

Using *in vitro* data in quantitative risk assessments (QRAs)

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Teeguarden et al., 2016

NIEHS workshop on In Vitro to In Vivo Extrapolation for High Throughput Prioritization and Decision Making

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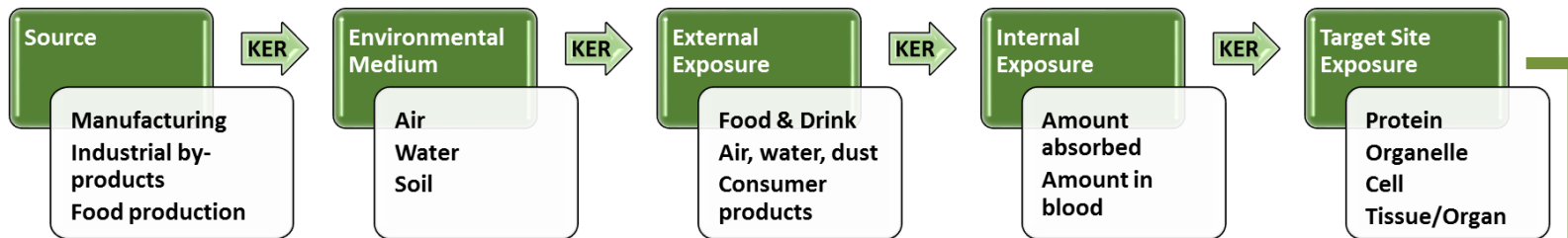
QRA and *in vivo* toxicity data

- For more than 80 years, toxicologists have sought to use *in vivo* data to guide risk decisions. Over this time, a system has been developed for interpreting and translating toxicological findings into decisions on safety:
 - The system is designed to account for the strengths and weaknesses of *in vivo* data.
 - The system has been codified in our laws and regulatory guidance documents and in the worldview of regulatory toxicologists.
- The process to create a similar system for *in vitro* data began only 10 years ago:
 - NAS report Toxicity in the 21st Century
 - High through-put screening risk assessments (Judson et al. 2011; Wentmore et al., 2015; Wambaugh et al. 2015; Shah et al. 2015; Rotroff et al , 2014; Kavlock et al. 2012)
 - This meeting is a step in building that system.

What would an a higher tiered *in vitro* based QRA look like?

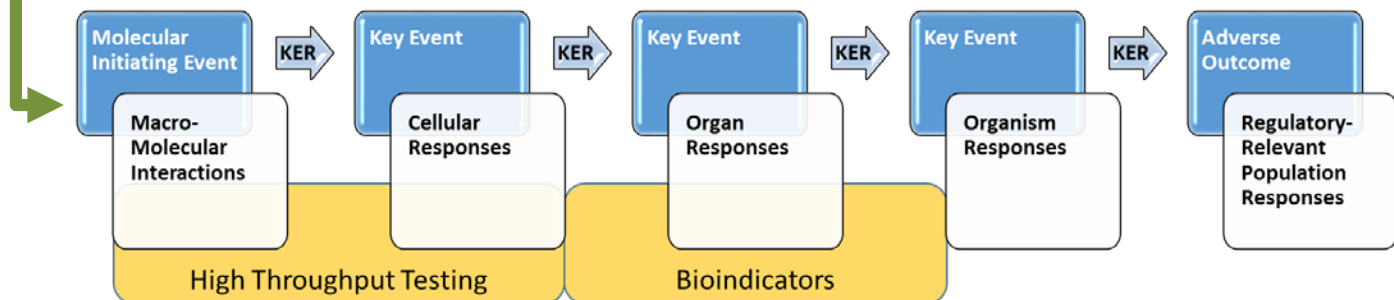
- To discuss this we need to discuss the concepts of:
 - Aggregate Exposure Pathways (AEP) and
 - Adverse Outcome Pathways (AOPs)
- Two approaches:
 - Forward-dosimetry based models of hazard index/margin of exposure (lower tier QRA)
 - AOP-based Biologically Based Dose Response (BBDR) models (higher tier QRA)

