National Centre
for the Replacement
Refinement & Reduction
of Animals in Research

Workshop report:

Applying exposure science to increase the utility of non-animal data in efficacy and safety testing

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Pioneering Better Science

Background

There are many non-animal approaches currently available with the potential to provide useful information on the biological effects of drugs and chemicals, which can be used to inform decision-making, both in product development programmes and in regulatory assessment. However, the usefulness of data from these approaches is often limited as exposure considerations are not adequately taken into account. For example, it is frequently not clear how the concentrations tested in *in vitro* assays relate to the amounts that humans or environmental species would be exposed to in real life situations. It can be difficult to determine how much of the chemical applied to the model system reaches the site of action (i.e. measured versus nominal or applied concentrations). This can affect interpretation of the test data. Better consideration of exposure within toxicological testing is necessary to ensure that the data generated is relevant to answer the scientific questions being asked. In this way data from non-animal approaches will be more useful for decision-making purposes, and support a reduction in the current reliance on data from animal tests. The need for exposure science to support and influence a '21st century' approach to efficacy and safety assessment has been increasingly recognised in recent years [1,2,3].

A scientific workshop was held in February 2017 with the aims of:

- Increasing awareness and building confidence in the application of exposure-driven approaches to support decision-making based on data from non-animal approaches across sectors;
- Building a community of scientists working in the area of exposure-driven safety assessment;
- Identifying gaps and challenge areas that need to be addressed to advance the application of exposure science; and
- Informing the development of future guidance to facilitate the use and wider acceptance of exposure-driven non-animal approaches to inform and improve efficacy and safety decision-making.

The NC3Rs

The NC3Rs has been working to support scientists to incorporate exposure-driven approaches more widely into non-animal methods for almost ten years. This began with a cross-sector workshop in 2008 which explored the potential application of toxicokinetic information in chemical hazard identification and characterisation, and its role in chemical risk assessment. The NC3Rs also has an ongoing programme supporting companies to utilise microsampling techniques for toxicokinetic analyses, which can reduce and refine animal use. The potential 3Rs benefits of applying exposure science were re-visited in 2014 in an expert cross-sector workshop. Here over 20 delegates from regulatory bodies and industry identified 'embedding exposure-driven approaches into dose selection' as one of four key priority areas necessary to ensure a paradigm shift in chemical safety assessment[4]. It was recognised that incorporating exposure considerations within safety assessment offers refinement opportunities, can increase the biological relevance and utility of both animal and non-animal data, and can be useful for informing the prioritisation and waiving of animal studies. Subsequent discussions within an NC3Rs-led cross-sector expert working group in 2016 resulted in the recommendation that a multidisciplinary workshop be held to explore the barriers and potential solutions for utilising exposure-driven approaches more widely to support the use of non-animal data in efficacy and safety testing.

Unilever

Unilever recognises that a greater understanding of levels of exposure will be of fundamental importance in the transition to pathways-based approaches to risk assessment (so called Next Generation Risk Assessment). Current approaches such as estimation of external applied dose and application of exposure-based waiving will continue to be used, however, higher tier exposure tools will be required to better understand the bioavailability of chemicals in the human body, the environment and in *in vitro* testing systems. Physiologically-based pharmacokinetic (PBPK) modelling can be used to integrate chemical-independent (physiological) and chemical-dependent (absorption, distribution, metabolism and excretion - ADME) parameters and predict plasma and/or tissue concentrations. These concentrations can then be used to inform the design of or interpret the results of *in vitro* studies. The growing emphasis on the use of *in vitro* systems also raises a key challenge in the need for tools to model, measure and control the free concentration of chemicals, rather than relying on nominal doses, which in current practice act as a surrogate in establishing dose-response relationships. Depending on the physicochemical properties of a chemical, substantial variability between the nominal and the freely dissolved concentration can exist. This represents a key challenge with respect to the development of approaches reliant on quantification of *in vitro*-to-*in vivo* extrapolation. Unilever has a long-standing programme of research activities across this area (see www.tt21c.org for more details).

The workshop

Recognising a joint interest in this area, the NC3Rs and Unilever came together to host this workshop in February 2017. A copy of the workshop programme can be found in Annex I. The presentations covered four key themes:

i. The current landscape of exposure science and exposure-based decision making (across efficacy and safety testing);
ii. Increasing the physiological relevance of *in vitro* assays and understanding of human-relevant exposures;
iii. Quantifying exposure-response relationships in *in vitro* assays: modelling, measurement and dosing; and
iv. Increasing confidence in and acceptance of new exposure-based computational models.

Breakout group discussions were held on day 1 of the workshop to explore the current status of exposure science and the barriers to its uptake (see page 11 and Annex II), and on day 2 to discuss how to overcome challenges and barriers across four key priority areas identified from the discussions on day 1 (see pages 12-15). A pre-meeting survey was also completed by delegates to help inform the breakout group discussions (see pages 9-10).

The workshop was attended by 83 scientists drawn from the pharmaceutical, industrial chemical, consumer products and agrochemical industries (36%) and academia (37%), as well as other relevant organisations (27%) including: consultancy companies, small and medium enterprises (SMEs) and contract research organisations (CROs), the European Commission, the US Environmental Protection Agency (EPA), the National Toxicology Program (NTP) Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), and the UK Health and Safety Executive (HSE). The majority of attendants were based in the UK (75%), followed by other European countries (19% across Belgium, Denmark, Ireland, Italy, the Netherlands, Norway and Switzerland), and North America (5% in the USA and 1% in Canada).

This report summarises the main themes presented and discussed at the workshop, and provides a basis to inform future activities in this area, to enable the wider application of exposure-based approaches to support the use of non-animal data in efficacy and safety testing.

1. The current landscape

Recent efforts to shift towards the use of more mechanistic approaches in safety assessment have been supported by conceptual frameworks such as Mode of Action (MOA) and Adverse Outcome Pathways (AOPs)[a]. Such frameworks play a key role in the development of non-animal methods (or combinations thereof) suitable for assessing whether chemicals could cause adverse effects at an organism or population level. They allow existing knowledge on biological pathways of toxicological interest to be organised in such a way that it can be integrated and evaluated, for use for either research or regulatory purposes. It is critical that fundamental knowledge of the physicochemical properties of compounds and the biological processes involved in the activation of a pathway of interest are understood and integrated when using mechanistic information for decision-making. These include how and when a chemical is metabolised (e.g. to or from a substance of concern), and how it gains access to the cellular site of action through biokinetic processes. The generation of information on chemical exposure within biological systems would ideally take place ahead of, or at least at the same time as, data are generated to determine the mechanisms by which the chemical causes toxicity. This would allow experiments to be designed to achieve intended concentrations at the site of action, and for the two data streams to be integrated, leading to decisions on chemical safety that are more accurate, relevant and risk-based (rather than solely hazard focused), with an increased physiological basis. Exposure considerations, that is quantitative considerations of dose, do not explicitly form part of a MOA or an AOP. However, the evolution of the initial International Programme on Chemical Safety (IPCS) MOA framework into the MOA-human relevance framework resulted in a much greater focus on quantitative dose-response relationships. AOPs deposited within the OECD initiative have been described as 'chemically agnostic' (i.e. they describe downstream pathways once a molecular initiating event (MIE) has been triggered by a chemical but do not consider kinetics or metabolism that may occur prior to the MIE, or quantitative dose-response relationships). Application of such AOPs to the assessment of specific chemicals and uses will require considerations analogous to those undertaken when assessing the relevance of a MOA. Unfortunately, to date, when developing and applying non-traditional means for assessing chemical safety, focus has been placed on the nature of the biological responses observed rather than also considering the exposure levels causing these effects; this has the added disadvantage of making comparisons of effects across different model systems more difficult.

