Toxicity testing in the 21st Century: Challenges and Opportunities

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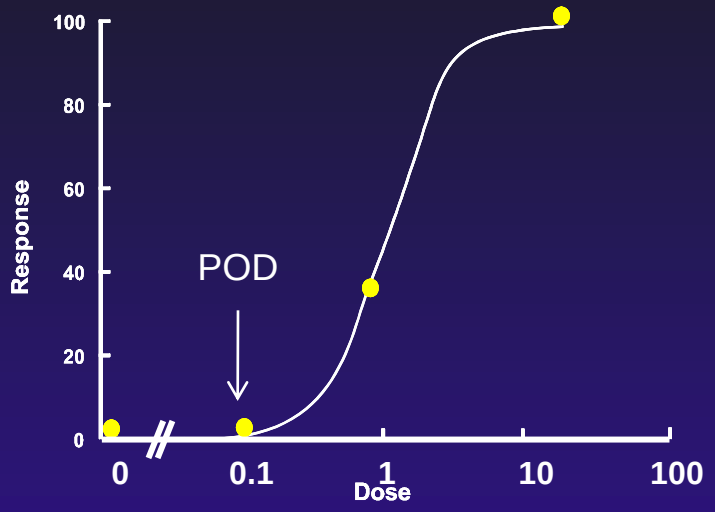
Risk assessment

Hazard ID
Hazard characterisation



Uncertainty factor

Reference value (e.g. ADI)
[RV] = POD/UF

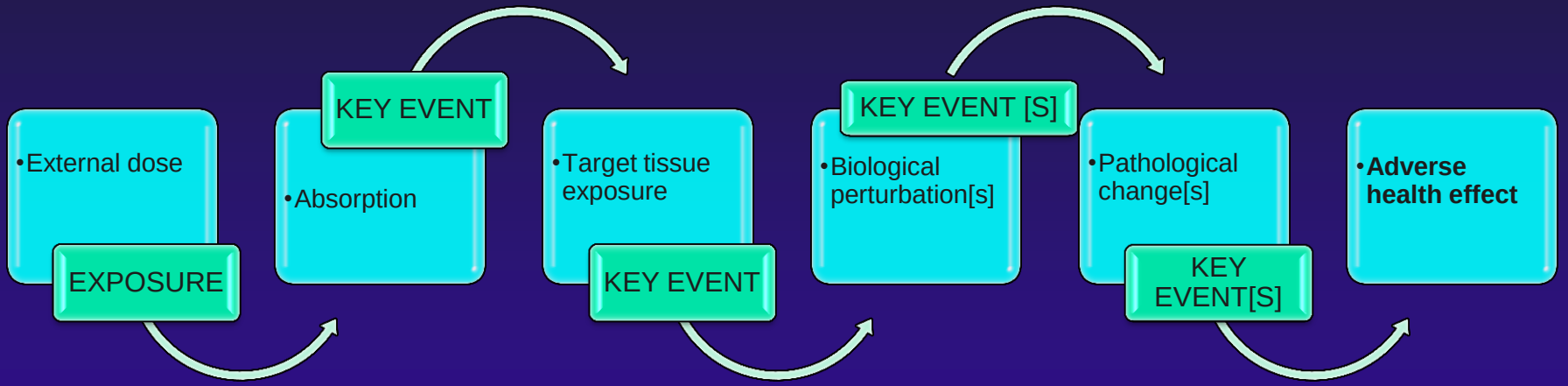
Exposure assessment
Risk characterisation

MOE = POD/Exposure

Current hazard (toxicity) assessment

- ADME and toxicokinetics
- Identify toxicity of compound over range of doses
- Include biochemical, physiological and morphological perturbations
- All body systems (cells and tissues) in both sexes
- Include age groups with potentially unique or quantitative differences in sensitivity
- Consider single to lifetime exposure
- Direct or heritable damage
- Confirm and characterise effects on specialised systems in studies designed for this purpose

