Comparing the Role of Time in *In Vitro* and *In Vivo* Toxicity Tests

Dr. Nynke Kramer | nynke.kramer@wur.nl | 07 November 2023

ECETOC Workshop 'Chronos and Kairos: Understanding Time in NGRA', Brussels

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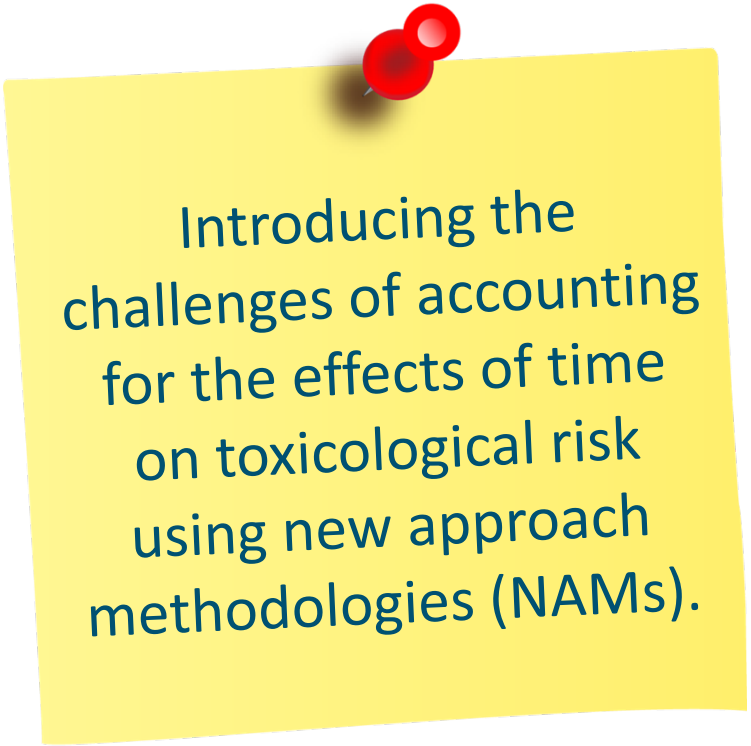
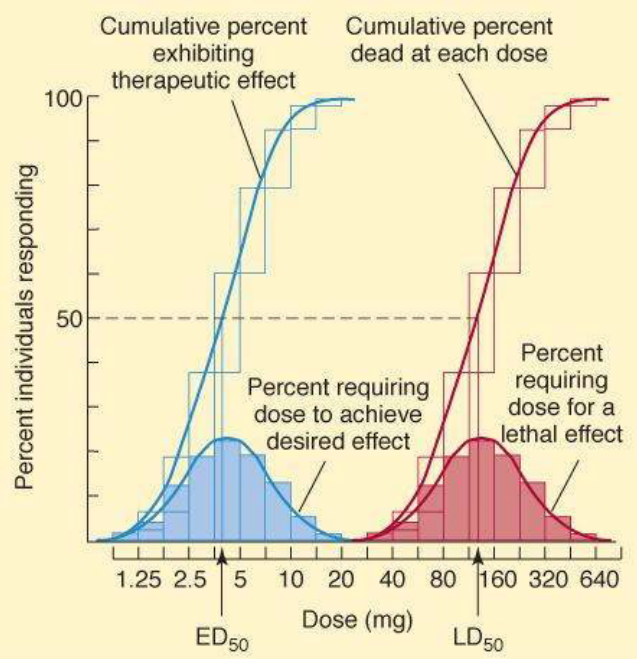
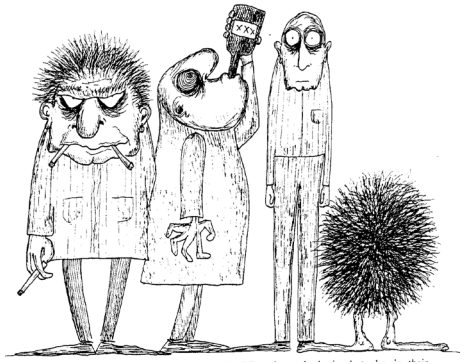
Presentation Aim