There are many current opportunities for companies to use exposure information internally - for screening and prioritisation purposes when developing new products, to help determine whether compounds of interest are likely to have activity at human or environmental biological targets. For pharmaceuticals, such activity may be desired to achieve a therapeutic outcome, whereas for other chemical types such as industrial chemicals and agrochemicals this may result in the disruption or termination of the development process. Such decisions could help to ensure that only the most promising of candidates (from an efficacy and/or safety perspective) enter mandatory animal tests in industry sectors where animal testing is still required. The use of exposure data to support regulatory decision-making is currently largely limited and varies by jurisdiction and chemical sector. Information on likely human exposures generated is starting to be considered in the screening and prioritisation of toxicity data for regulatory purposes, for example to support the high-throughput models used in the ToxCast programme in the United States. There are also some instances where exposure-based waiving of regulatory animal tests occurs across different sectors, following consideration of the likely exposure scenario: who may be potentially exposed (e.g. workers versus consumers); route of exposure; and how long might they be exposed for (e.g. single occasion; every day over years). A current lack of biomonitoring/epidemiological data on human and environmental exposure to chemicals (particularly for non-pharmaceuticals) is limiting the implementation of exposure-based waiving on a large scale.

The characterisation of exposure to drugs and other chemicals within non-animal test systems is critical to ensure that the effects observed in such assays can be confidently related to effects in humans or environmental species, as the difference in concentration can vary by many orders of magnitude. This is achieved through a process known as *in vitro* to *in vivo* extrapolation (IVIVE). This provides a more physiologically-relevant basis for decision-making when assessments of efficacy or safety are conducted and ensures that the new approaches to assessing toxicity are designed and used appropriately.

a. Whilst the main focus of such activities to date has been on safety assessment, the same conceptual approaches can be applied to the assessment of efficacy.

2. Increasing the physiological relevance of *in vitro* assays and the understanding of human-relevant exposures

Understanding how chemicals interact with their biological targets

New high-throughput technologies are emerging to enable the binding kinetics of compounds (i.e. how much a compound actually interacts with the biological target of interest) to be assessed, for example time-resolved fluorescence energy transfer (FRET) approaches capable of simultaneously measuring the kinetics of hundreds of compounds in cellular systems. These approaches demonstrate that compound binding, and thus action, is a complex process, reliant not only on the amount of compound applied (i.e. nominal concentration) but also on its physicochemical properties, and is time dependent. Such approaches will be useful for the pharmaceutical industry to screen for compounds with the appropriate binding (and thus exposure) characteristics and to better estimate risk of adverse effects from exposure to chemicals. Efforts are also being made into understanding local concentrations, which are necessary to fully interpret the kinetics of receptor-ligand binding. Different concentrations can be observed throughout the 'micro' environment between the cells and buffer/culture medium; this is influenced by the presence and quantity of the receptor of interest within cell membranes, which in turn determines how much of a compound is bound or freely available in the buffer at any given time.

Understanding how biological effects can be altered by the time course of chemical exposure

Consideration of the time course of chemical exposure and concentrations used within a cell-based assay are necessary in terms of determining whether any adverse effects observed are relevant under both acute and chronic exposure conditions. Such considerations are also essential to determine what concentrations are likely to be encountered by cells in real-world situations, to avoid non-relevant effects such as cell death resulting simply from unrealistically excessive concentrations. For example, it is possible that chemicals which are classified as having genotoxic potential under standard (i.e. acute or one-off) dosing regimens in typical cell-based assays may not show such effects when the same concentration is instead delivered over several days. There are likely explanations of this change in effect related to underlying homeostatic mechanisms and it is important that these are understood before accurate safety decisions can be made.

Using mathematical modelling to increase the physiological relevance of non-animal approaches and improve IVIVE

Mathematical modelling approaches are invaluable for improving IVIVE from both an effects and exposure perspective. Such *in silico* models can be used to help predict the characteristics necessary to replicate normal physiology in cells or 3D biological structures to be used as tools in toxicity testing. In this way, models can be improved to not only better mimic the physiology of the real-life situation, but also to ensure the accurate incorporation of components that influence how much of a chemical cells are exposed to. Physiologically-based pharmacokinetic (PBPK) models are an example of a mathematical modelling technique used routinely in the pharmaceutical industry to predict the absorption, distribution, metabolism and excretion (ADME) of drugs in humans and other species, including at a population level. These can be used in a 'top-down' approach, where information on biokinetics from *in vivo* studies, such as clearance in human volunteers, or information from human biomonitoring, is used to inform human-relevant concentrations for testing non-clinically (also known as reverse dosimetry); or in a 'bottom-up' approach, to predict biokinetics in the population of interest, and to support IVIVE. However, their application depends critically on adequate knowledge of the physiological factors involved. For example, this is currently limited when ADME is reliant on drug transporters[7].

3. Quantifying dose-response relationships in *in vitro* assays: modelling, measurement and dosing

There is a need for more sophisticated techniques and approaches to ensure the accurate understanding and quantification of *in vitro* bioactive test concentrations achieved in model systems. This is necessary to inform study design and data interpretation, enabling translation to human or animal toxicological equivalent doses (i.e., quantitative IVIVE) and for cross-assay comparisons. These include those used to determine *in vitro* biokinetics, true free/unbound/bioavailable concentrations in *in vitro* assays (as opposed to a continued reliance on nominal concentrations), and the consequences of repeated dosing (also see section 2).

Use of computational tools to design studies

Interactive web-based tools, such as steady state mass balance models, are now available to support the design and interpretation of *in vitro* assays[8,9]. These allow determination of the potential implications of different assay formats on the cells' exposure to the chemical, for example the dimensions of the well plate used and media serum content. Sensitivity and uncertainty analyses can also be incorporated into such models, and can be used to inform future research needs, for example efforts to expand the applicability domain of the models. There is also scope to incorporate more dynamic components into such models which, for example, could take variations in chemical concentration over time into account.

Consideration of factors affecting free and intracellular concentrations

The physicochemical properties of test chemicals will profoundly influence the *in vitro* biokinetics and effects of repeated dosing. For example, for charged complex chemicals such as surfactants, it has been shown that the membrane-water partition coefficient, pH, serum and calcium levels in the exposure medium can have an effect on partitioning, and on cytotoxicity[10]. Also, when dosed repeatedly, chemicals with a high affinity for cells will accumulate in the absence of clearance mechanisms and may lead to observation of increased toxicity. Even where free concentrations can be accurately predicted or measured, there remains the caveat that not all of the free chemical will necessarily be available to act at the target site, as a result of metabolism or the activity of transporters. Therefore, it may be timely to move towards assessment of 'cell-associated' concentrations, although this would be technically more challenging and is likely to be of lower throughput.