Mode of action and key events

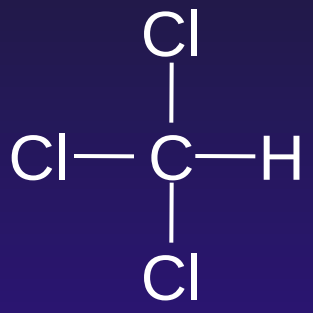


MOA in risk assessment

Oxidative CYP2E1 Metabolism

Phosgene

Chloroform



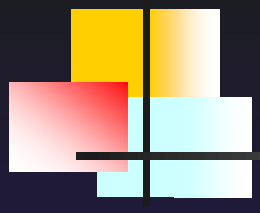
Sustained cytoxicity

Regenerative cell proliferation

Tumour development

Key events

MOA and human relevance



Key Events established based on “Hill Criteria”

Step 1

- Is the weight of evidence sufficient to establish a mode of action (MOA) in animals?

NO → Proceed with risk assessment

YES

Step 2

- Can human relevance of the MOA be reasonably excluded on the basis of fundamental, qualitative differences in key events between animals and humans?

YES → MOA not relevant

NO

Step 3

- Can human relevance of the MOA be reasonably excluded on the basis of quantitative differences in either kinetic or dynamic factors betweenanimals and humans

NO → Proceed with risk assessment

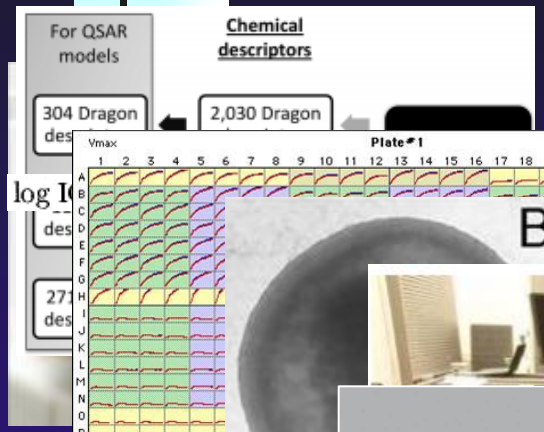
YES → MOA not relevant

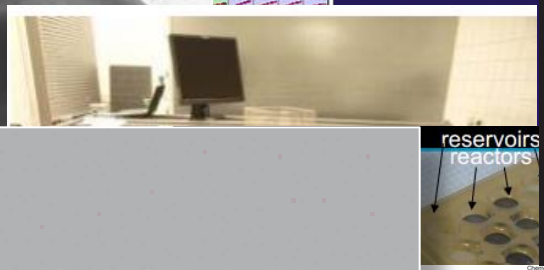
Implications of kinetic & dynamic data for Dose-Response

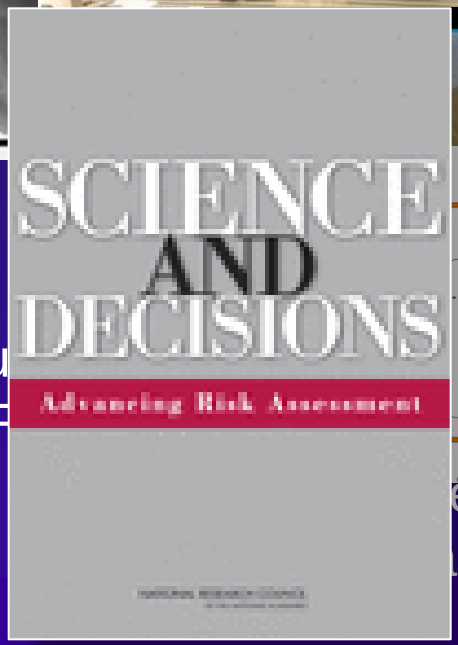
Why does toxicity testing have to change (further)

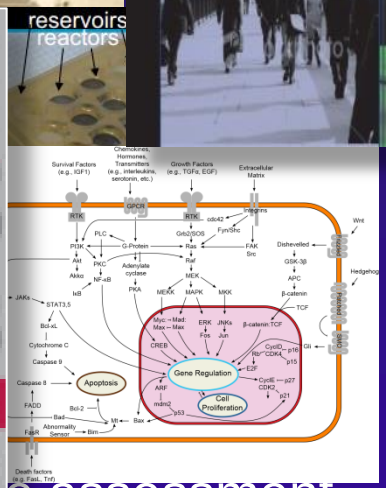
- Large numbers of chemicals with limited toxicity information
  - HPVs, REACH, etc
  - 90,000 chemicals on the EPA TSCA inventory; 140,000 chemicals preregistered under REACH, ~70,000 will require toxicity data
  - Metabolites and degradation products, process intermediates, mixtures
- Novel materials and processes, e.g. nanomaterials
- Accuracy of risk assessments, based on laboratory species
  - Coverage of all relevant endpoints and sub-populations?
- Use of laboratory animals in toxicity testing
  - 3R's – reduction, refinement and replacement

