *in vitro* characterisation of kinetics e.g. metabolism and clearance.

WoE frameworks are essential for the successful use of NAMs and integration of the information they provide. A number of approaches were presented which could be built upon. There is a need to **understand and characterise uncertainty** from NAM data and how these will affect WoE.

Future use of NAMs: practical application

The case studies presented in the workshop were valuable to illustrate how NAMs can and are being used, with some limitations and shortcomings noted. There is a need for further **case studies to demonstrate the practical application of NAMs** and clarify issues relating to potential regulatory uptake.

There are a number of clear examples where case studies could add value to the use of NAMs, demonstrate the different regulatory applications, define how NAMs could be standardised and better used etc. (Section 4.3); such progress could be initiated within on-going European or other worldwide projects, focusing on specific topics and endpointsWhilst these areas for case studies have been identified, there is a need to properly consider all aspects of case studies and prioritise these.

The workshop noted on numerous occasions a need for **capacity building** in terms of personnel and facilities. There is a need for training and education in the use and meaning of NAMs across all stakeholder groups. There is also an opportunity for cross-

fertilisation of knowledge e.g. developers of NAMs to better understand regulatory needs.

New cooperation paradigm for NAM development

The "overall outcomes and suggestions" above reflect the needs and actions at different levels of concept, discussion, purpose of application and implementation. Common needs of different regulatory frameworks as well as their specific needs should be identified.

Where the development of NAMs is to inform current regulatory needs and processes, regulators, academia and industry need to cross-fertilise their work through targeted cooperation and capacity building, across the borders of their organisations. Finally, the creation of new networks and tools has to respond to the complexity of the undertaking to make better toxicology through the use of NAM.

Appendix 1 List of presentation titles

Day 1, 19 April 2016

Theme 1: Definitive hazard assessment: improvement of read-across

- Setting the scene: Critical aspects in the assessment of read-across adaptations: the role of supporting evidence, Norbert Fedtke, ECHA, Finland
- Case study from SEURAT-1: Read-Across for 90-Day Rat Oral Repeated-Dose Toxicity for Selected Perfluoroalkyl Acids: A Case Study, Terry Schultz, University of Tennessee, USA
- Case study from SEURAT-1: Read-Across for 90-Day Rat Oral Repeated-Dose Toxicity for Selected β-Olefinic Alcohols: A Case Study, Mark Cronin, Liverpool John Moores University, UK
- Case study from BASF: Metabolomics as read-across tool: a case study with phenoxy herbicides, Bennard van Ravenzwaay, BASF, Germany
- RAAF assessment: Perfluorinated alkyl acids: direct acting toxicant category supported by ToxCast evidence, Sharon Stuard, Procter & Gamble, USA
- RAAF assessment: β-Unsaturated alcohols: indirect acting toxicant category supported by SEURAT-1 data, Andrea Richarz, European Commission, Joint Research Centre, Italy
- RAAF assessment: Read-across with metabolomics for phenoxy herbicides, Bennard van Ravenzwaay, BASF, Germany

Day 2, 20 April 2016

Theme 2: Screening and priority setting

- The NICNAS IMAP Program, Kerry Nugent, National Industrial Chemicals Notification and Assessment Scheme, Australia
- Application of computational and high-throughput in vitro screening for prioritization, Richard Judson, Endocrine Disruptor Screening Program, US EPA
- Integrating New Approach Methodologies under Canada's Chemicals Management Plan, Christine Norman, Health Canada, Canada
- A common screening approach for REACH and CLP processes, Panagiotis Karamertzanis, ECHA, Finland

Theme 3: Prospects for regulatory science

- Moving Towards Version 2.0 of Toxicity Testing in the 21st Century and Application to Regulatory Decision-Making, Russell Thomas, US EPA
- How to overcome limitations of new approach methodologies in the context of regulatory science, Romualdo Benigni, Istituto Superiore di Sanità, Italy
- Analysing Data: Towards a framework for transcriptomics and other Big Data analysis for regulatory application, Timothy W Gant, Public Health England, UK
- Using new approach methodologies in regulatory science: tools and methods for integration of evidence, George Fotakis, ECHA, Finland