Consideration of intracellular concentrations of chemicals, in addition to measuring free concentrations in media, will prove valuable, although it is not practical or currently possible to assess chemical concentrations in miniaturised systems (i.e. 384 or 1536 well plates). Instead, extended mass balance models can be applied to determine free concentrations and intracellular exposure for neutral and ionogenic organic chemicals. The degree of partitioning between protein (e.g. serum albumin), lipid (e.g. cell membranes) and water can be estimated using various software including QSAR models. Modelling conducted in this way on data from the US EPA ToxCast database has revealed that the distribution and bioavailability of chemicals is highly dependent on the lipid and protein composition of media used in the assays, which can vary substantially. Therefore, in addition to physicochemical properties such as hydrophobicity and degree of ionisation of a chemical, exposure concentrations are also highly dependent on the bioassay set up, and this must be considered when comparing the biological effects seen in such assays.

Controlling delivery of test substances

There is also the option, through use of techniques such as passive dosing, to control the concentrations of a chemical delivered *in vitro*, as opposed to only characterising the levels of exposure retrospectively. Silicone O-rings have been used successfully to passively dose cells over time. However this type of approach is currently not suitable for high-throughput screening due to technical aspects and the need to use smaller rings when scaling up towards 1536 well plates. Adaptations also need to be made to allow for closed-well testing of volatile chemicals and the testing of mixtures, as unequal depletion of individual components will unduly affect the mixture composition.

4. Increasing confidence in, and acceptance of, new exposure-based computational models

As described in section 2, exposure-based computational models are currently used for the purposes of predicting biokinetics and dosimetry. Such approaches have two primary aims: a) to support IVIVE and data interpretation, by predicting how concentrations used *in vitro* and the effects observed relate to doses and effects encountered by humans/environmental species; and b) to prospectively calculate exposure concentrations for use in *in vitro* tests that have human or environmental relevance, based on exposures detected in humans or environmental species from samples taken in biomonitoring/epidemiological studies, or using data from *in vitro* or *in silico* approaches that assess ADME. Traditionally, samples used for biomonitoring purposes have been primarily blood and urine, although other non-invasive matrices, such as saliva, breath and hair can be used. It should be noted that although these two approaches are the inverse of each other, this does not necessarily mean that the models can simply be used in reverse.

PBPK models can be used for these approaches, although this relies on the ability to reconstruct *in vivo* exposures from biomonitoring data to 'validate' them – for example demonstration that biomonitoring outputs can be used to accurately predict *in vivo* exposure levels. To achieve this, targeted studies are required which capture both biomonitoring outputs and actual exposures. In the future, it is critical that scientists and modellers conducting the exposure reconstructions work in parallel with those collecting biomonitoring samples, to ensure that the most informative data at the necessary level of detail (i.e. level and time course of exposure) is captured.

The effective use of PBPK models is also very dependent on understanding the fidelity of the model in terms of the biological system it represents, incorporating the appropriate level of biological detail, and understanding the uncertainty and variability within the models. This has led to the ongoing development of an open access modelling platform, RVis, which aims to be a user-friendly *in vitro* and *in vivo* exposure predictor for predicting equivalent human oral, dermal or inhalation exposures consistent with measured *in vitro* target tissue concentrations.

As biokinetic measurements are not often a feature of *in vitro* assays, the US EPA's RED (Rapid Exposure and Dosimetry) project is developing new high throughput PBPK tools to characterise, simulate and evaluate chemical biokinetics to enable extrapolation from *in vitro* concentrations to target tissue or blood concentrations. For chemicals where human or animal *in vivo* data are available it can be assessed where the models perform well and where other effects (such as those mediated by transporters) may impact on the model assumptions. The chemical types for which the models can be used with sufficient confidence can be identified, and conversely for which chemical types different approaches or further data may be needed. Where the model assumptions have proven to be appropriate, virtual simulation of quantitative chemical-specific effects in tissues is possible. This type of approach holds promise for use in prioritising chemicals for further toxicity testing and hazard characterisation, based on comparing information on concentrations shown to cause toxic effects *in vitro* (such as that generated in ToxCast) and the modelled information on the likelihood of those concentrations being encountered in target tissues.

Summary of the future opportunities for applying exposure science identified from the workshop presentations

It is clear that much progress is being made to develop and apply new approaches and techniques, be they computational or laboratory-based, to increase the understanding of chemical exposures both within cellular systems and at a human/environmental population level.

However, there remains a need for the further development of these novel approaches to produce more relevant exposure information, and for improvements in the understanding and characterisation of relationships between human- or environmentally-relevant exposures and those used in toxicity testing systems. There is also a need to more widely incorporate this type of information into MOA/AOP-driven assessments, which by their nature will require the use of integrated approaches to ensure that the appropriate information is generated in the absence of data from a whole organism. To focus efforts in this regard the field would benefit from an organisational and predictive framework to support the generation, application and integration of exposure data. With this in mind, an 'Aggregate Exposure Framework'[5] has recently been proposed and is currently in development.

One of the key actions necessary for ensuring wider use of approaches that integrate both biological effects and exposure data is increasing recognition of the benefits of considering exposure information in a decision-making context by regulatory agencies. The combination of hazard characterisation and exposure assessment is axiomatically the prerequisite for risk assessment, as opposed to safety decisions made purely on the hazard potential of a chemical. In this way, public health decisions can be made on more of a cost/benefit analysis basis, and/or acceptable margins of exposure can be more easily determined. The use of biokinetic information has many potential advantages in the safety and risk assessment process, including informing dose selection for mandatory *in vivo* studies so that unrealistically high doses are avoided (thus avoiding unnecessary systemic toxicity which can cause suffering in experimental animals and artefacts caused by excessively high doses in *in vitro* tests) and ensuring that doses tested are more relevant to humans or the environment. There is also scope for *in vivo* or *in vitro* biokinetic information to inform read-across approaches, which could help to support arguments to waive mandatory *in vivo* studies. It is also important to consider whether testing chemicals *in vivo* through multiple routes of exposure is always necessary and whether these studies add value to the safety risk assessment process. For example, it has recently been recognised by the US EPA following a retrospective data analysis exercise that conducting dermal as well as oral acute toxicity tests for pesticide formulations is not necessary, as in 95% of cases dermal toxicity occurs at higher exposures than oral toxicity, so that oral data alone will be sufficient to inform the risk assessment.

The potential utility of computational models to support the application of exposure science has become increasingly apparent in recent years. For example, PBPK approaches have the potential to be used for both *in vitro* and *in vivo* dose setting and their value to support IVIVE for environmental chemicals is being increasingly recognised. PBPK models form a major component of the large-scale Horizon 2020 project EU-ToxRisk which aims to provide proof-of-concept for a mechanism-based chemical safety testing strategy. One of the major limitations of using PBPK models in the prediction of ADME for non-pharmaceutical chemicals is related to the lack of real-world data on chemical exposure, such as epidemiological and biomonitoring data, to inform model development and validation, although there are now examples of PBPK models that have been built solely based on *in vitro* and *in silico* data (e.g.[6]). Although widely used in drug discovery, results from PBPK models are not yet widely used in regulatory decision-making. A recent EURL-ECVAM (European Union Reference Laboratory for alternatives to animal testing) workshop 'Physiologically-Based Kinetic Modelling In Risk Assessment – Reaching A Whole New Level In Regulatory Decision-Making' discussed issues related to the regulatory acceptance of these models and a working group is currently devising next steps to promote their development and acceptance, including an OECD (Organisation for Economic Co-operation and Development) Guidance Document.

