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Challenge 27: DoCE

NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

Dosing for Controlled Exposure (DoCE)

Sponsors: Unilever & Shell

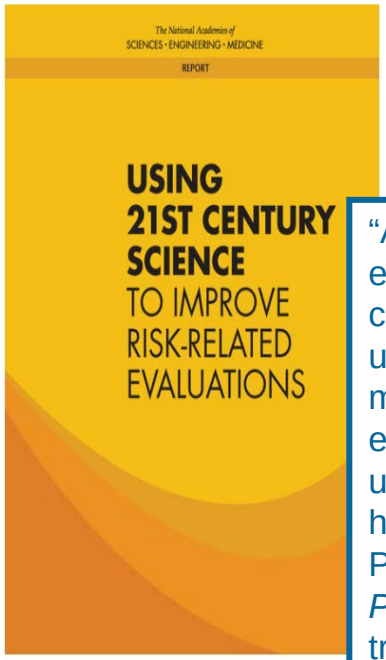
Launch Meeting

7 September 2017

The Challenge – Overall Aims

To establish improved and increased throughput methods and approaches to better account for bioavailability through development of dosing and measurement strategies of test chemicals in *in vitro* assays that are reflective of human and environmental species *in vivo* exposure conditions, to enable robust Quantitative *in vitro* to *in vivo* extrapolation (QIVIVE).

Why was this Challenge developed?

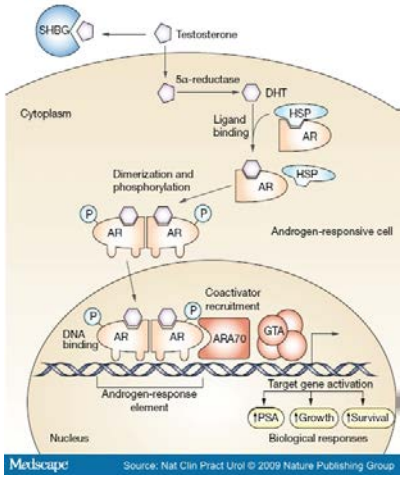


"A primary objective for improving exposure science is to build confidence in the exposure estimates used to support risk-based decision-making, be enhancing quality, expanding coverage and reducing uncertainty ……An important focus has been on the development of PBPK (*Physiologically Based PharmacoKinetic*) models for translating exposures between test systems and human exposure scenarios" 2017

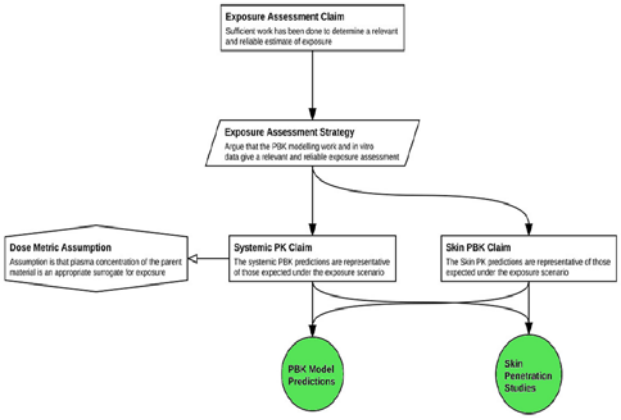
Next Generation Risk assessment (NGRA)

- Using new tools and approaches to build environmental and human health risk assessments to enable decisions to be made (without animal tests)
- An exposure-led risk assessment solution to biological pathway-indicated hazard concerns