Appendix 2 List of poster titles

Theme 1: Definitive hazard assessment: improvement of read-across			
Poster number	**Organisation / Country**	**Submitter**	**Title / Authors**
1	European Chemicals Agency, Finland	Niklas ANDERSSON	**The read-across assessment framework under REACH** Niklas Andersson, David R. Bell, Ingo Bichlmaier, George Cartlidge, Karel De Raat, Norbert Fedtke, Anneli Kojo, Agnes Kovari, Tatiana Netzeva, Eric Stilgenbauer
2	European Commission, Joint Research Centre, Italy L'Oreal Unilever SEAC Procter & Gamble	Elisabet BERGGREN	**SEURAT-1 Proof-of-Concept: The ab Initio safety assessment case study for daily exposure to an active ingredient in a body-lotion** Elisabet Berggren, Gladys Ouedraogo, Alicia Paini, Andrea Richarz, Andrew White and Catherine Mahony
3	Ideaconsult Ltd, Bulgaria Clariant Produkte (Deutschland) GmbH, Germany CEFIC, Belgium	Nina JELIAZKOVA	**Linking LRI AMBIT Chemoinformatic system with the IUCLID Substance database to Support Read-across of Substance endpoint data and Category formation** N. Jeliazkova, V. Koch, Q. Li, U. Jensch, J. Schneider-Reigl, R. Kreilingb, I. Georgiev, B. Hubesch
4	Eupoc GmbH, Germany Department of Statistics, Ludwig-Maximilians-University Munich, Germany	Uwe KÖNIG	**Exploring Uncertainty in Exposure Thresholds to help Downstream Companies in Acquisition, Analysis and Evaluation of Exposure Data to Implement adequate**

			Activities in their Daily Work Uwe König, Malte-Matthias Zimmer, Göran Kauermann
5	United States Environmental Protection Agency, USA	Jason C. LAMBERT	**Adverse Outcome Pathway 'footprinting': an integrated read-across approach to the assessment of mixtures** Jason C. Lambert
6	CAAT-Europe, University of Konstanz, Germany CAAT- Environmental Health Sciences, USA Rutgers University at Camden, USA	Thomas HARTUNG	**REACH-across - making the publicly available safety data for 9,801 substances registered under REACH (2008-2014) a resource for read-across** Thomas Luechtefeld, Alexandra Maertens, Daniel Russo, Hao Zhu, Costanza Rovida and Thomas Hartung
7	Lhasa Limited, UK	Donna S. MACMILLAN	**Predicting skin sensitisation using a decision tree integrated testing strategy with an in silico model and in chemico/in vitro assays** Donna S. Macmillan, Steven J. Canipa, Martyn L. Chilton, Richard V. Williams and Christopher G. Barber
8	European Chemicals Agency, Finland	Gesine MÜLLER	**Predicting hazardous properties of substances from related substances - some case reports** Gesine Muller, Jonas Nygren, Silvia Lapenna, Ari Karjalainen, Fabrice Broeckaert, Chiara Perazzolo, Linda Spjuth, Alexis Nathanail, Ricardo Simoes