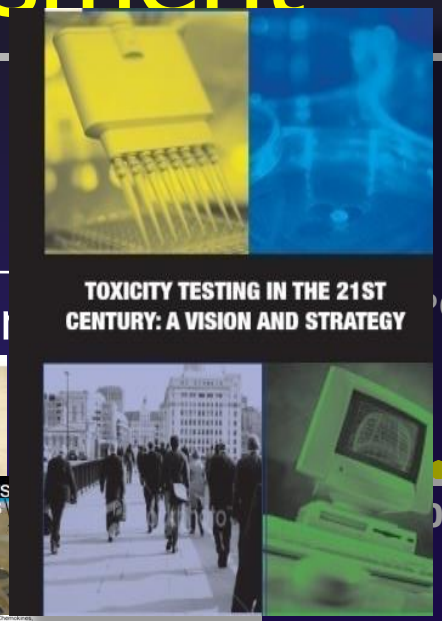
Risk assessment

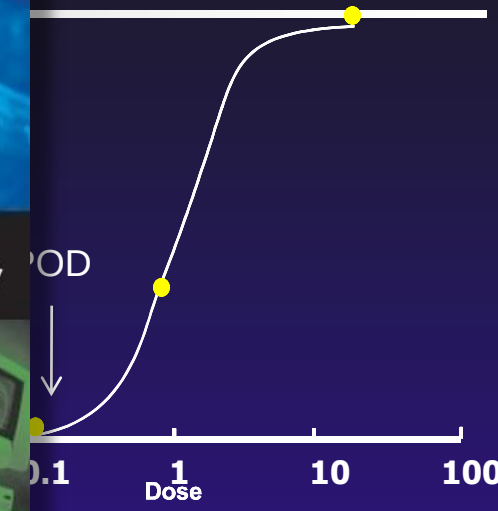












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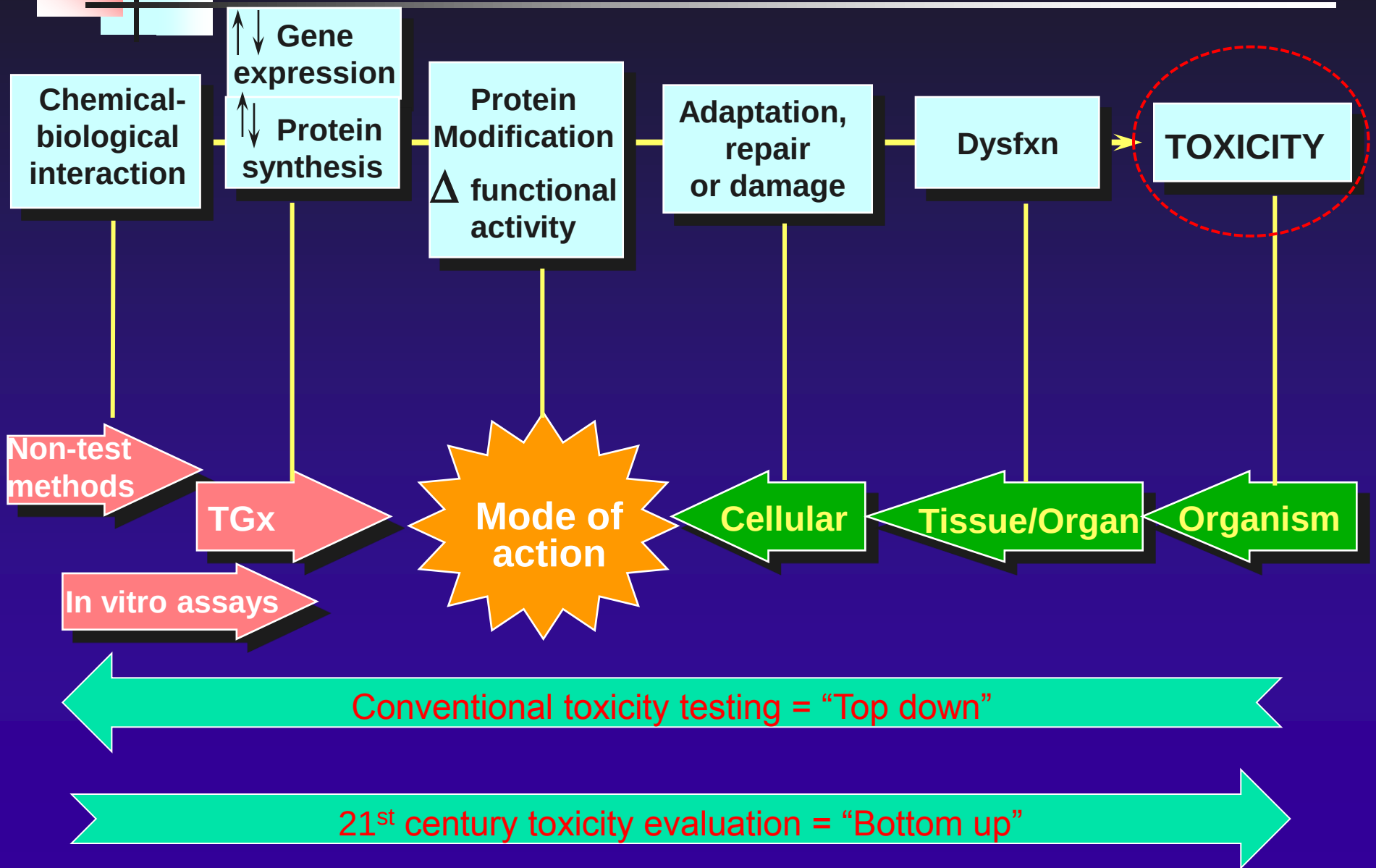
Use of the MOA concept

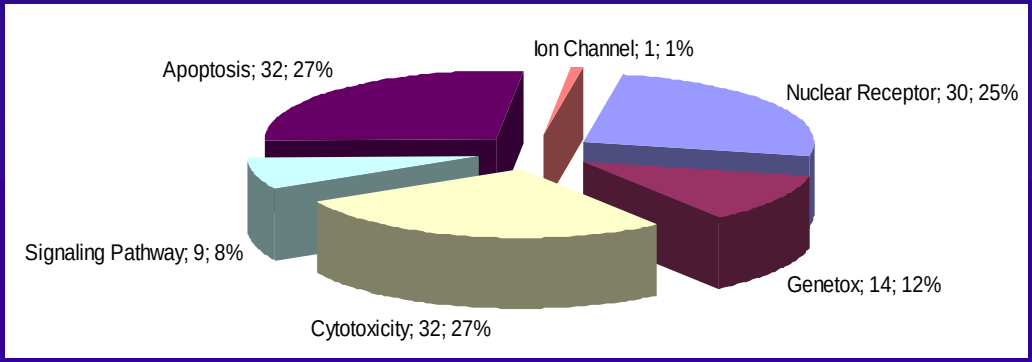


Tox21 assays

General toxicity

- Cytotoxicity assays
  - Cell viability assay (measures ATP)
- Apoptosis assays
  - Caspase assays (measure activity of caspase 3/7, 8, 9)
- Membrane integrity assay
  - LDH release
  - Protease release
- Mitochondrial toxicity assay
  - Mitochondrial membrane potential
- Gene tox assays
  - Micronucleus
  - DNA repair

- "Tox Pathways"
  - CREB
  - ER stress
  - HRE/hypoxia
  - NFκB
  - P53
  - NRF2/ARE
  - HSE (heat shock)
- Targets
  - Nuclear receptor assays: AR, AhR, ERα, FXR, GR, LXR, PPARδ, PPARγ, PXR, RXR, TRβ, VDR, RORα
  - hERG channel
- Inter-individual variation
  - 87 HapMap lines