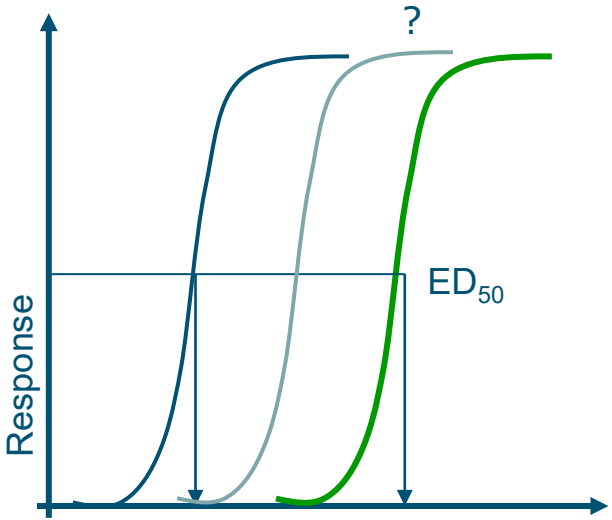
The Central Tenant in Toxicology

- The dose makes the poison



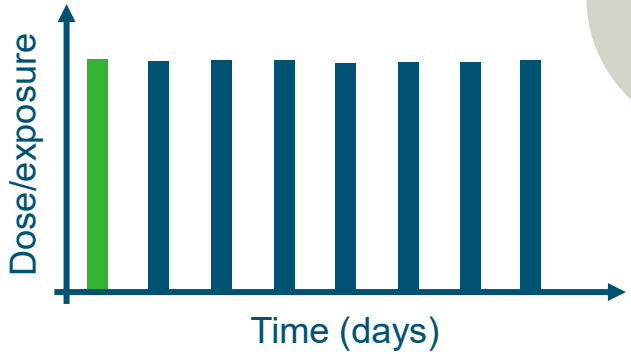


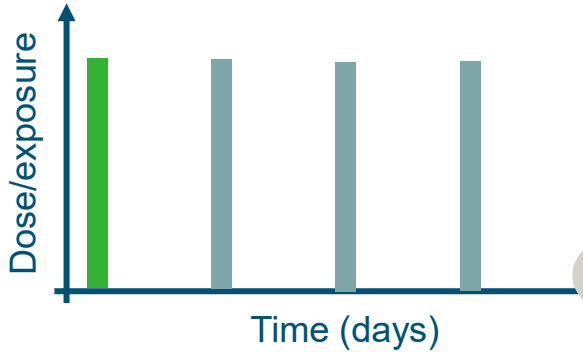
But What is the Dose?



Dose (mg/kg bw after single dose)

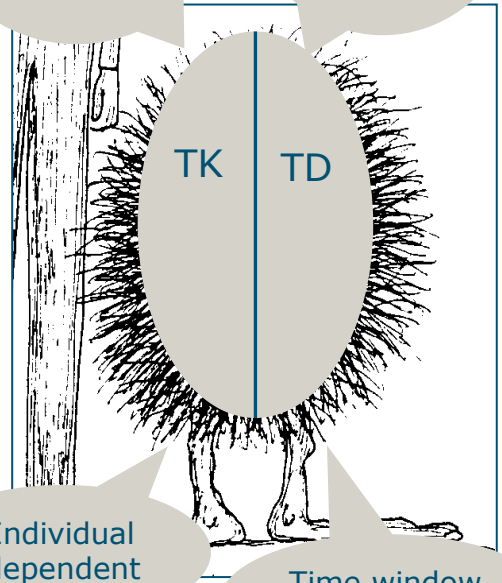
Dose (mg/kg bw/day over a lifetime)





Full elimination before repeat dose?

Damage reversed before repeat damage event?



Individual dependent

Time window dependence

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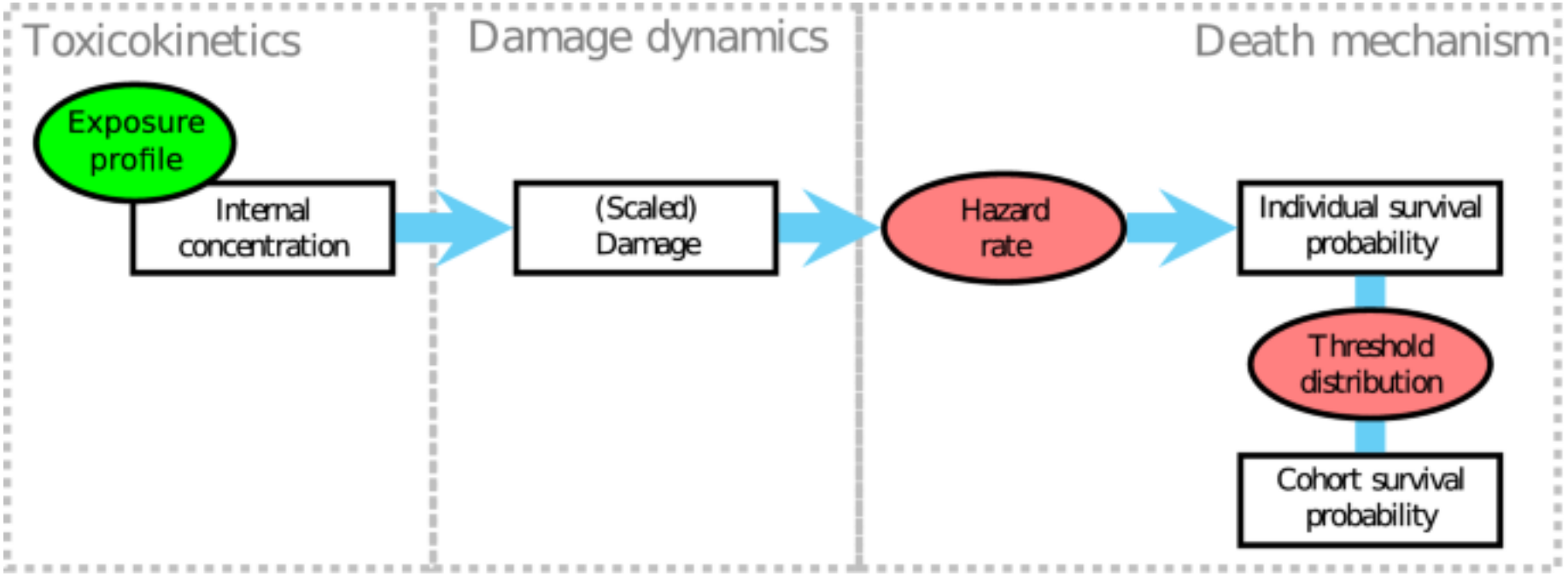
But What is the Dose?