The presentations at the workshop described in this report highlighted the exciting ongoing initiatives to increase the application of exposure science in efficacy and safety testing, but also raised a number of issues and barriers that exist which currently inhibit its widespread uptake. These included the need for greater cross-disciplinary collaboration and an increased recognition of the importance and benefits of incorporating exposure considerations into decision-making processes. Such issues were further discussed during the workshop's breakout sessions.

Summary of pre-meeting survey results

Prior to the workshop, delegates were asked to complete a pre-meeting survey to gain an understanding of their views on the challenges and barriers to the advancement of the application of exposure science capability, including:

- Current research needs;
- Challenge areas that need to be addressed; and
- How confidence can be built in new approaches to enable their wider use and acceptance in practice.

The questions posed were:

1. What is the biggest basic research need that must be addressed to advance the application of exposure science capability to support the use of non-animal approaches in efficacy and safety testing?
2. What is the biggest need or challenge that must be addressed before industry can take up and utilise exposure-based approaches to support the use of non-animal approaches in screening, prioritisation and regulatory testing?
3. What do you think is needed to enable information generated using exposure-based approaches (in combination with non-animal efficacy or hazard data) to be accepted by regulators?
4. What is needed to build a community of experts in exposure-driven non-animal safety assessment?
5. What would be the one thing needed to move this field forward towards wider industry application?

We received 53 full responses from scientists working for consumer products, pharmaceuticals, agrochemicals and industrial/general chemicals companies (43%), academics (36%), and scientists from other organisations (21%). This was largely representative of the spread of delegates present at the workshop.

The responses were grouped under different themes and were presented at the workshop prior to the day 1 breakout session to help guide and inform discussion. The main themes for each question are outlined below, ranked in order of the frequency they were mentioned.

Questions 1-3: The biggest basic research, industry and regulatory needs to advance the application of exposure science capability.

- Improved/better understanding of the relationship between *in vitro* and *in vivo* exposures i.e. IVIVE through basic research;
- Development of new tools and models;
- Validation, transparency, accuracy and confidence in the reliability and reproducibility of the models;
- Increased application of existing knowledge;
- Standardisation of methods and guidance to support this;
- Increased funding;
- Harmonisation of regulatory requirements;
- Regulatory acceptance;
- Incorporation of internal exposure considerations;
- Data sharing, reference databases and the generation and sharing of case studies; and
- Training and education.

Question 4: Needs for building a community of experts.

- Collaboration;
- More workshops, working groups and meetings;
- Data sharing;
- Transparency and openness;
- Dedicated funding;
- Attitude and vision; and
- Training.

Question 5: The one thing that is needed to move exposure science forward for wider industry application.

- Collaboration;
- Increased confidence in and 'validation' of approaches;
- Regulatory acceptance, guidance and standards;
- Data sharing and case studies; and
- Improved understanding.

Day 1 breakout discussions took place across four mixed sector groups (the participants in each of these groups were pre-selected with the aim to reflect the spread of expertise present at the workshop), and aimed to:

- Identify the current application of exposure-based approaches to support the use of non-animal methods in efficacy and safety testing and what makes this possible.
- Identify the barriers which limit the wider uptake and acceptance of exposure-based approaches to support decision making.

All four groups were asked to consider the same four questions:

1. Where in efficacy and safety testing is exposure science currently used to support non-animal approaches?
2. At what level are data derived using exposure science accepted by (a) regulators or (b) internally?
3. Can exposure science be applied more broadly in efficacy and safety testing, and if so, where/how? What are the incentives to do so?
4. What are the barriers preventing broader application of exposure science in safety and efficacy testing?

Responses to each of the questions posed to the breakout groups are summarised in Annex II. The output from the session was used to identify areas for more focused discussion in the day 2 breakout sessions. These were voted for during the networking session at the end of day 1.

The barriers identified in question 4 were grouped in order of the following priority 'needs'. These are ranked according to the number of votes each area received; each delegate was allowed up to five votes and were asked to vote for the area or 'need' they felt required addressing most strongly. The number of votes is presented in parentheses below. The top four areas (in bold), based on the numbers of votes cast, were taken forward for discussion during the day 2 breakout session.

Barriers preventing broader application of exposure science in efficacy and safety testing

1. **Regulatory acceptance and harmonisation of requirements (48).**
2. **Increased confidence/uncertainty in reliability and reproducibility of the models (44).**
3. **Collaboration, need to integrate knowledge and work together (31).**
4. **Increased understanding of *in vitro* doses and *in vitro* models (31).**
5. Increased expertise and communication across end-user communities (29).
6. Clarity on what the novel exposure approaches are and how they support decision making (22).
7. Increased availability of relevant data (data sharing, case studies etc.) (20).
8. Better analytical chemistry methods to support high throughput screening (20).
9. Provision of standards and guidance for *in vitro* methods (10).

Summary of breakout group discussions: Day 2

These discussions took place across the four key priority areas selected by delegates at the end of day 1, and aimed to develop an approach to address the selected need and identify the required resources and external partners which might be required to facilitate this, covering:

- Advances in science required;
- Change in practice needed;
- Possible incentives;
- Resources required; and
- Partners/people to engage with.

Delegates had the opportunity to join discussions on two of the priority areas. Responses to the questions posed on each of the priority areas are summarised below.

Current status of exposure science and barriers to uptake (1)

1. Where in safety and efficacy testing is exposure science currently used to support non-animal approaches? i.e. informing next steps, critical aspects of a safety/efficacy argument, inform clinical study design, etc.

- In ecotoxicology assessments, where fate models are used to model which compartment chemicals partition to.
- When determining cellular uptake of nanoparticles in genotoxicity assays.
- The Multiple-Path Particle Dosimetry (MPPD) Model is used to predict particle inhalation.
- The application of PBPK modelling to support toxicity assessment.
- In chemical read across and assessment of toxicokinetics.
- To support the Tox21 programme in the verification of the concentrations being tested in the *in vitro* assays
- To prioritise which ToxCast chemicals should be taken forward for further investigation.
- To inform the top dose that should be tested *in vivo* by determining metabolism saturation.