9	European Commission, Joint Research Centre, Italy Liverpool John Moores University, UK The University of Tennessee, USA	Andrea RICHARZ	**Essential Aspects of Read-Across for Repeated-Dose Toxicity Predictions** Andrea-N Richarz, Mark TD Cronin, Terry W Schultz
10	European Commission, Joint Research Centre, Italy Cosmetics Europe, Belgium Procter & Gamble, UK	Andrea RICHARZ	**SEURAT-1 Proof-of-Concept Read-Across Case Study for Repeated-Dose Toxicity** Andrea-N Richarz, Elisabet Berggren, Martina Klaric and Catherine Mahony
11	CAAT Europe, Germany REACH mastery	Costanza ROVIDA	**Practical needs to implement advanced strategies for a proper justification of the read across/category approach** Costanza Rovida, Monica Locatelli,
12	The University of Tennessee, USA Iowa State University, USA Liverpool John Moores, UK	Terry W. SCHULZ	**Read-Across for 90-Day Oral Repeated-Dose Toxicity for Low or No Toxicity Substances: The Importance of Toxicokinetic Similarity** Terry W. Schultz, Steven P Bradbury and Mark T.D.Cronin
13	Escola Superior de Tecnologia da Saúde de Lisboa – IPL, Portugal Escola Nacional de Saúde Pública, ENSP, Universidade Nova de Lisboa, Portugal	Susana VIEGAS	**How to deal with uncertainties regarding the occupational exposure to antineoplastic mixtures – Additive effect should always be considered?** C. Ladeira, S. Viegas, A. Costa-Veiga
14	Novartis Institutes for Biomedical Research, Switzerland Toxicodynamix International LLC, USA	Gian C. WINKLER	**In Silico and Read-Across Mutagenicity and Carcinogenicity Assessment to Close Data Gaps for the Pharmaceutical Intermediate Trans-1,4-dibromobut-2-ene** Gian C. Winkler, Phil Bentley, Susanne Glowienke, Ester Lovsin Barle

Theme 2: Screening and priority setting			
15	American Chemistry Council, USA US EPA National Center for Computational Toxicology Procter & Gamble Ted Simon LLC	R.A. BECKER	**Integrating the threshold of toxicological concern (TTC) with high throughput exposure assessment for risk-based screening of several thousand commodity chemicals** R.A. Becker, J. Wambaugh, G. Patlewicz, S. Felter, T.W. Simon
16	North Carolina State University, USA	David M. REIF	**Leveraging the power of high-dimensional data for integrated screening and prioritization decisions** David M. Reif
17	XCellR8 Ltd, UK	Carol TREASURE	**Adaptation of Human Cell Based Safety Tests to Animal Product Free Conditions** Carol Treasure, Nathalie Belot, Bushra Sim
18	University of Birmingham, UK	Mark R. VIANT	**Metabolomics: a tool for mechanistic toxicology** Mark R. Viant
19	Technical University of Denmark (DTU), Denmark	Eva B. WEDEBYE	**New free Danish online (Q)SAR predictions database with >600,000 substances** Eva B. Wedebye, Marianne Dybdahl, Trine K. Reffstrup, Sine A. Rosenberg, Nikolai G. Nikolov
Theme 3: Prospects for regulatory science			
20	US National Institute of Environmental Health Sciences, USA	Warren CASEY	**Developing a US National Strategy and Roadmap for the Replacement of Animal-Based Toxicity Testing** Warren Casey

21	ScitoVation, Research Triangle Park, USA Unilever, SEAC, UK	Rebecca A. CLEWELL	**Using 21st Century tools to identify point of departure for safety assessment of genotoxic compounds** Rebecca A. Clewell, Bin Sun, Salil Pendse, Patrick D. McMullen, Yeyejide Adeleye, Paul L. Carmichael, Melvin E. Andersen
22	University of Birmingham, UK	John COLBOURNE	**PhyloToxicology: exploiting evolutionary concepts to improve toxicity testing** John Colbourne, Mark Viant
23	Parker Doe Partnership LLP, UK	John DOE	**Removing Blockers to the Acceptance of New Methodology in Regulatory Science** John Doe
24	Parker Doe Partnership LLP, UK	John DOE	**A 21st Century Roadmap for Human Health Risk Assessment** John Doe
25	Douglas Connect GmbH, Switzerland	Barry HARDY	**Integration into risk assessment of open source human omics data from in vitro studies** Lucian Farcal, Thomas Exner, Barry Hardy
26	Humane Society International, Belgium	Jarlath HYNES	**Integrated Approaches to Testing and Assessment (IATA) can facilitate acceptance and regulatory use of non-animal methods** Jarlath Hynes, Kate Willett
27	Health Board, Estonia Tallinn University of Technology, Estonia	Kaja ILMARINEN	**The scientific background for identification of selected substances** Kaja Ilmarinen, Merike Nugin, Riina Aav, Dzmitry Kananovich, Sandra Kaabel, Maria Fomitšenko, Arvo Mere