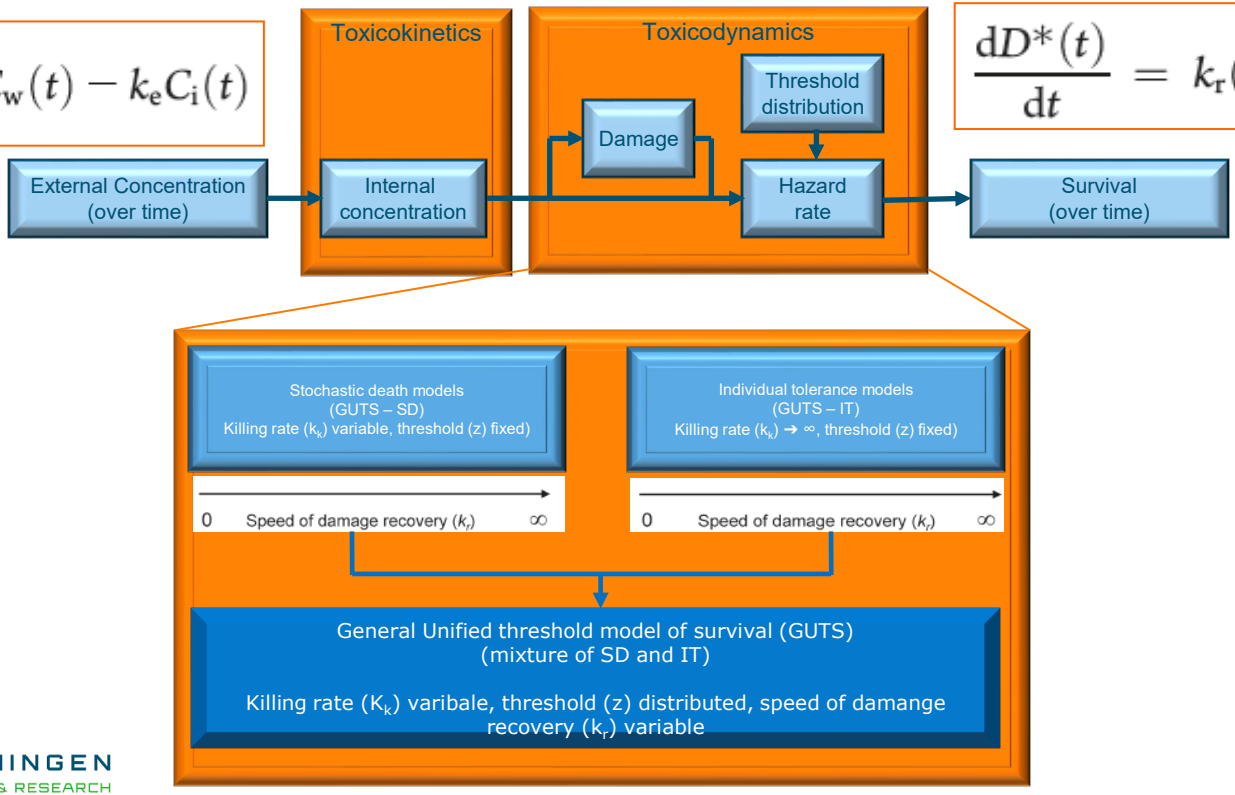
General Unified threshold model of survival (GUTS)

- Absorption
- Distribution
- Biotransformation
- Elimination

- Damage acrual (toxic mechanism)
- Damage recovery (biochemical and physiological compensation process)

$$\frac{dC_i(t)}{dt} = k_i C_w(t) - k_e C_i(t)$$

$$\frac{dD^*(t)}{dt} = k_r(C_i(t) - D^*(t))$$



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Jager *et al.* (2011) Env. Sci. Techn. 45:2529

Chemical Risk Assessment

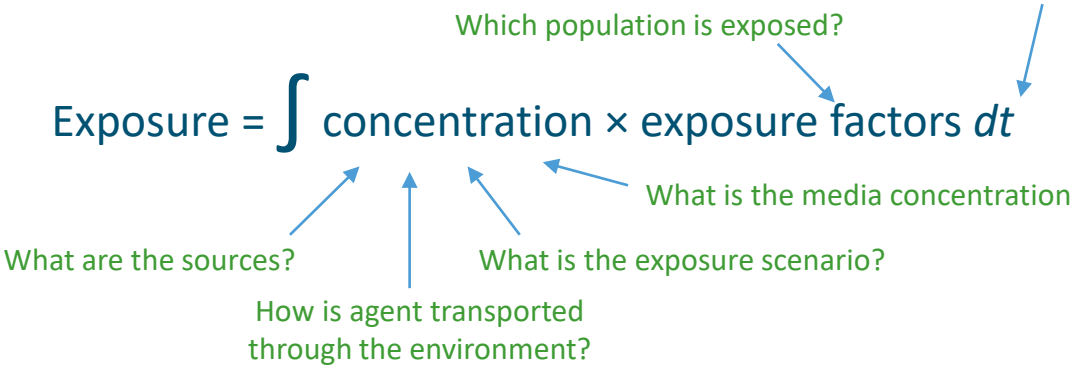
The systematic scientific evaluation of potential adverse health effects resulting from exposures to hazardous agents or situations.