Austin, 2010

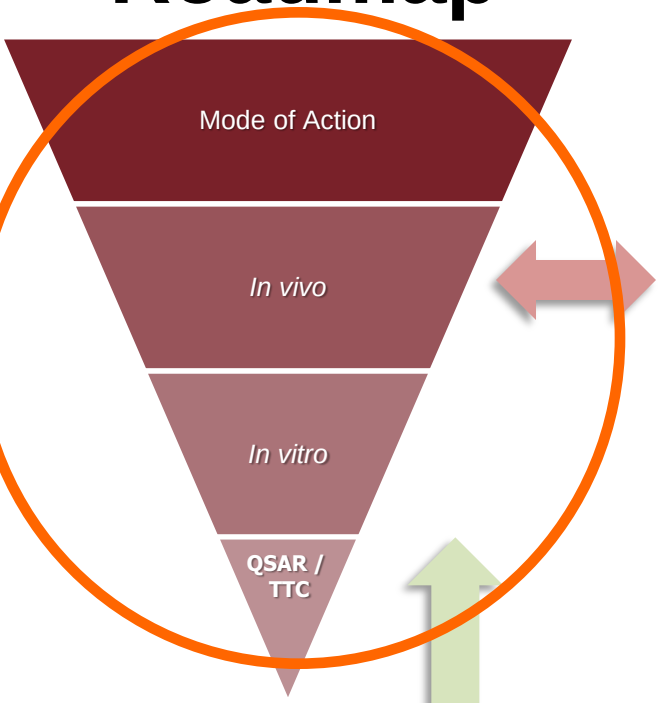
Some challenges in achieving a paradigm shift in toxicity testing

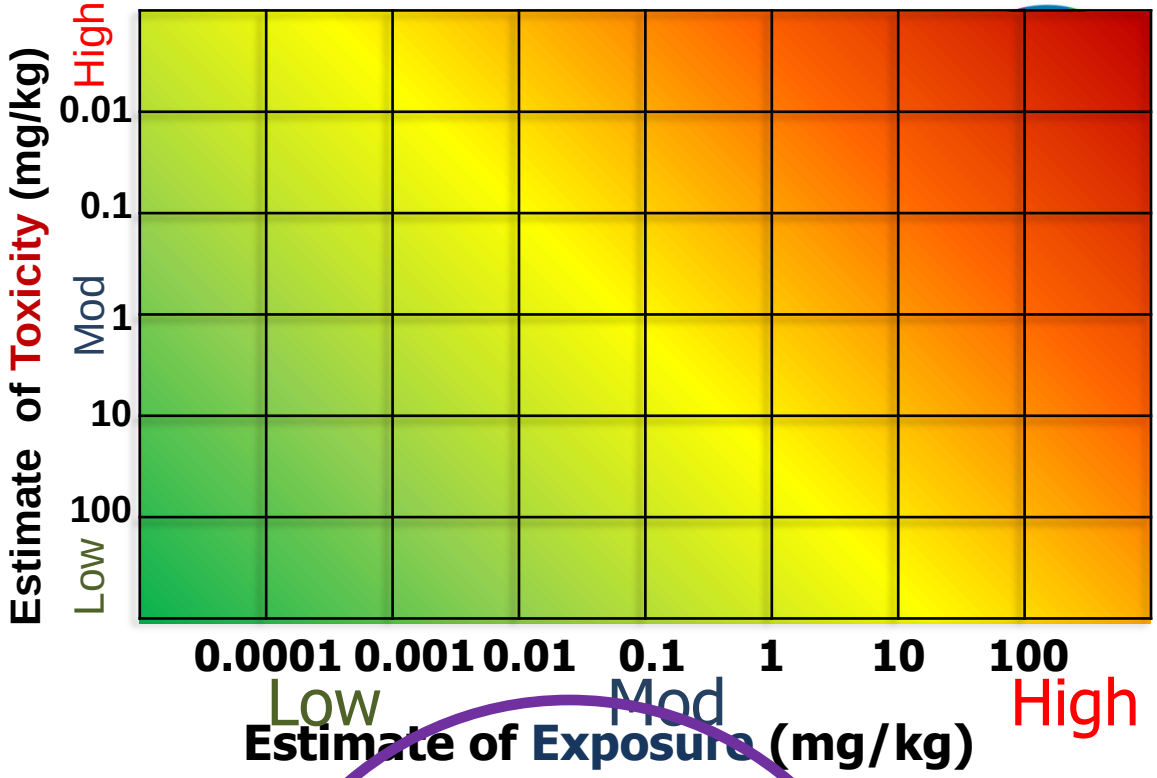
- Adequacy of coverage of toxicological/biological space?
  - Knowledge gap?
- Reliability of extrapolation from effects on *in vitro* toxicity pathways to biologically relevant hazard?
  - Cell models
  - Exposure duration
- Establishing fitness-for-purpose of new methods (who and how)
  - Use of human-derived cell systems
  - Toxicological anchoring to data from laboratory species?
- Quantitative accuracy of *in vitro* – *in vivo* extrapolations?
- Domain of applicability?