Exposure led

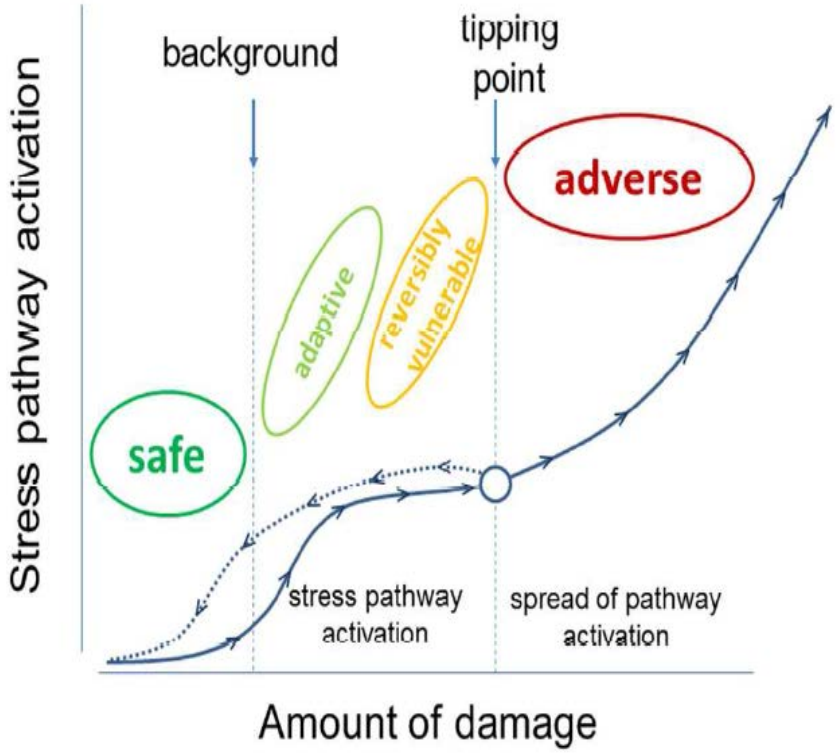


Mechanistic



Hypothesis driven

‘Tipping points’ *in vitro*

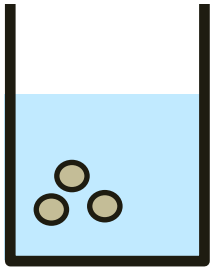


From Middleton *et al.,* (2017). Applied In Vitro Toxicology, 3 (2), 199-210
‘*Workshop report from case studies in cellular stress: Defining Adversity/Adaptation tipping points*’

With thanks to Kim Boekelheide

In vitro assays in risk assessment

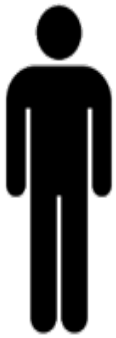
effect in vitro



0-10% serum
single cells / microtissues
high concentrations
short exposure time
no bioaccumulation
limited metabolism

target site
intracellular
free concentration

effect in vivo



100% serum
connected cells / tissues
low concentrations
long / pulsed exposure
tissue bioaccumulation over time
full metabolic pathways

What metrics can we use to compare effects *in vivo* to effects *in vitro* to improve QIVIVE?