2. At what level are data derived from using exposure science accepted by regulators?

- Currently non-animal data is rarely accepted by regulatory bodies. In principle it should be utilised under regulatory frameworks as part of integrated approaches to testing and assessment but there is currently an obstacle regarding the lack of validation of non-animal test methods.
- Accepted in human and environmental exposure-based waiving arguments and when applying the threshold of toxicological concern (TTC) concept.
- Used to determine acceptable use conditions through understanding of exposure scenarios in the agrochemical and pharmaceutical industries, biomonitoring conducted post-marketing to understand unforeseen uses.
- In US-based regulatory agencies exposure drives safety assessment.
- The European REACH regulation works on a tonnage-based prioritisation system.
- In bioaccessibility models (for food packaging and mining industries).
- In anti-microbial assessments for some veterinary drugs.
- OECD Test Guideline 428 (Skin Absorption: *In Vitro* Method) is the only method of assessing exposure *in vitro* that is accepted by regulators.
- When assessing enzyme induction (especially the US FDA)

2. At what level are data derived from using exposure science accepted internally (within companies)?

- Translating data across different model systems.
- PBPK modelling to inform in vitro doses (internal) and inform dose setting for *in vivo* tests (reducing the need for dose range finder studies).
- In the building of PBPK models, which are fed with *in vitro* data.
- To increase understanding and improve predictions.
- When determining the chemical concentration in cell culture medium.
- To inform chemical design and synthesis, using fate models.
- It is common practice in the pharmaceutical sector:
  - PBPK modelling
  - Investigations of metabolism and the actions of transporters
  - For efficacy assessments, when relating *in vitro* concentrations to human exposures
  - Estimating retained doses by covalent protein binding
  - In the development of better *in vitro* screens to help direct preclinical testing
  - Determining the doses used for cardiotoxicity *in vivo* studies based on results from the hERG assay.
- In the agrochemical industry for prioritisation. There is perhaps better acceptance of these approaches in agrochemical environmental risk assessment than for human health assessment.
- The cosmetics industry.
- Screening to predict the outcome of regulatory studies and prioritise candidate development.

Current status of exposure science and barriers to uptake (2)

3. Can exposure science be applied more broadly in safety and efficacy testing, and if so, where/how? What are the incentives to do so?

- Yes!
- Greater exposure-based prioritisation for testing and understanding of real life exposure scenarios using biomonitoring data.
- Concentration selection for *in vitro* assays, providing more relevant biology, relevant mechanisms and dose responses, and avoiding artefacts caused by too high concentrations.
- In the technology development of *in vitro* assays to provide better ADME, bioactive concentrations, dosing and measurement.
- For determining exposure in test systems and relating cellular dose to cellular effects.
- As the driver of for development of quantitative AOPs.
- To move from qualitative to quantitative assessment e.g. for endpoints such as genotoxicity which currently give 'binary' readouts.
- For facilitating the acceptance of PBPK modelling.
- Priority setting/screening of molecules.
- In IVIVE to support *in vitro*-based risk assessment
- Better experimental design and exposure-driven approaches – start with low dose exposures and move up, to find thresholds of toxicity.
- To increase understanding of the unknowns and identify outliers with regard to toxic responses and idiosyncratic responses.

Incentives:

- The 3Rs.
- Increase the biological relevance, the human health relevance and the accuracy and applicability of risk assessment.
- New product discovery, better characterisation of efficacy and potency.
- Decreased cost of product development
- Decreased uncertainty.
- Could decrease time products take to reach the market by increasing throughput.
- Maximisation of available data.

4. What are the barriers preventing broader application of exposure science in safety and efficacy testing?

- Lack of confidence in modelling and monitoring approaches.
- Cost.
- Legislation and regulatory silos, and differences in regulatory practice in different parts of the world.
- Dogma of hazard driven approaches (cultural/mindset issues), continued application of precautionary principles (conservatism), regulatory inertia and requirements within OECD Test Guidelines.
- Lack of training/education/communication.
- Lack of available tools including open source tools.
- Lack of data availability and sharing which could help to overcome perceived complexity.
- Lack of a focused framework or standards/guidance.
- The complexity of most current solutions, a disconnect between needing to produce novel of science vs. practical and simple solutions.
- A shortage of experts/skills (regulators, academics and industry scientists) and funding to train them).
- Public scepticism.
- The unknown uncertainties.
- Analytical chemistry not catching up and a lack of (bio)analytical chemists.
- Lack of understanding of *in vitro* doses and models.
- Lack of integration of knowledge.
- Lack of knowledge on transporters.
- Overconfidence in and more comfort associated with *in vivo* approaches.
- Lack of precision of methods (due to maturity of methods).
- Lack of knowledge on Cmax/AUC driving effects.
- Lack of capability within contract research organisations.
- The community is currently fragmented and working in silos.
- Uncertainty around interpretation and how the data will be used, and applied in weight of evidence approaches.

What is needed to overcome the following barrier:

1. Regulatory acceptance and harmonisation of requirements

Science

Where do advances in the science and its application need to occur?

The identification of key mechanisms, followed by the building AOPs, development and identification of relevant assays, validation of the assays and integrated approaches necessary using case studies. Agreement of when it is pragmatic to stop adding to AOPs and avoid excess detail.

Better biomonitoring data for setting doses/interpreting data from *in vitro* assays.

Increased confidence that the model works – for this there is a need for high quality data.

Practice/incentives

What changes in practice (company, regulatory, funder, academic, etc.) are needed?

Include exposure in tiered testing strategies.

Greater combining of knowledge on mechanisms.

Better definition on 'margins of comfort'.

Direct funding (more of this as fewer overall less studies would be needed).

Alignment of basic research with regulatory requirements.

Change of mindset – focus on how much of an improvement on current practice the approaches offer, rather than how good they are in themselves.

Cross-validation using multiple *in silico* and *in vitro* models and comparing the results of these different approaches.

Incentivising uptake by regulators, particularly where not a regulatory requirement for exposure data/relation of effects to likely human or environmental exposures.

Characterisation of the variability in animal studies to provide a 'performance metric' against which to assess the new approaches.

Resources

What resources will be needed?

Better structure-based predictive models.

More value of information analysis exercises to determine whether certain tests/exposure routes are redundant and not adding value to the safety assessment process.

Use environmental exposure models to inform human exposure-driven approaches.

Conduct of an information gathering exercise to define regulatory requirements by chemical sector and region, to help ascertain what to prioritise.

Communication of these regulatory requirements.

Partners

Who else do we need to engage with?

International industry stakeholders.

Legislators.

The public.

Policy makers and scientists in countries developing new regulations e.g. China.

Guidance for funding bodies.

Top tier journals to support greater acceptance of papers on toxicity studies which include exposure considerations.

Improved industrial collaboration and data sharing.

Other

What other recommendations would help?

Incorporation of exposure in classification and labelling regulations.

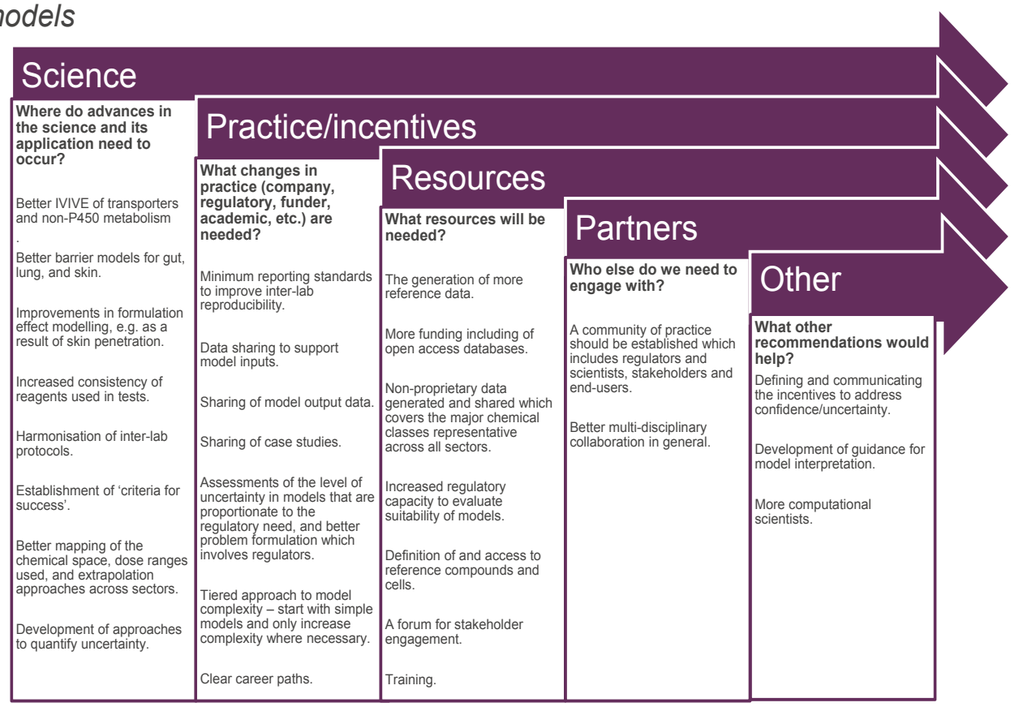
Emphasis on the benefits of human/environmental relevance.