4 steps

- Exposure assessment
- Hazard identification
- Hazard characterisation (dose-response)
- Risk characterisation

Exposure Assessment

The process of estimating or measuring the magnitude, frequency and duration of exposure to an agent, along with the number and characteristics of the population exposed. Describes the sources, transport, routes, and the uncertainties in the assessment.



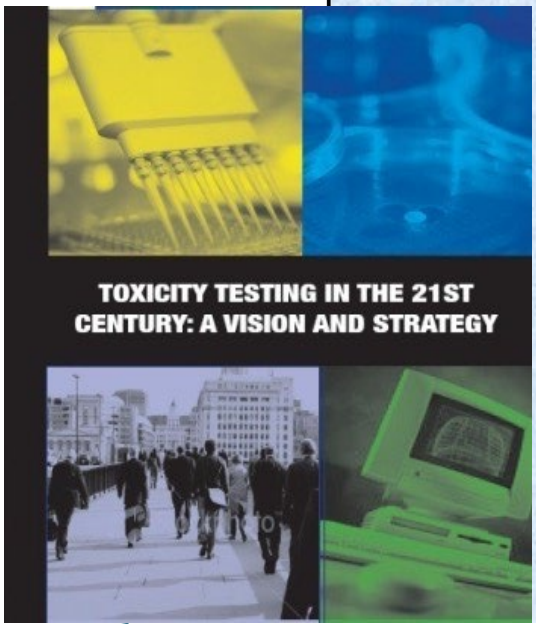
$$\text{LADD} = \frac{\text{Concentration of the toxicant in the exposure media} \times \text{Contact rate} \times \text{Contact fraction} \times \text{Exposure duration}}{(\text{body weight})\,(\text{lifetime})}$$

Lifetime average daily dose

Hazard Characterisation

- Chemical Characterization
- Literature Review
- Structure-Activity Assessment
- (Sub)Acute Toxicity Test
  - Subchronic Toxicity
    - Chronic Toxicity
    - Reproductive/ Teratology
  - Toxicokinetics
  - Oncogenicity…

A Change in Paradigm



Toxicity Testing in the 21st Century: A Vision and Strategy

Committee on Toxicity Testing and Assessment of Environmental Agents

Board on Environmental Studies and Toxicology

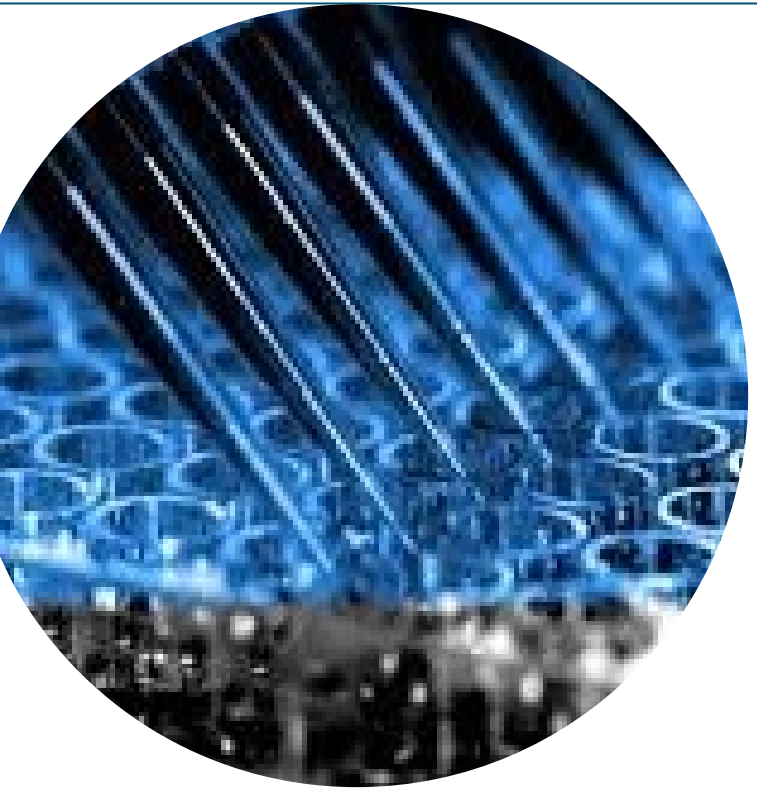
Institute for Laboratory Animal Research

Division on Earth and Life Studies

National Research Council

THE NATIONAL ACADEMIES
Advisers to the Nation on Science, Engineering, and Medicine

In Vitro Toxicity Assays



- High throughput
- Little waste
- Human tissue
- (Ethically) sound science
- Mechanistic approach