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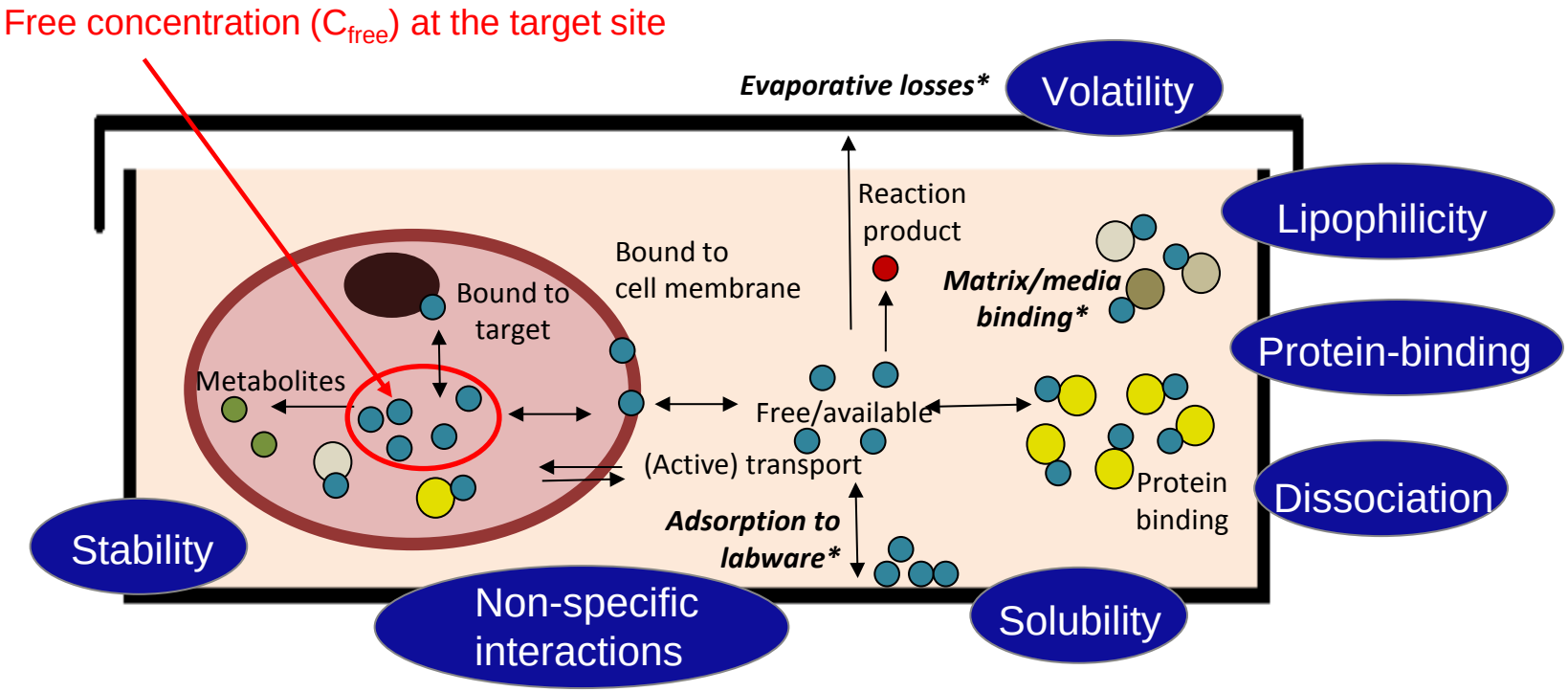
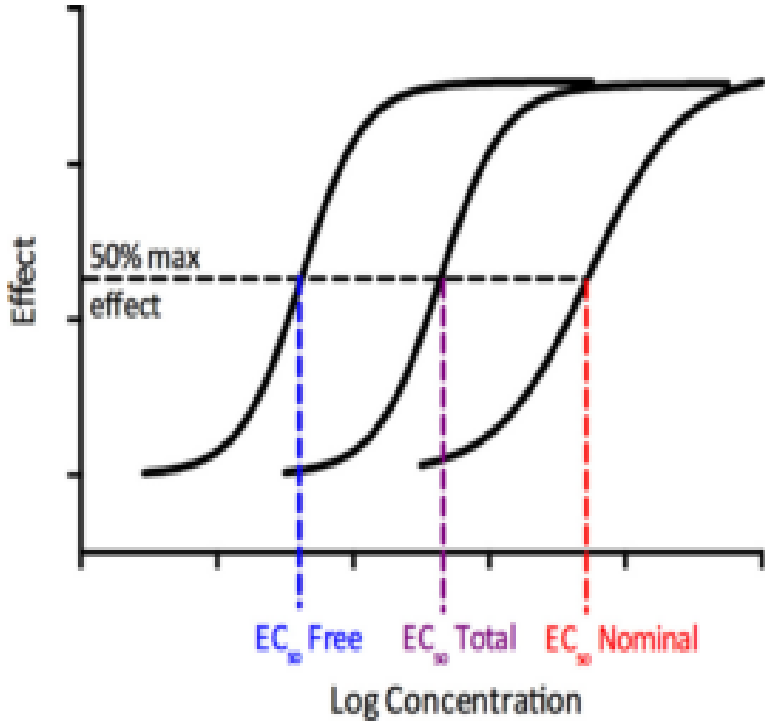
Relationship between nominal / total dose and biologically effective dose *in vitro*



A compound's fate *in vitro* (adapted from Heringa et al., 2004 and Kramer, 2011).