Aggregate Exposure Pathway



Teeguarden et al., 2016

Adverse Outcome Pathway



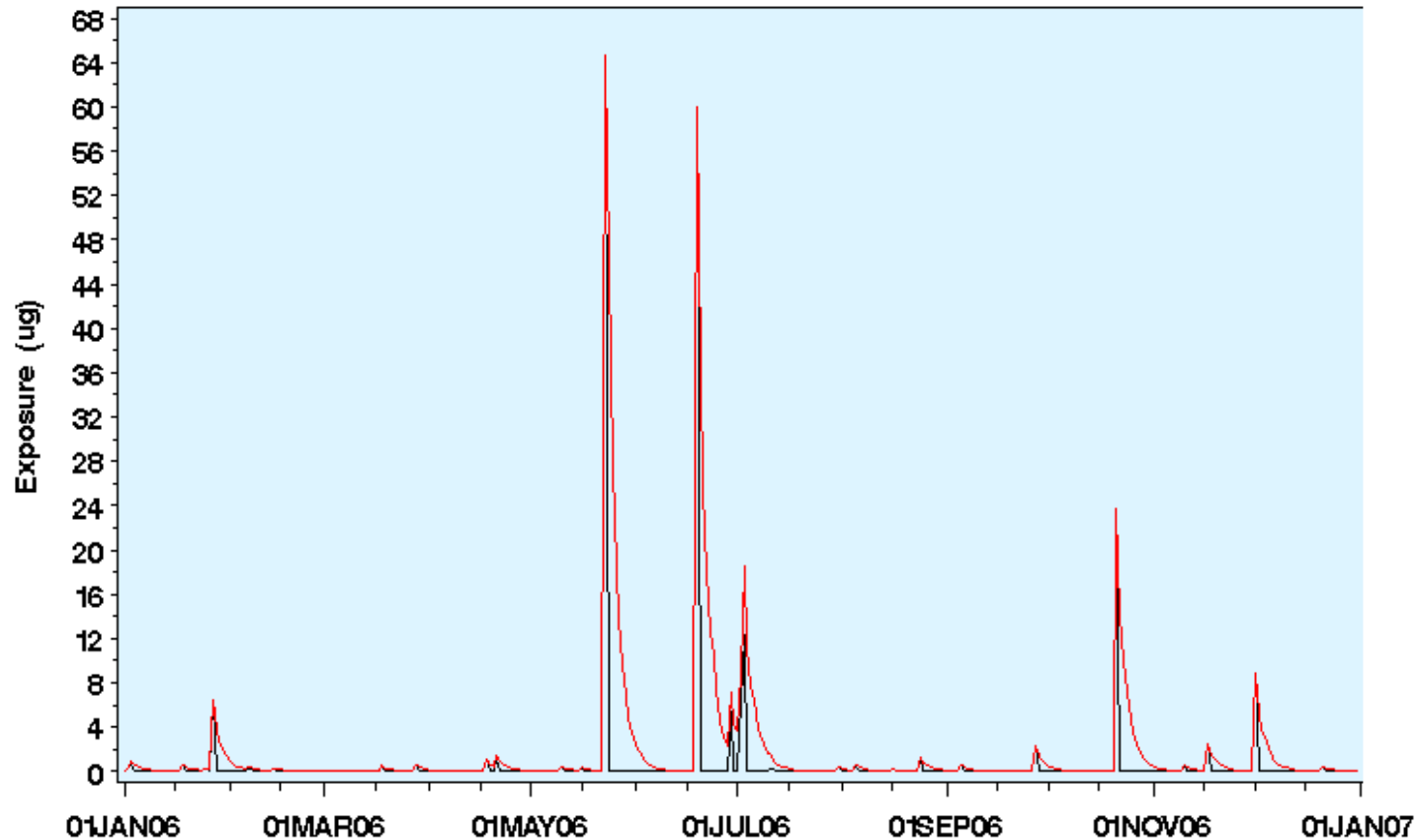
Courtesy of S. Edwards (NCCT/ORD/EPA)