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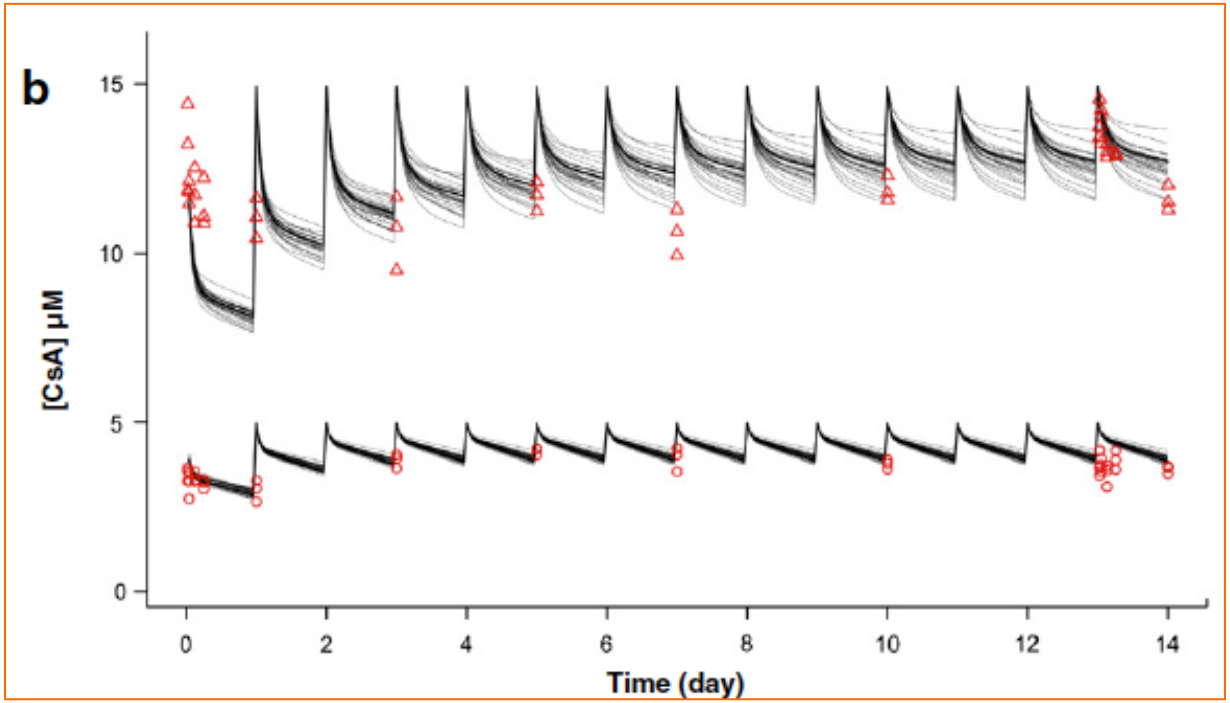
Repeat Dose *In Vitro* Representative for *In Vivo*?