28	Laboratory of Mathematical Chemistry, Bourgas "Prof. As. Zlatarov" University, Bulgaria ECHA, Finland	Ovanes MEKENYAN	**QSAR Toolbox as read-across/category building platform suitable for combining in-vivo experimental results with mechanistic data and expert knowledge** O. Mekenyan, S. Dimitrov, T. Pavlov, C. Kuseva, T. Sobanski, D. Hirmann,T. Netzeva, A. Gissi, A. Martin Aparicio
29	US Army Engineer Research and Development Center, USA	Edward PERKINS	**Driving Risk Decisions Through Information Integration and Visualization Using Systems Biology, Ontologies and the AOPXplorer** Edward Perkins, Natalia Garcia-Reyero, Kurt Gust, Mitchell Wilbanks, Natalie Barker, and Lyle Burgoon
30	3Rs Management and Consulting, Denmark Adriaens Consulting Oroxcell Yves Rocher University of Milan, Italy MB Research Laboratories CellSystems Pierre-Fabre Vrije Universiteit Medical Center, Belgium MatTek Cooperation, USA SenzaGen, Sweden Eurofins MatTek IVSL Stiefel	Erwin L ROGGEN	**Potency ranking of skin sensitizers using the Reconstituted Human Epidermis (RhE) IL-18 test and the Genomic Allergen Rapid Detection (GARD) test** Erwin L Roggen, Els Adriaens, Eric Andres, Fanny Boisleve, Sun-A Cho, Emanuela Corsini, George DeGeorge, Horst W Fuchs, Pierre-Jacques Ferret, Sue Gibbs, Patrick Hayden, Henrik Johansson, Dagmar Lehmeier, Silvia Letasiova, Jean-Philippe Therrien
31	ECEAE/CrueltyFreeInternational, UK	Katy TAYLOR	**The need to ADAPT to new methodologies** Katy Taylor
32	EBTC at the Johns Hopkins Bloomberg School of Public Health, USA	Katya TSAIOUN	**Evidence-Based Toxicology – the missing link between the advancements of science and the confidence of regulatory decisions** Katya Tsaioun

Appendix 3 List of participants

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JUTILA	Arimatti		

Appendix 4 Speech from the European Partnership for Alternative Approaches (EPAA) presented by Renate Weissenhorn

Dear Ladies and Gentlemen. Thank you for giving me this opportunity to introduce EPAA, the European Partnership for Alternative Approaches to animal testing to you.

EPAA is a voluntary partnership dedicated to the promotion of alternative approaches to animal testing, between the European Commission, European trade associations and companies from seven industry sectors. Its vision is to replace, reduce and refine (3Rs) animal use for meeting regulatory requirements through better and more predictive science. Last year we celebrated the tenth anniversary and are hence one of the "oldest' actors in the field. There are now 35 companies from 7 industry sectors: Animal Health, Chemicals, Cosmetics, Crop Protection, fragrances, Pharmaceuticals and Soaps and Detergents. from the Commission 5 Directorate Generals co-operate: DG Health and Consumer Protection (SANCO), Joint Research Centre with EURL-ECVAM, DG Research and Innovation (RTD), DG Environment (ENV) and DG Internal Market, Industry, Entrepreneurship and SMEs (GROW).

The strategic decisions on the EPAA projects, support to third-party activities and EPAA membership are taken by the Steering Committee consisting of members from both industry and the respective Commission services. The co-chairs from industry and the Commission convene the quarterly meetings. The Steering Committee is advised by the Mirror Group that consists of experts from the civil society including academia, animal welfare, laboratory animal science, innovation actors and third party organisations.

One of the strengths of EPAA is that this co-operation is based on working together on concrete projects, which stem from the mutually agreed 5 year Action programmes that set the strategic orientation.

With its new Action Programme EPAA will concentrate on facilitating regulatory acceptance for the use of effective alternative methods in research and science.