Purpose of toxicity prediction

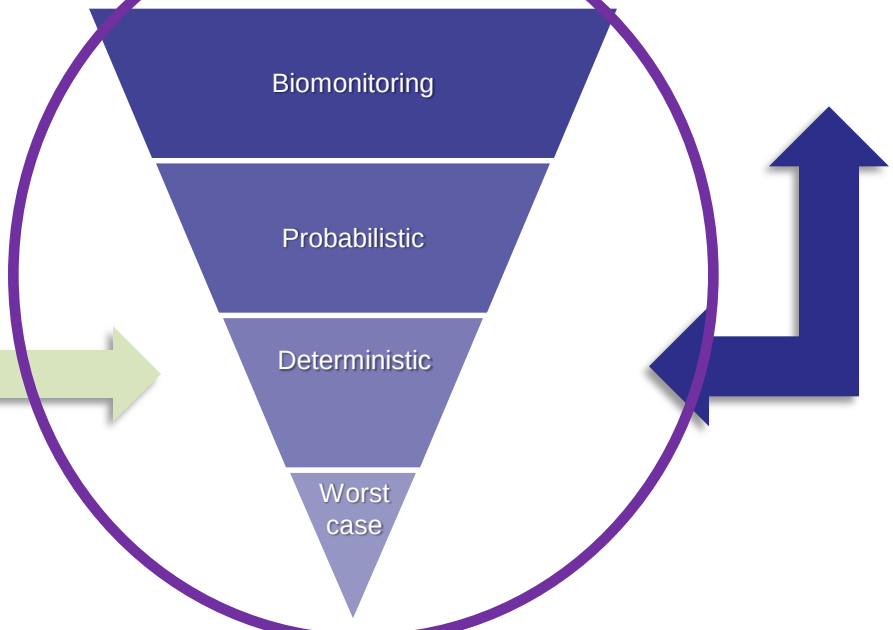
- Importance of problem formulation
- Product development
  - Screening to design or select least hazardous substances for further development
- Prioritisation of substances for further evaluation
- Classification and labelling
  - Indication of worst case effects (e.g. for emergencies during transport and other accidents)
- As part of an approvals or authorisation process
  - Intentional exposure (e.g. drugs, personal care products)
  - Incidental exposure that can be controlled (e.g. occupational)
  - Incidental exposure of general public (e.g. from water, food, air)
- As part of risk assessment of compounds to which people are already being exposed