Forward and reverse dosimetry

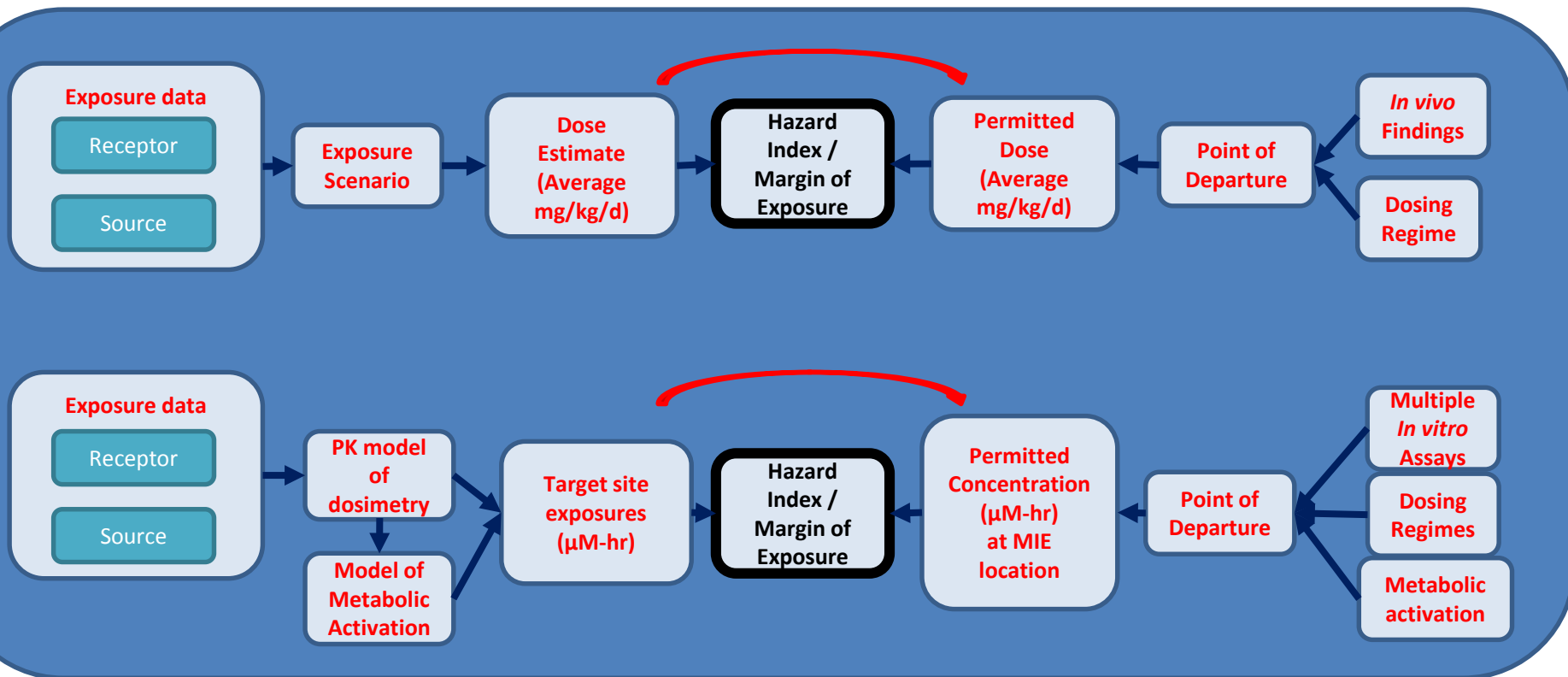
- Reverse dosimetry is a useful tool for screening HTS risk assessments, since it allows the hazard and exposure assessments to be performed on the basis of the historically used “administered dose”.
- Reverse dosimetry’s assumption of steady-state exposures makes it less useful for higher tiered risk assessments.
- Forward dosimetry allows the capturing of temporal variation in exposure, uptake, and clearance in the risk assessment process.

Forward dosimetry of body burdens from variable dietary intakes of a pesticide

Daily doses and resulting daily body burdens in an individual over 1 year
SHEDS Multimedia model of dietary exposure to a pyrethroid