Relationship between nominal / total dose and biologically effective dose *in vitro*



Mechanistically, the most relevant dose metric is the freely available concentration at the target site (target concentration / Biologically Effective Dose) as a function of time, which is directly linked to the primary effects / responses at the target site *in vitro* and *in vivo*.

Failure to understand this issue has hampered uptake of *in vitro* tests bacause results have been based on nominal concentrations and tests appear to lack sensitivity.

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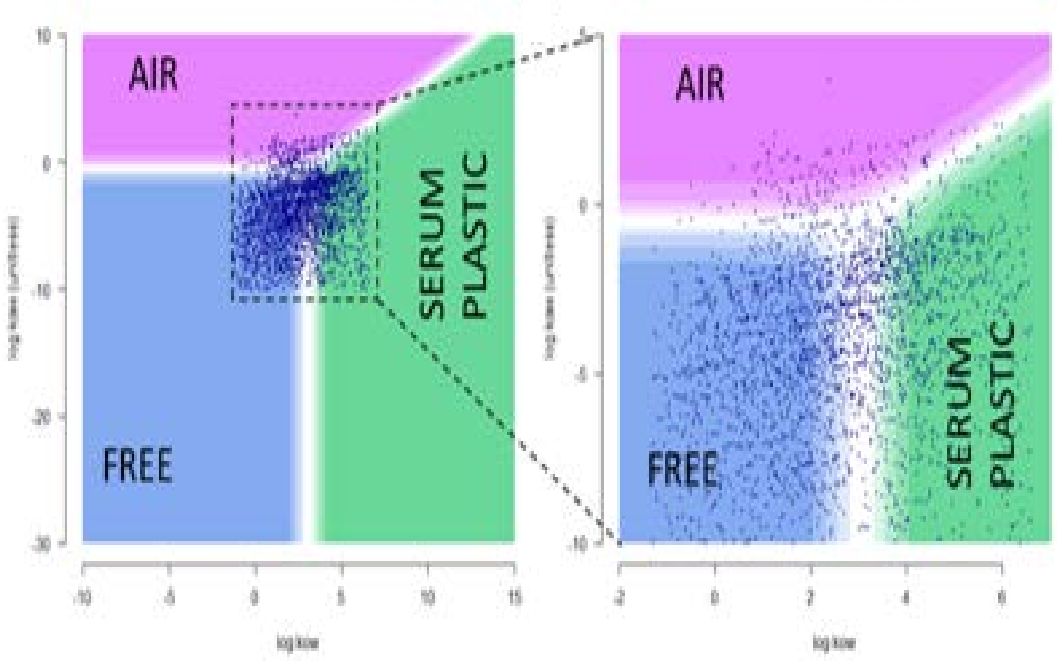
The importance of understanding the dose / kinetics *in vitro*



Generic *in vitro* assay (5% serum in a 96 plastic well plate with a total medium volume of 0.1 mL).

- A significant fraction of some chemicals will partition into the serum and test system components (*e.g.* plastic).
- As such, the use of nominal concentrations for these chemicals makes extrapolating exposure concentrations from dose-response curves intrinsically flawed.
- This increases uncertainty in defining effect concentrations and decreases their value and credibility for use in subsequent risk assessments.