Sharing of case studies, be they successful or not.

Sharing of information with regulators e.g. through workshops.

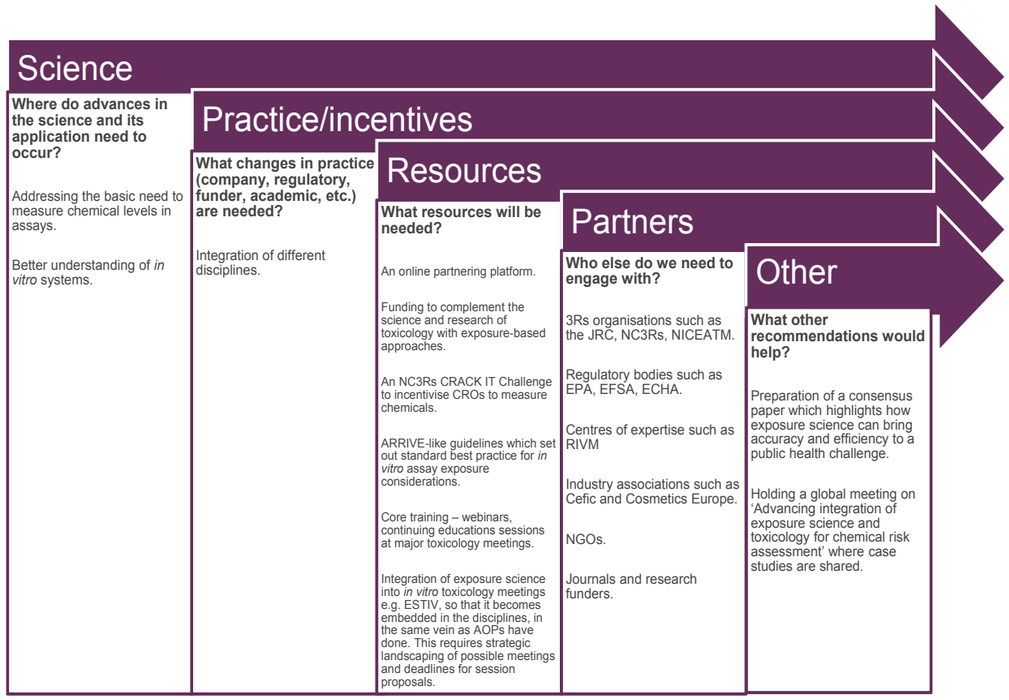
What is needed to overcome the following barrier:

2. Increased confidence/uncertainty in reliability and reproducibility of the models



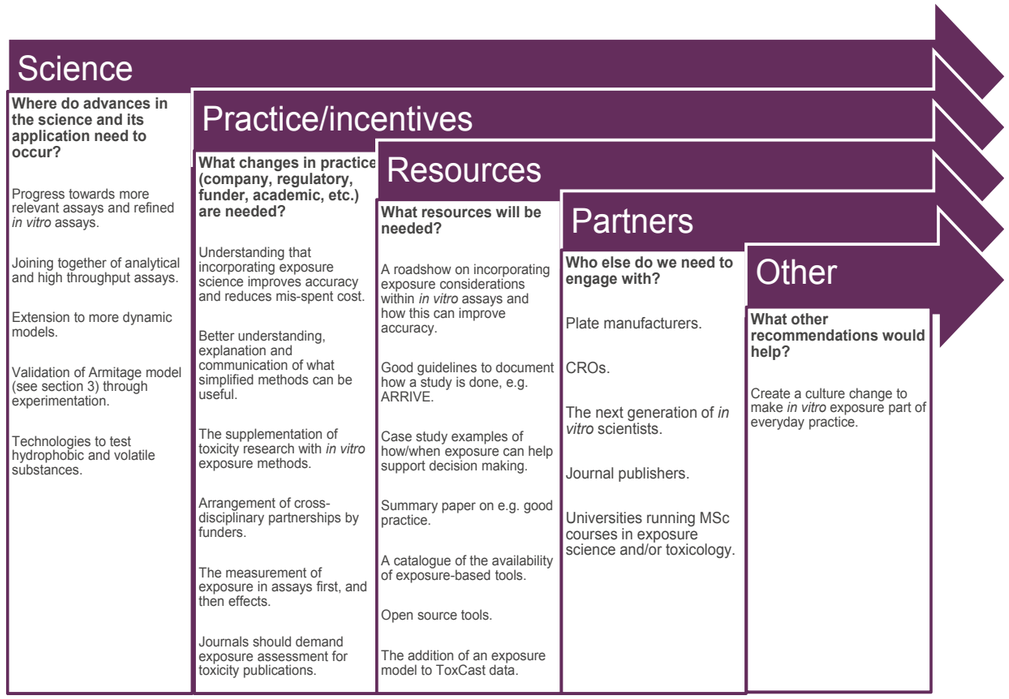
What is needed to overcome the following barrier:

3. Collaboration, need to integrate knowledge and work together



What is needed to overcome the following barrier:

4. Increased understanding of in vitro doses and in vitro models



Increasing the application of exposure science in efficacy and safety testing: key themes

A number of themes were identified during the course of the workshop in terms of future needs to ensure an increase in the application of exposure-driven approaches in non-animal efficacy and safety assessments, as well as the potential opportunities to address these.

Key themes included, but were not limited to, the need for:

1. Better understanding of the incentives, benefits and implications of incorporating exposure science into non-animal approaches across sectors/disciplines, including the regulatory community;
2. Creating a culture change whereby the incorporation of exposure science becomes the norm; and
3. A forum for multidisciplinary collaboration/cross-talk between the relevant scientific communities.

Better understanding of the incentives, benefits and implications of incorporating exposure science into non-animal approaches across sectors/disciplines, including the regulatory community

These include the potential improvements in the accuracy, science and physiological relevance underlying efficacy and safety assessment. This in turn could increase the predictive value of new biological effects models and approaches, contributing to a decrease in the current reliance on traditional animal (toxicity) tests and thus advancing the 3Rs. In a wider sense, this could lead to a reduction in the cost and time needed to bring new products to the market, even if only utilised to support internal screening and prioritisation. This would provide marked societal benefits, with new, safe and efficacious products entering the market more quickly, and ensuring that those products or constituents that are unsafe are identified as early as possible and do not enter the market to pose a risk to humans and the environment. Regulators and policy makers in particular need to be more widely engaged in this respect, considering that the benefits of incorporating exposure science are not currently uniformly recognised or required by legislation set out in each geographical region. There would also be a benefit to a better understanding of cross-sector differences in requirements and practice, to facilitate the translation of knowledge and techniques between them.