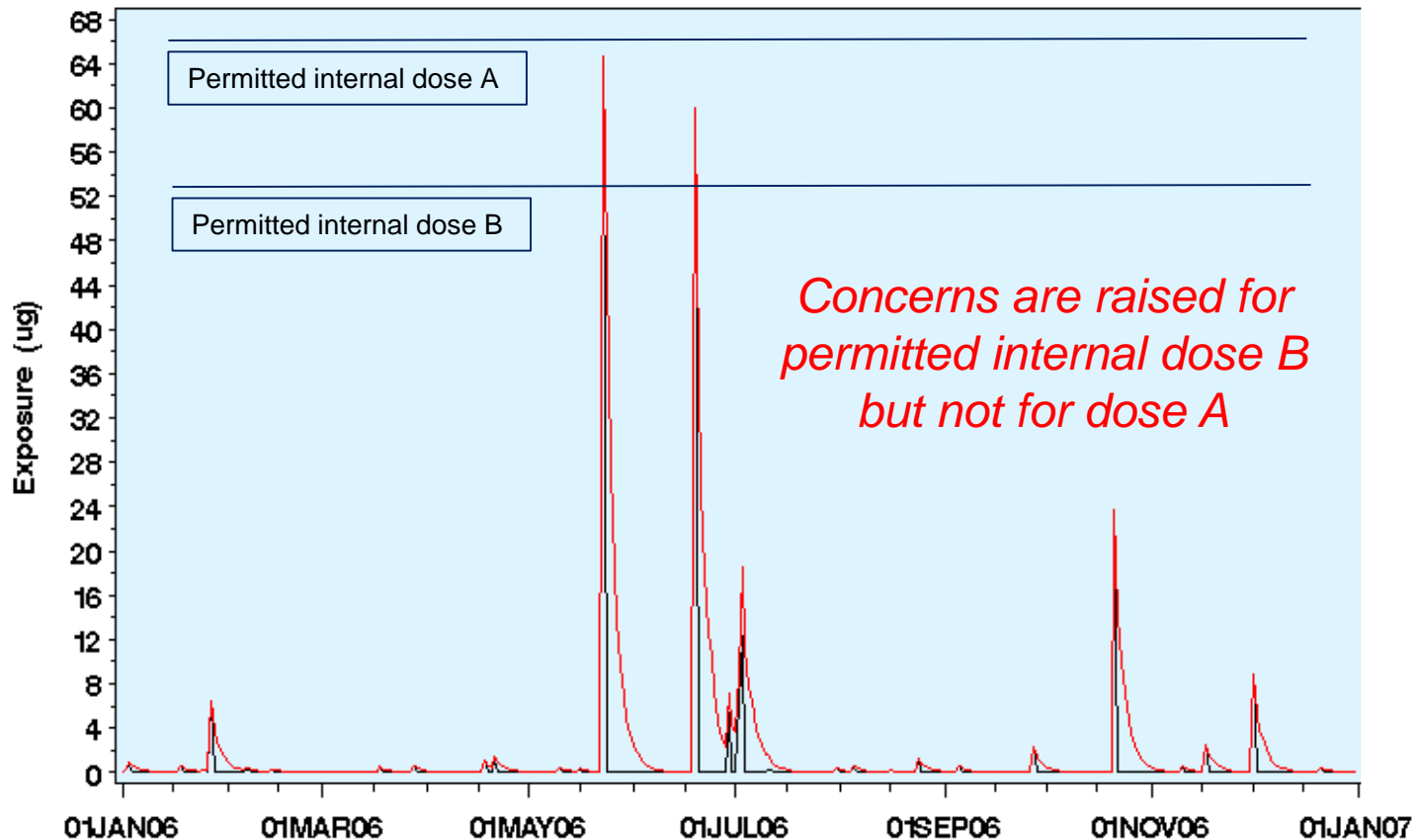


Approach 1. *In vivo* and forward-dosimetry *in vitro* risk assessments processes