We report on our achievements over the last year and the outlook for the next to a wider 3R-concerned and interested public during the EPAA Annual conference. This will take place this year on 5 December afternoon under the working title "Science based regulation". Some of its outcome might be fed in the ECI Conference just the day after. You are welcome to attend and you can register later via the EPAA website!

Let me now give you a few examples of concrete projects that EPAA is working on:

1. Optimised Strategies for Skin Sensitisation
The aim of the skin sensitisation project is to share information about existing non-animal test methods that can be used in Integrated Approaches on Testing and Assessment (IATA) for regulatory decisions on skin sensitisation for hazard classification in time for REACH 2018.

In order to help address this challenge, the EPAA organised a skin sensitisation workshop with Cefic LRI and Cosmetics Europe in April 2015 in ECHA. This was the fourth workshop on this topic by EPAA and Cefic in the last years. The participants including representatives of OECD, ECHA, EC, national competent authorities and industry discussed integrated non-animal skin sensitisation strategies on the basis of several case studies and looked at their use for hazard classification, potency and weight of evidence.

Important conclusions from the workshop were the need for greater clarity in the

definition of applicability domains and that no single method but a tiered strategy based on AOP for skin sensitisation may help to better characterise the skin sensitisation potential. Other key issues identified included the feasibility of using the new approaches by SMEs, especially, when operating through CR0, monitoring uptake in regulatory filings and ECHA acceptance.

2. Carcinogenicity (In vitro (genotoxicity) and in vivo (3- or 6-month rat subchronic toxicity) **studies may provide sufficient data to waive the need for a 2-year rat carcinogenicity study**
Building on the outcome of an earlier review and workshop held by EPAA in 2013, this project aims at collecting scientific evidence through an extended database that could convince regulators to accept waiving of the 2-year carcinogenicity study on rats. This study forms part of the regulatory package for pharmaceuticals, additives and chemicals (mainly agrochemicals) and it entails the use of large numbers of animals, high cost and time.

Within this project conducted by researchers at the University of Wageningen, data for 364 compounds has been completed and stored in the open access TOXREf database in an anonymized way. A peer-reviewed publication with the conclusions of this database will be delivered soon. On basis of this publication a team from EPAA and EFPIA will possibly develop a concept paper to be presented at the next ICH Conference and agree concrete steps for talking to the regulators.

3. Acute Toxicity (Replacement of death as an end-point and waiving of the dermal route)
The aim of this project is to improve animal welfare and facilitate reduction in animal use for meeting information requirements for acute toxicity under REACH. Based on technical progress and recently established 3Rs "Best practices" the EPAA Acute toxicity technical expert group and the Humane Society International submitted proposals to the European regulators in 2012, recommending to waive acute toxicity testing via the dermal route for substances which are non-toxic via the oral route. The proposal has been accepted by the REACH Committee and will be adopted soon.

The EPAA project has identified further opportunities to waive acute animal testing requirements completely or, where this is not possible, to refine the decision-making steps or assessment strategies to minimize suffering of test animals. Additional evidence in support of this framework is being developed through data mining of acute oral toxicity studies in collaboration with the UK National Centre for the 3Rs (NC3Rs) and the UK Chemicals Regulation Directorate. Data from previously filled acute toxicity studies are collected and will be analyzed to confirm that clinical signs are an appropriate alternative to death as an endpoint. ECHA expressed interest in participating in this project.

4. Conclusion
As you have seen from these examples, EPAA concentrates on new methodologies and, like we also discussed today, works not only on full replacement methods, but also on refinement and reduction approaches. With its new Action Programme 2016- 2020 EPAA has moved towards the implementation or application side of alternatives, concentrating on "facilitating regulatory acceptance", hence also closer to regulatory agencies like ECHA.

What do we need to do to ensure the applicability and acceptability of the new approaches? This is the main question we discuss during our workshop now and it is equally EPAA's major concern for the next 5 years.

Our ultimate aim in the long term is to have testing methods for regulatory decision

making which are scientifically robust, faster and cheaper than those currently used. Once we understand human toxicology better, we can better predict and need less and hopefully one day no animals at all.