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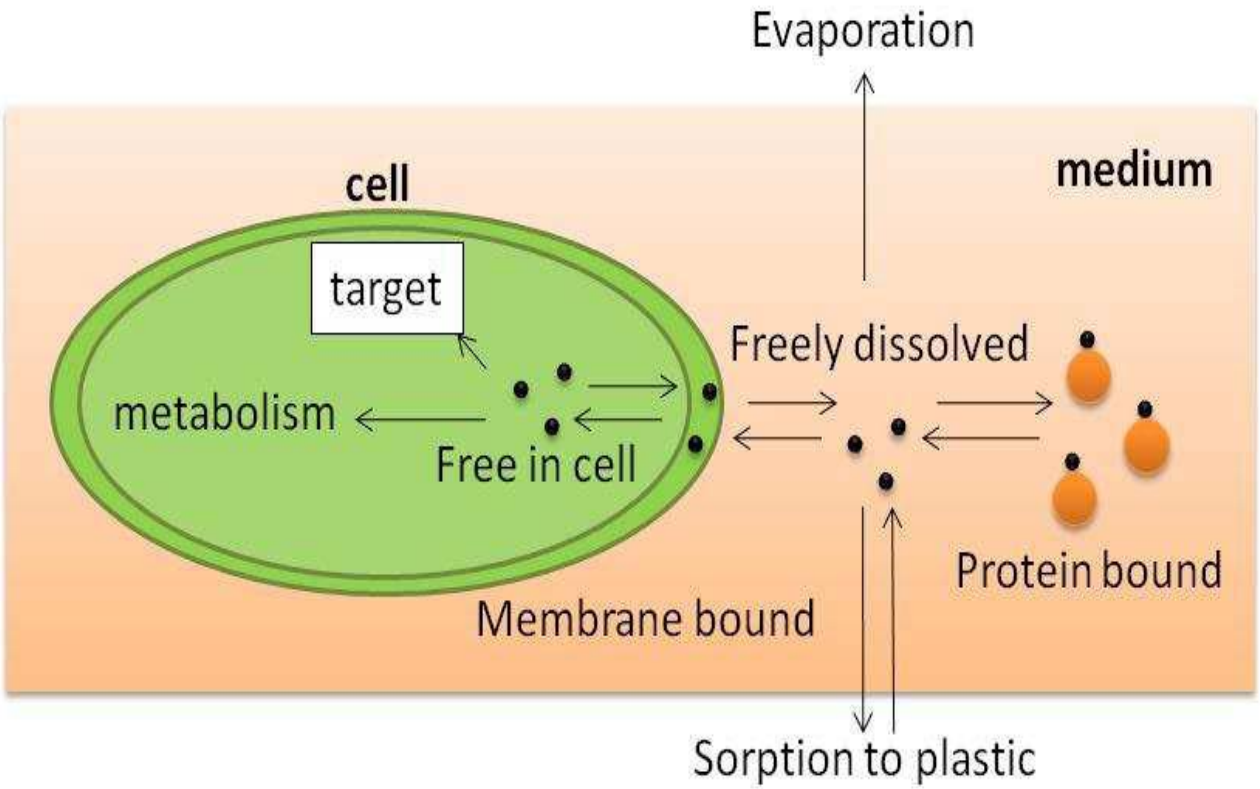
Phenanthrene Example (Kramer et al, 2012 *Chem. Res. Toxicol.*)

- The free concentration of phenanthrene has been demonstrated to be reduced in basal cytotoxicity assays (mouse fibroblast (Balb/c 3T3) and rainbow trout gill (RTgill W1)) because the chemical evaporates into the headspace and binds to serum constituents, plastic, and cells.



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Current state of the art

- Approaches are available to define and control exposure for Difficult to test Substances (DttS), *e.g.* passive dosing, but not in multi-well format.
- Repeated dosing approaches have been reported, typically involving split dosing.
- Automated dispenser technologies are available for more accurate addition of compounds *e.g.* acoustic dispenser – preventing losses to plastic surfaces.
- Headspace free set-ups for volatile compounds.
- Automated measurement systems are in a development stage.
- Modelling approaches can be used to predict the cell internal concentration in a well plate format.

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Gaps

- Development of technology solutions to allow dosing and measurement of chemicals *in vitro*, with emphasis on increased throughput methods.

- Flexibility to adapt these approaches to use in longer duration assays, with more complex cell systems *e.g.* 3D tissue models, and with more complex substances.

- Ultimately, solutions must be commercially feasible – compatible with routine testing.
  - Workable protocols are needed for routine application across broad chemical space.
  - Defined applicability domains and clarity on when to use what approaches (*e.g.* based on physico-chemical properties and test system design).

- Proof of concept/validation of models to predict $C_{free.}$

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3Rs drivers

- Recent developments have led to improved *in vitro* dosing systems. However, their routine use remains limited to low throughput assays due to
  - technical challenges when used in multi-well format (dosing and measurement)
  - wider acceptance of these approaches by the scientific and regulatory communities.