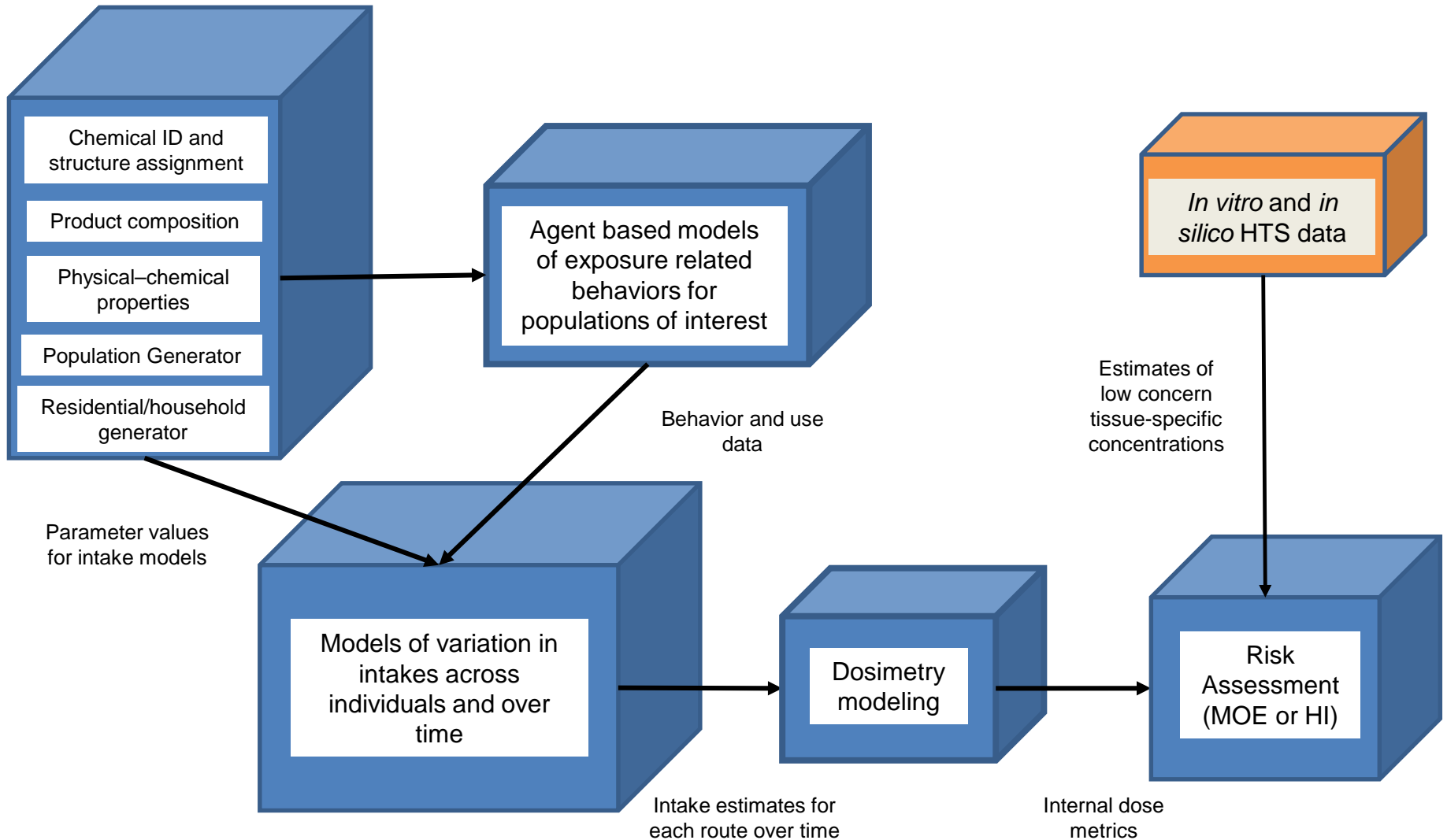


Forward dosimetry based risk assessment

Daily doses and resulting daily body burdens over 1 year
SHEDS modeling of dietary exposure to a pyrethroid