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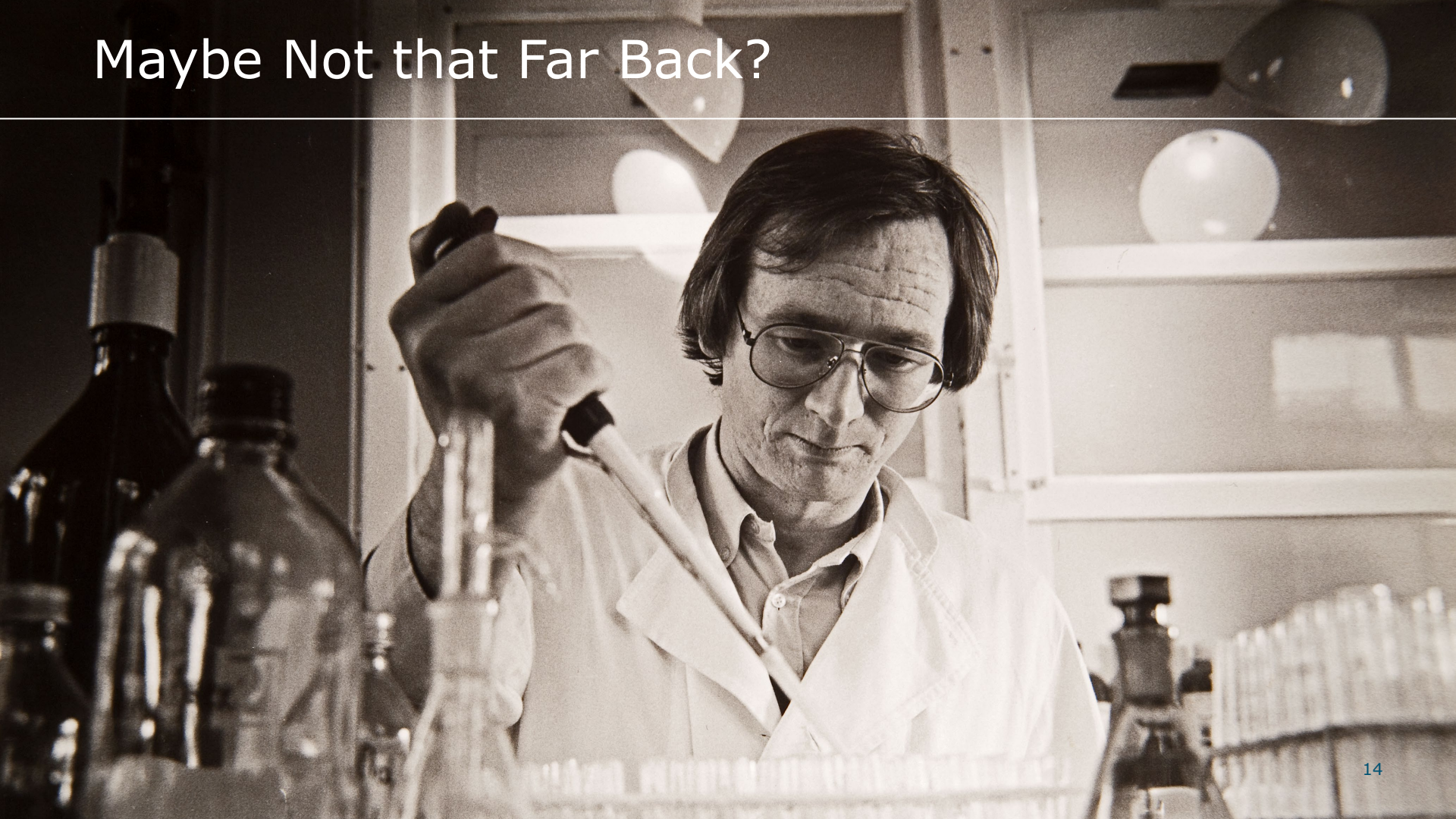
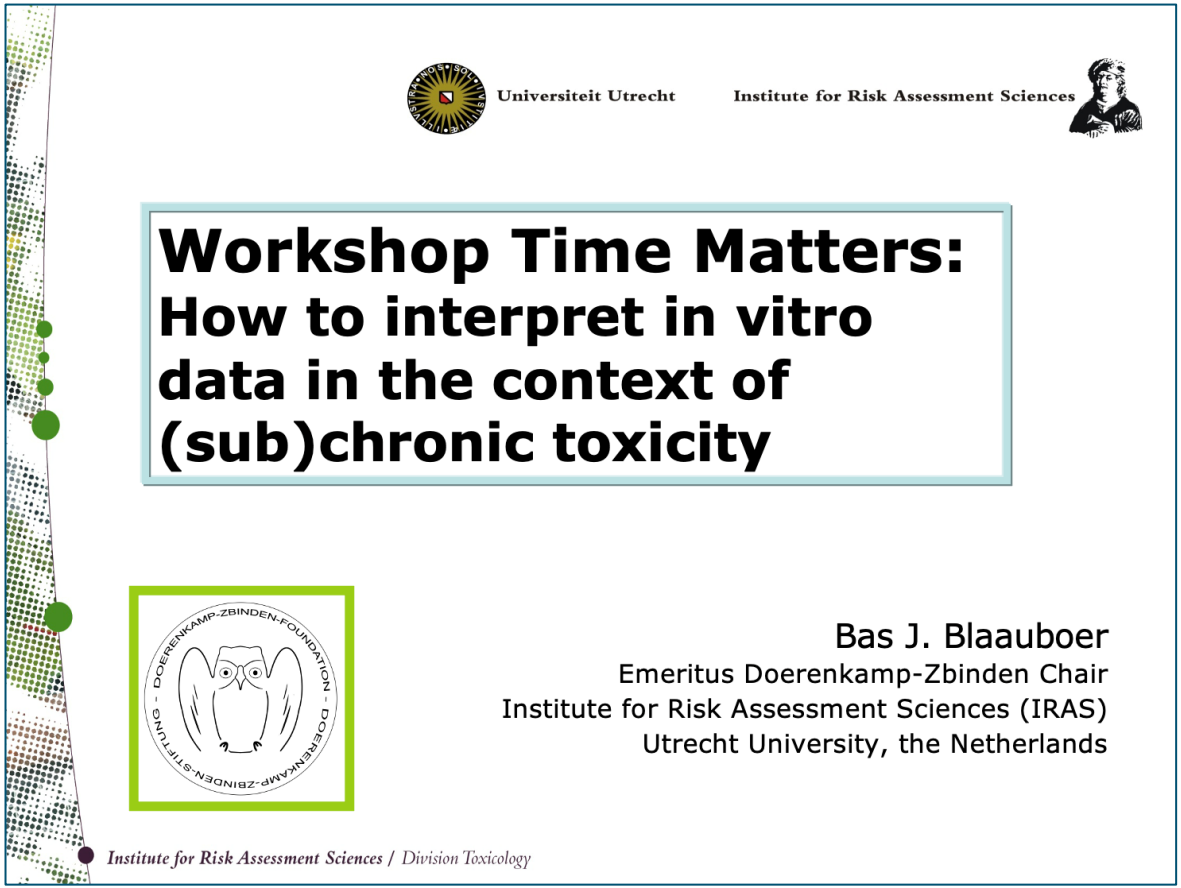
Wilmes et al. (2013) J Proteomics 79, 180-194 (Predict-IV)

Going Back in Time

Maybe Not that Far Back?