The RISK21 Roadmap

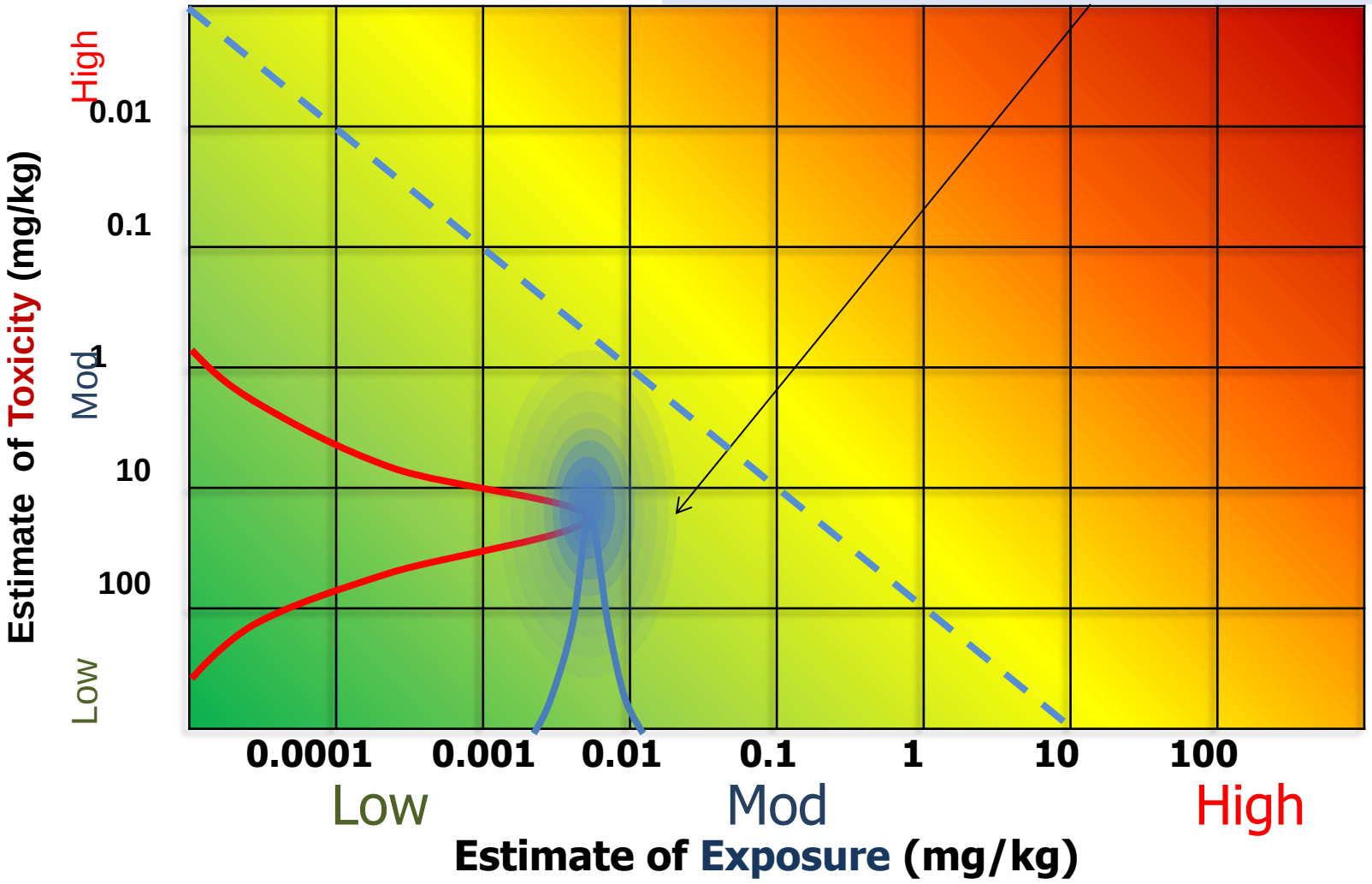




Problem Formulation:
- What is it?
- Where used?
- How used?
- How much?
- What do we already know?



“Mix” of toxicity and exposure probability estimates indicating position of the situation



Changing the paradigm

- One size does not fit all
  - Critical importance of problem formulation
    - Sufficient precision for problem to be addressed
- It should not be a competition!
  - Balancing adequate protection of public health whilst ensuring societal benefit requires fit-for-purpose solutions
  - Need mechanisms to reach consensus on method acceptability and applicability domain
  - The appropriate solutions are not yet known; they may be a hybrid of the “old” and the “new”
- Over-optimism is natural, but should not drive policy:
  - An inherent belief that change is essential
  - Research funding is limited - grantsmanship
  - Current R&D is very expensive and time consuming
- Inherent conservatism of many risk managers

The future of toxicity prediction

- Four futures, all likely to be quite different from each other
  - The future we would like ("The Vision")
  - The future we are investing resources in (e.g. ToxCast, SEURAT-1)
  - The future we convince ourselves has been achieved
  - The future we eventually find ourselves in
- We need to recognise which future it is that we are most likely to achieve, based on:
  - Resources committed
  - State of knowledge
- The timescale for the contribution of scientific advances to risk assessment is almost always substantially under-estimated