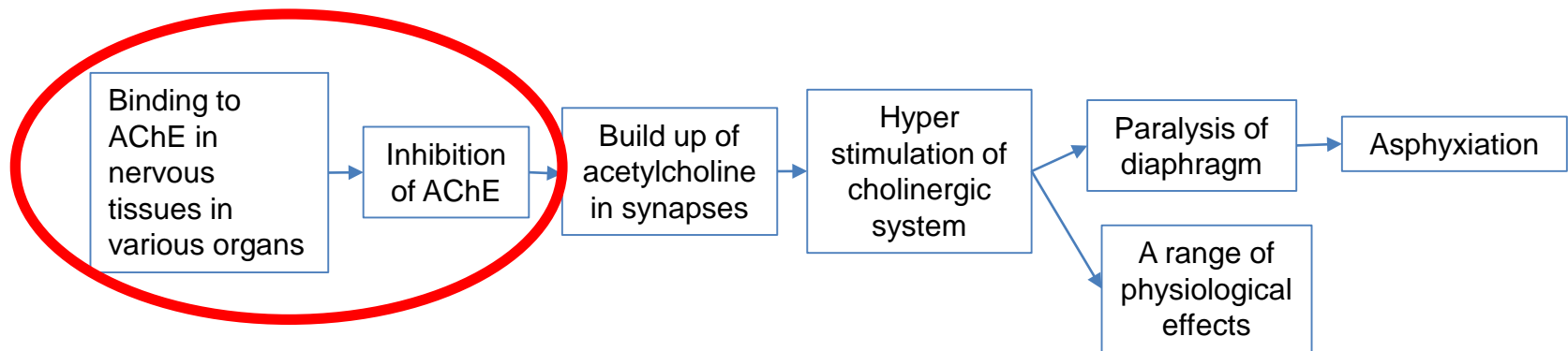


Human Exposure Model in the CSS project, LC-HEM



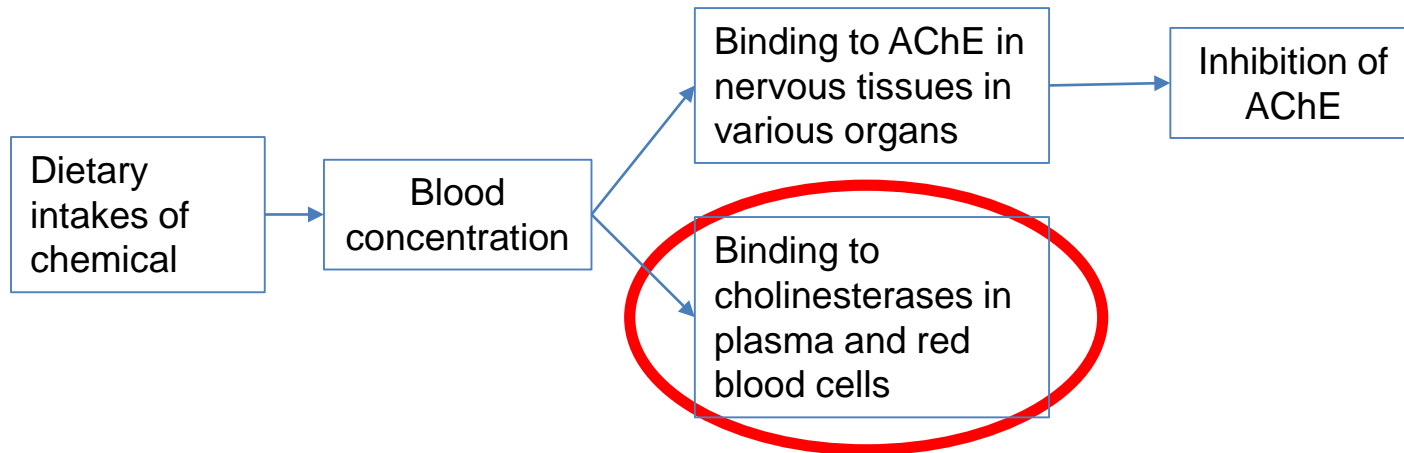
Approach 2. AOP-based BBDR models

AOP for Acetyl cholinesterase inhibition (AChEI)



This AOP has been used for decades to regulate chemicals that cause AChE

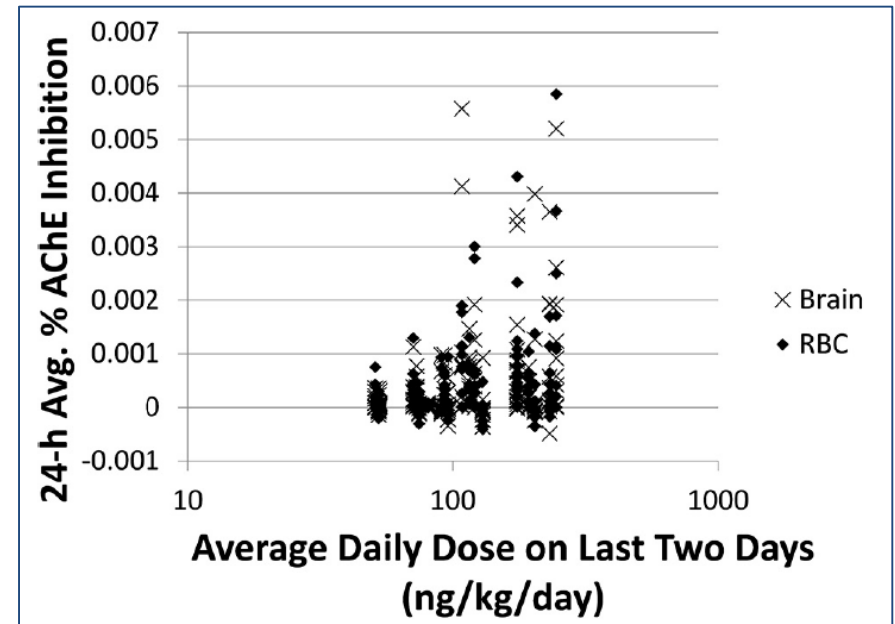
Case study: carbamates



- AChE is easier to measure in red blood cells than nervous tissues.
- Consensus has been reached that when RBC AChE inhibition is <10%, then inhibition of AChE in brain and other tissues are negligible.