Time Matters Workshop, Utrecht, 2015



- Since then, NAMs in risk assessment have really taken off…
- Understanding how to incorporate the effects of time on toxicological risk is critical

ECETOC Workshop Aim

- Explore the need and approaches to study the influence of time in toxicity in NAM-based (human) risk assessment.
- Discuss how to integrate the influence of exposure time window, duration, frequency and damage accrual rate in developing and interpreting NAM data for risk assessment, incl.
    - *in vitro* assays and molecular biomarkers of toxicity
    - quantitative adverse outcome pathways (qAOP)
    - quantitative *in vitro* to *in vivo* extrapolation (QIVIVE).

How do we relate an early molecular effect *in vitro* to a latent toxic effect *in vivo?*

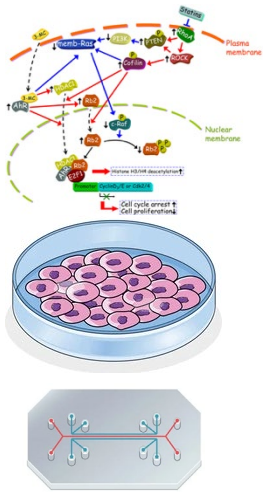
External dose

Interaction with target

Effect on organism(s)

in vitro

e.g. fold gene expression change



in vivo

e.g. neuro-degeneration

Toxic Concentration in Cells ≠ Toxic Applied Dose

External dose

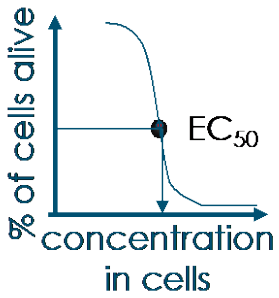
Interaction with target

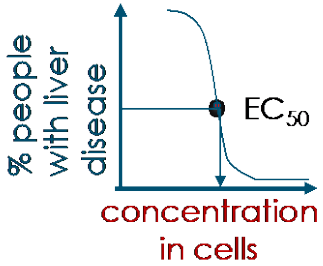
Effect on tissue

Effect on organism(s)