Creating a culture change whereby the incorporation of exposure science becomes the norm

Responsibility for this will lie with a number of parties – with regulators and policy makers to ensure this occurs within industry research and testing settings; with journals publishing studies and funders of research to influence the behaviour of academic scientists; and with universities to ensure the training and building of awareness within early career scientists destined for careers in academia, industry or regulatory bodies. There are organisations and universities implementing their own training schemes in this area, but no coordinated efforts are underway at the current time. Clearer guidance from regulatory authorities will also help to steer future academic research and may support a transition towards research that is more aligned with regulatory needs. Ultimately, the culture change will need to be driven by the scientists themselves who are involved in the generation and interpretation of data.

A forum for multidisciplinary collaboration/cross-talk between the relevant scientific communities

Until now the *in vitro* efficacy/safety science and exposure science communities have remained relatively disparate with limited interactions between the two. There is also a need to ensure that expertise from computational and mathematical modelling, chemistry and engineering are included to enable genuine advances and sustainable collaborations. The recognition of the benefits to *in vitro* science, as raised in the first point above, will be critical and could be better achieved through engagement of relevant scientific societies, and the provision of sessions or training at their scientific meetings. Research funders could play a role in encouraging and facilitating cross-disciplinary partnerships. The sharing of data and case studies on the impact of exposure-driven approaches on the interpretation of non-animal data and on regulatory decision-making will be useful in demonstrating the benefits and identifying gaps in future research needs.

Implications for the 3Rs

There was a general consensus that the timing of this workshop captured a swell in opinion regarding recognition of the importance of exposure science in ensuring that efficacy and safety testing continues to move forward, and that the time is right for the *in vitro* toxicology and efficacy fields to face the challenge of embedding exposure science into everyday practice. This paradigm shift complements the vision of moving efficacy and safety assessment towards a reduced reliance on animal tests, in parallel with improvements in the underlying science and the efficiency of decision-making.

Increasing the application of exposure science in efficacy and safety testing: key themes

We propose the following recommendations be taken up by the scientific community, to address the key needs and opportunities identified at the workshop:

1. Preparation of guidance for the appropriate reporting of *in vitro* studies and data, particularly in the peer-reviewed literature. This would support the reporting of measured, in addition to nominal, concentrations used in *in vitro* assays, and consideration of how doses used relate to those likely to be encountered by humans and the environment. This should be prepared in collaboration with journals and funding bodies and would ultimately aim to seek endorsement/enforcement of the guidance by the journals and funders. At the same time a co-ordination of training materials for scientists a) in early career stages (under- and postgraduate students) and b) already working in the field of *in vitro* science should be prepared and become a routine component of training courses. It would also be useful through this exercise to further encourage the early stage use of *in silico* exposure prediction tools to guide and inform experimental design.

2. Holding a global cross-disciplinary workshop on the theme of 'advancing the integration of exposure science and toxicology for chemical risk assessment' where case studies are presented demonstrating the implications of incorporating exposure-driven approaches on internal company decision-making (i.e. at the scientific project or internal risk assessor level) and the potential impact this could have on regulatory safety and policy decisions. Case studies could be identified from ongoing projects already exploring the incorporation of exposure considerations (e.g. ToxCast, EU-ToxRisk) or could be initiated for the purpose of the workshop.

Concluding remarks

It is clear from the presentations and discussions that took place at this workshop that the time is right to work with the scientific and regulatory communities towards routinely applying exposure science capabilities in efficacy and safety assessment. The delegates demonstrated that there is an increasing appetite across all of the relevant scientific communities to come together and make this a reality. There remains a genuine need to increase the awareness of the importance and benefits of using exposure science to increase the utility of data from non-animal approaches for decision-making, be that internally within companies or at the regulatory level.

This is an area that has been slowly gaining momentum in recent years, with several ongoing but disparate activities focusing on different aspects. One of the key next steps should be to take forward the recommendations made here, which will require focused leadership. To be successful, this will also rely on effective collaboration between safety/efficacy and exposure scientists, as well as multidisciplinary input into the next steps taken - ensuring that consideration of exposure within non-animal approaches (at both the individual assay and IVIVE level) can be used to inform the accurate interpretation of the data they generate. This will increase their physiological relevance and ultimately support the transition away from the current reliance on traditional animal tests, towards a more scientifically and mechanistically-driven approach to product development and decision-making.

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Annex I: Workshop Programme

DAY ONE	
09.30 – 10.00	**REGISTRATION AND REFRESHMENTS**
09.00 – 09.10	**Welcome and introduction** Professor Alan Boobis OBE; Imperial College London (Chair), UK
10.20 – 10.30	**Introduction to the NC3Rs** Dr Natalie Burden, NC3Rs, UK
The current landscape	
10.30 – 11.00	**Enhancing acceptance of and confidence in predictive models of AOPs** Dr Bette Meek, University of Ottawa, Canada
11.00 – 11.40	**Beyond the axis of ignorance: How 21st century exposure science will transform chemical safety assessment** Dr Justin Teeguarden, Pacific Northwest National Laboratory, USA
11.40 – 12.00	**REFRESHMENTS**
Increasing the physiological relevance of *in vitro* assays and understanding of human-relevant exposures	
12.00 - 12.25	**Measuring binding kinetics and local drug concentration to develop "micro PK-PD" relationships** Professor Steve Charlton, University of Nottingham, UK
12.25 - 12.50	**Development of chronic and passive dosing systems *in vitro* for genotoxicity assessment** Professor Gareth Jenkins, Swansea University, UK
12.50 - 13.45	**LUNCH AND POSTER VIEWING**
13.45 - 14.10	**CRACK IT Challenge: Improved *in vitro* to *in vivo* extrapolation in chemical safety risk assessment of human systemic toxicity** Dr Steve Webb, Liverpool John Moores University, UK
14.10 - 14.50	**Population-based physiologically-based PK/PD simulators** Dr Iain Gardner, Certara, UK
14.50 - 15.20	**REFRESHMENTS**

Breakout groups and discussion	
15.30 - 15.40	**Introduction to day 1 breakout groups and results of the pre-meeting survey** Dr Fiona Sewell, NC3Rs, UK
15.40 - 16.40	**Current status of exposure science and barriers to uptake** Facilitated session to explore the barriers to acceptance and uptake of exposure-based science to increase the utility of non-animal data in efficacy and safety testing. Themes identified in this session will be used to inform the breakout group sessions on day 2. Discussions will centre on the following questions: ▪ Where in efficacy and safety testing is exposure science currently used to support non-animal approaches? ▪ At what level are data derived using exposure science accepted internally or by regulators? ▪ Can exposure science be applied more broadly in efficacy and safety testing, and if so, where/how? What are the incentives to do so? ▪ What are the barriers preventing broader application of exposure science in efficacy and safety testing?
16.40 - 17.20	**Feedback from breakout groups** Discussion
17.20 - 17.30	**Wrap up and opening of networking reception**
Networking reception	
17.30 - 19.00	**Networking reception and poster viewing**