BBDR model predictions

- Linked dietary, PBPK model of dosimetry, and PBPD model of binding to cholinesterases
- Temporal variation in dose and variation across one year for 500 individuals
- Brain and RBC AChE have <0.01% inhibition at current exposures (Phillips et al., 2014)



Phillips et al., 2014

In vitro based QRAs and the issue of variability

- Inter-individual variation in tolerance of chemical stressors
- Temporal variation in dose and response

Addressing interindividual variability in QRAs

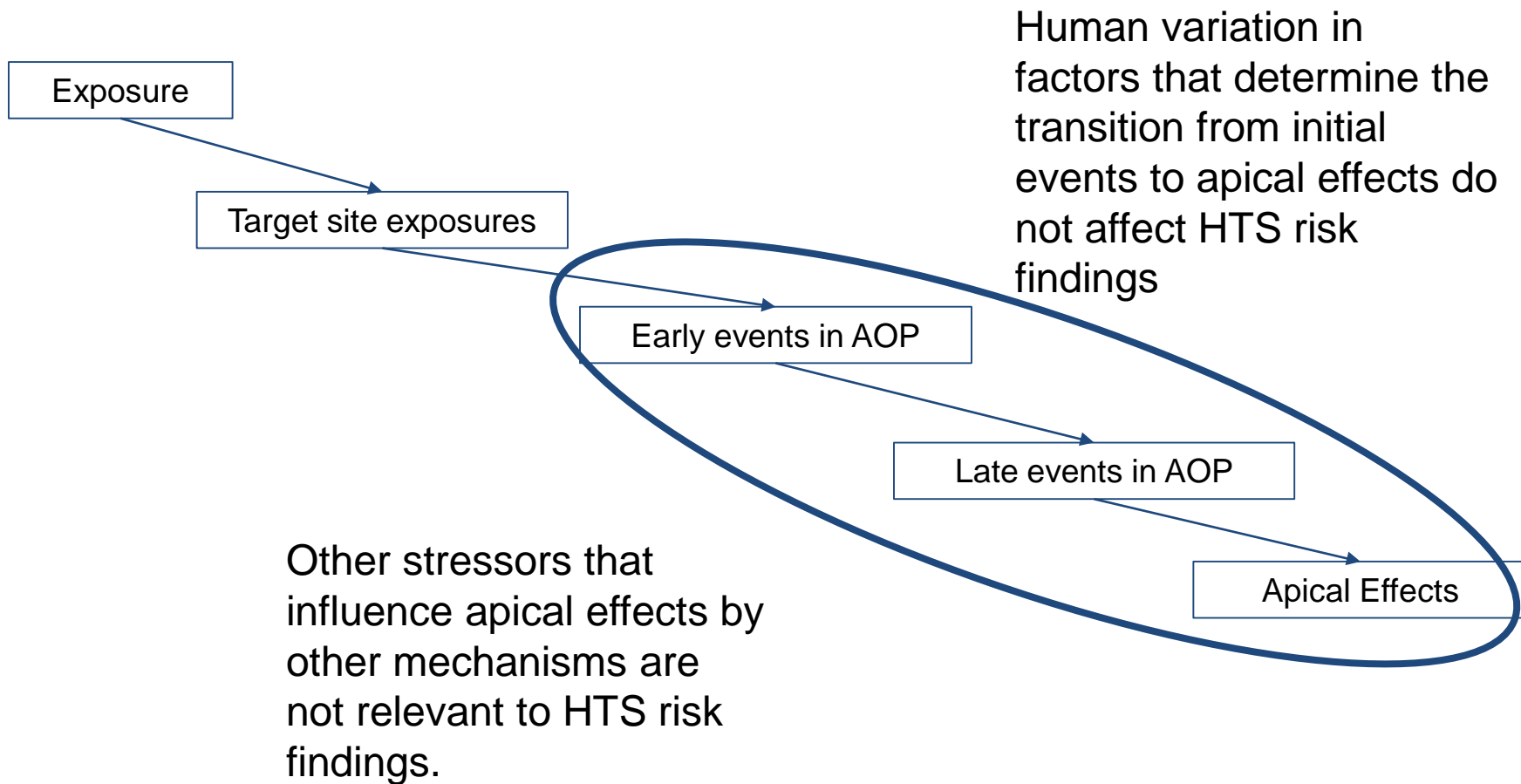
In vivo assays

- No finding is made in animal studies that is relevant to human variation in response
- Animal testing treats variation in response across test animals at a dose level as noise (measurement uncertainty in an idealized animal species/strain)
- Compensation for human variability is addressed using uncertainty factors