- Developing more robust approaches that can be routinely and easily incorporated into assays would increase their uptake. As more people start reporting and publishing relevant exposure data this should ultimately lead towards regulatory acceptance.

- If successful long term, the Challenge will provide essential tools in the evaluation of safety in the pharmaceutical, chemical, petroleum and consumer product sectors significantly reducing reliance on *in-vivo* animal tests.

Deliverables – Key Deliverables

- A framework to describe when and how to select appropriate *in vitro* dosing technologies for a broad range of chemicals with diverse physico-chemical properties (*e.g.* including hydrophobic, volatile, and unstable chemicals) and chemical mixtures (*e.g.* UVCBs).

- Development of dosing and measurement technology to improve and understand exposure of chemicals/chemical mixtures within *in vitro* studies, thereby enabling their increased use in risk-based decision making. Emphasis on more reliable, increased throughput dosing; and approaches to maintain exposure concentrations *in vitro*.

- The technology developed will be used to provide measured C_{free} and/or cellular concentration data for a range of case study chemicals/chemical mixtures with diverse physico-chemical properties. Consideration will be given to how this data informs the development of *in silico* approaches to aid the prediction of concentration-response relationships.

Deliverables – Phase 1

- **Framework development:** Based on an understanding of physico-chemical properties of chemicals, which influences their bioavailability in *in vitro* test systems, describe a framework strategy to enable the controlled exposure of chemicals for relevant *in vitro* toxicity assays.
  - *Consideration should be given to the current landscape of on-going research in the area, identifying gaps and key technology development needs to facilitate their use in routine in vitro toxicity assay systems.*

- **Technology development:** Provide proof-of-concept data showing improved control of dosing and measurement of C_{free} and / or cellular concentration *in vitro* towards the adoption of increased throughput methods.

- Robust plans to deliver Phase 2 of the Challenge including commercialisation, dissemination and steps to enhance regulatory acceptance.

Deliverables – Phase 2

- **Framework development:** Finalisation of the chemical-specific framework and protocols for dosing and measurement of chemicals *in vitro* based on physico-chemical properties (ideally in collaboration with a contract research organisation (CRO) to enable commercialisation and uptake of the approach).

- **Technology development:** Development / application of a range of dosing technologies (*e.g.* direct spiking, repeat dosing, partition-controlled dosing) as required by the proposed framework (see Phase 1).
  - *Consideration should be given to study design to enable higher throughput control of exposure allowing better understanding of C_{free} and cellular concentration over study duration to inform risk-based decision making.*

- *In silico* prediction of exposure and concentration-response relationships: Use measured data in a reiterative process to improve current models for predicting *in vitro* bioavailable concentrations.

- A clear dissemination plan to not just inform, but actively engage with relevant industry, academic and regulatory stakeholders on key learnings from the project to maximise the potential for acceptance of the approaches developed.

What is not wanted for this Challenge…

- Solutions need to address the challenges presented by chemicals with diverse physico-chemical properties and chemical mixtures, rather than focusing on a single chemical class.

- The primary focus of the challenge is on improving dosing procedures to control exposure *in vitro*, rather than measurement and modelling approaches *per se* as these are currently being developed in other exposure focused projects (*e.g.* CEFIC-LRi ECO-36).

Sponsor in-kind

Phase 1

- Identification of case study chemicals that have challenging physico-chemical property profiles for controlled dosing and testing.
- Scientific advice and modelling experience.

Phase 2

- In house assessment of the approaches developed through this Challenge, as appropriate, to facilitate industry uptake.
- Access to relevant findings from ongoing research programmes focussing on Next Generation Risk Assessment approaches (*e.g.* www.tt21c.org).
- Provision of risk assessment expertise for a range of relevant chemicals used in personal and home care, and petrochemical contexts, and understanding of their chemistries.
- Provision of expertise / knowledge gained from (i) in-house experimental approaches currently employed for improved characterisation of concentration-response relationships, and (ii) relevant external collaborative activities and initiatives.

Thank you for listening

The Sponsors are happy to discuss the challenge and potential applications with people in the run up to the submission deadline

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