DAY TWO	
08.30 - 09.00	**REGISTRATION AND REFRESHMENTS**
09.00 – 09.10	**Introduction to Day 2** Professor Alan Boobis OBE; Imperial College London (Chair), UK
09.10 - 09.20	**Physiologically-based kinetic models reaching a whole new level in regulatory decision making – At a glance** Dr Alicia Paini, European Commission Joint Research Centre, Italy
Quantifying exposure-response relationships in *in vitro* assays: modelling, measurement and dosing	
09.20 - 09.45	**Development of a modelling framework to help design and interpret *in vitro* dose-response relationships** Dr Cecilie Rendal, Unilever, UK
09.45 - 10.10	**Quantitative *in vitro* to *in vivo* extrapolation (QIVIVE), free concentration, toxicokinetics and repeated dosing** Dr Nynke Kramer, Utrecht University, the Netherlands
10.10 - 10.35	**Mass balance modelling for the assessment of internal exposure in cell based bioassays** Professor Beate Escher, Helmholtz Centre for Environmental Research (UFZ), Germany
10.35 - 11.00	**REFRESHMENTS**
11.00 - 11.25	**Passive dosing of hydrophobic organic chemicals to *in vitro* assays – controlling, defining and linking exposure** Professor Philipp Mayer, Technical University of Denmark (DTU), Denmark
Increasing confidence in and acceptance of new exposure-based computational models	
11.25 - 11.50	**Exposure reconstruction using Bayesian inference and reverse dosimetry** Dr George Loizou, Health & Safety Executive, UK
12.50 - 12,15	**Toxicokinetic and dosimetry modelling tools for exposure reconstruction: US EPA's RED Project** Dr Barbara Wetmore, Environmental Protection Agency, USA
12.15 - 12.40	**A toxicokinetic-toxicodynamic framework for *in vitro* to *in vivo* ecotoxicity extrapolation** Dr Roman Ashauer, University of York, UK

12.45 - 13.45	**LUNCH AND POSTER VIEWING**
13.45 - 14.15	**Incorporating exposure driven approaches and *in vitro* data into regulatory decision making** Dr Nicole Kleinstreuer, National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), USA
Breakout groups and discussion with refreshments	
14.15 - 14.30	**Introduction to day 2 breakout groups**
14.30 - 15.45	**Overcoming the challenges and barriers to uptake of exposure-driven approaches** Breakout groups will examine the barriers identified in the day 1 breakout sessions to develop a framework for expediting the acceptance of exposure-driven science to increase the utility of non-animal data in efficacy and safety testing.
15.45 - 16.00	**REFRESHMENTS**
16.00 - 16.45	**Feedback from breakout groups** Discussion
16.45 - 17.00	**Wrap up and meeting close**

Current status of exposure science and barriers to uptake (1)

1. Where in safety and efficacy testing is exposure science currently used to support non-animal approaches? i.e. informing next steps, critical aspects of a safety/efficacy argument, inform clinical study design, etc.

- In ecotoxicology assessments, where fate models are used to model which compartment chemicals partition to.
- When determining cellular uptake of nanoparticles in genotoxicity assays.
- The Multiple-Path Particle Dosimetry (MPPD) Model is used to predict particle inhalation.
- The application of PBPK modelling to support toxicity assessment.
- In chemical read across and assessment of toxicokinetics.
- To support the Tox21 programme in the verification of the concentrations being tested in the *in vitro* assays
- To prioritise which ToxCast chemicals should be taken forward for further investigation.
- To inform the top dose that should be tested *in vivo* by determining metabolism saturation.

2. At what level are data derived from using exposure science accepted by regulators?

- Currently non-animal data is rarely accepted by regulatory bodies. In principle it should be utilised under regulatory frameworks as part of integrated approaches to testing and assessment but there is currently an obstacle regarding the lack of validation of non-animal test methods.
- Accepted in human and environmental exposure-based waiving arguments and when applying the threshold of toxicological concern (TTC) concept.
- Used to determine acceptable use conditions through understanding of exposure scenarios in the agrochemical and pharmaceutical industries, biomonitoring conducted post-marketing to understand unforeseen uses.
- In US-based regulatory agencies exposure drives safety assessment.
- The European REACH regulation works on a tonnage-based prioritisation system.
- In bioaccessibility models (for food packaging and mining industries).
- In anti-microbial assessments for some veterinary drugs.
- OECD Test Guideline 428 (Skin Absorption: *In Vitro* Method) is the only method of assessing exposure *in vitro* that is accepted by regulators.
- When assessing enzyme induction (especially the US FDA)

3. At what level are data derived from using exposure science accepted internally (within companies)?

- Translating data across different model systems.
- PBPK modelling to inform in vitro doses (internal) and inform dose setting for *in vivo* tests (reducing the need for dose range finder studies).
- In the building of PBPK models, which are fed with *in vitro* data.
- To increase understanding and improve predictions.
- When determining the chemical concentration in cell culture medium.
- To inform chemical design and synthesis, using fate models.
- It is common practice in the pharmaceutical sector:
  - PBPK modelling
  - Investigations of metabolism and the actions of transporters
  - For efficacy assessments, when relating *in vitro* concentrations to human exposures
  - Estimating retained doses by covalent protein binding
  - In the development of better *in vitro* screens to help direct preclinical testing
  - Determining the doses used for cardiotoxicity *in vivo* studies based on results from the hERG assay.
- In the agrochemical industry for prioritisation. There is perhaps better acceptance of these approaches in agrochemical environmental risk assessment than for human health assessment.
- The cosmetics industry.
- Screening to predict the outcome of regulatory studies and prioritise candidate development.

Current status of exposure science and barriers to uptake (2)

4. Can exposure science be applied more broadly in safety and efficacy testing, and if so, where/how? What are the incentives to do so?

- Yes!
- Greater exposure-based prioritisation for testing and understanding of real life exposure scenarios using biomonitoring data.
- Concentration selection for *in vitro* assays, providing more relevant biology, relevant mechanisms and dose responses, and avoiding artefacts caused by too high concentrations.
- In the technology development of *in vitro* assays to provide better ADME, bioactive concentrations, dosing and measurement.
- For determining exposure in test systems and relating cellular dose to cellular effects.
- As the driver of for development of quantitative AOPs.
- To move from qualitative to quantitative assessment e.g. for endpoints such as genotoxicity which currently give 'binary' readouts.
- For facilitating the acceptance of PBPK modelling.
- Priority setting/screening of molecules.
- In IVIVE to support *in vitro*-based risk assessment
- Better experimental design and exposure-driven approaches – start with low dose exposures and move up, to find thresholds of toxicity.
- To increase understanding of the unknowns and identify outliers with regard to toxic responses and idiosyncratic responses.

Incentives:

- The 3Rs.
- Increase the biological relevance, the human health relevance and the accuracy and applicability of risk assessment.
- New product discovery, better characterisation of efficacy and potency.
- Decreased cost of product development
- Decreased uncertainty.
- Could decrease time products take to reach the market by increasing throughput.
- Maximisation of available data.

5. What are the barriers preventing broader application of exposure science in safety and efficacy testing?

- Lack of confidence in modelling and monitoring approaches.
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- Legislation and regulatory silos, and differences in regulatory practice in different parts of the world.
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