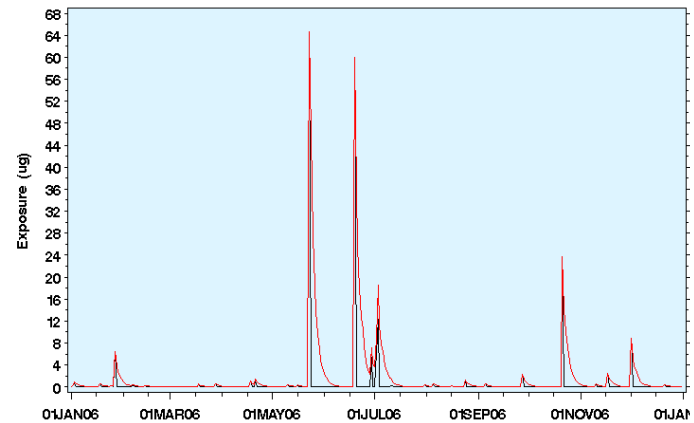
In vitro assays

- Assays have not investigated the impact of genetic variability on a consistent basis
 - Limited studies have been performed in certain assays (Abdo et al. 2015)
 - More could be done in the future
- Kinetic variation has been addressed in forward and reverse IVIVE modeling

Early versus apical effects and interindividual variation in response



Addressing temporal variability



In vivo assays

- Limited capacity to address temporal variability
 - Effects from longer durations of exposures are usually studied in assays where doses are kept constant
- Little data on recovery or on the impacts of episodic exposures
- Haber's law is generally assumed

In vitro assays

- Chronic effects are modeled based on knowledge of early events
- Data on recovery can be modeled
- Forward dosimetry models can address time varying exposures and chemical and individual rates of clearance

Toxicity data on 8,000 chemicals

- There is an indirect but very powerful effect on the assessment of risk and chemical management that results from having consistent data on chemicals.
- This results from the fact that there are two different decisions occurring:
 - Decisions related to technical findings – what did the study find?
 - Decisions relating to safety – how should a safety decision be changed based on the findings?
- In general, *in vivo* data are very powerful in demonstrating the existence of an effect but are much weaker in showing how the effect occurs or if it is relevant to humans.

Evaluation of chemicals in isolation

- In a “one at a time” approach, there is an asymmetry in the value placed on positive and negative technical data for a safety finding:
 - Positive data are weighted more heavily than negative data.
 - The occurrence of a false positive is viewed as more acceptable than a false negative.
 - Value of additional data in the decision making is reduced.
- The result is that once a chemical is in the queue of “chemicals of concern”, it is viewed as having little chance of escaping:
 - As a result, industry tends to move on to a replacement chemical.
 - The major advantage of the replacements are not that they are lower risk, only that they are not in the “queue”.
 - Society is now set up for a slow motion game of “whack a mole”.
- The net effect:
 - A societal decision is made and the chemical regulated or “deselected”.
 - The technical findings on actual impacts of health, however, are not resolved.
- It would be preferable to actually make a finding that a chemical was indeed acceptable or not acceptable for a specific use.

When a consistent set of data is available on multiple chemicals

- Both positive and negative data are both considered in screening and ranking.
- Chemicals are viewed as being more acceptable based on data rather than its absence.
- When a chemical has findings that indicate a concern, the findings can be linked to AOPs
- AOPs define mechanisms that can be tested to confirm a finding.

Summary

- The concept of the Aggregate Exposure Pathway and the Adverse Outcome Pathway provide a framework for use of *in vitro* data in both lower tier risk assessments (HI/MOE) and higher tier assessments (BBDR models)
- While much work remains to be done on issues such as the relationship between assay data and AOPs and metabolic activation the use of *in vitro* data for risk assessment provides advantages to QRA
- A new system of models, knowledge bases, and policies is needed for use of *in vitro* data in QRA.

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