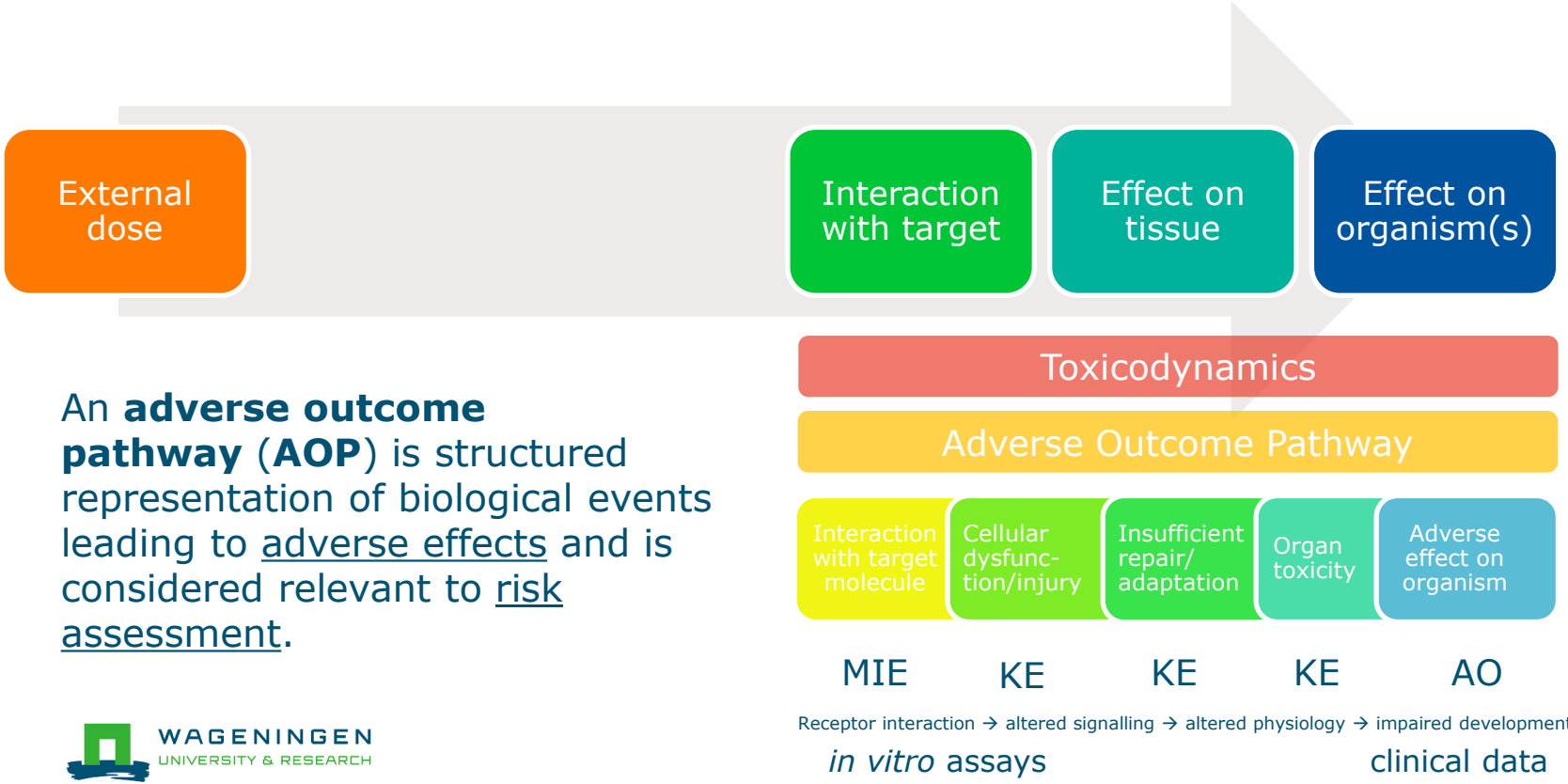
Toxicodynamics

Adverse Outcome Pathway





Adverse Outcome Pathways (AOP) Help



An **adverse outcome pathway** (**AOP**) is structured representation of biological events leading to adverse effects and is considered relevant to risk assessment.

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Quantitative Adverse Outcome Pathways (qAOP)

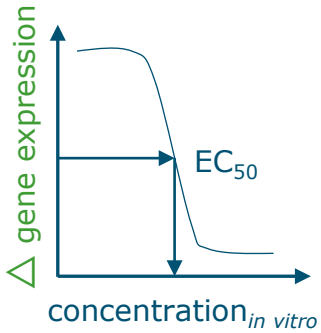
External dose

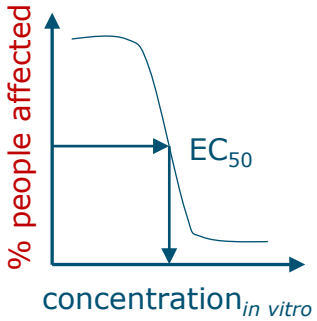
Interaction with target

Effect on tissue

Effect on organism(s)

Adverse Outcome Pathway





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Response-Response Modelling

External dose

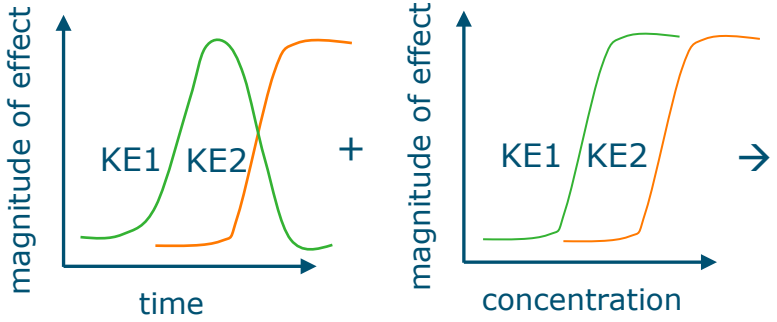
Interaction with target

Effect on tissue

Effect on organism(s)

Adverse Outcome Pathway

Causality +



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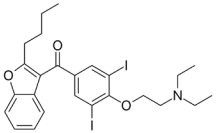
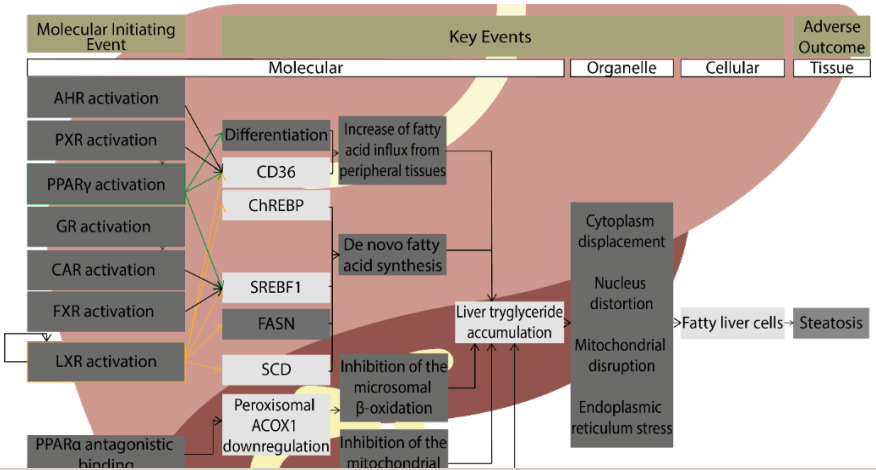
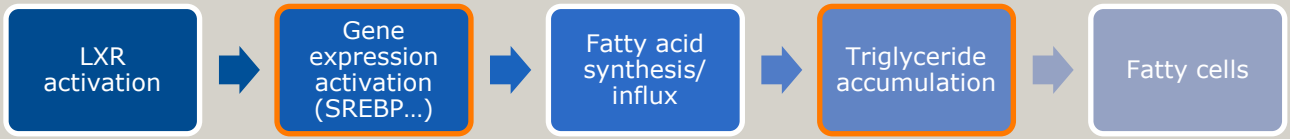
Liver steatosis by amiodarone

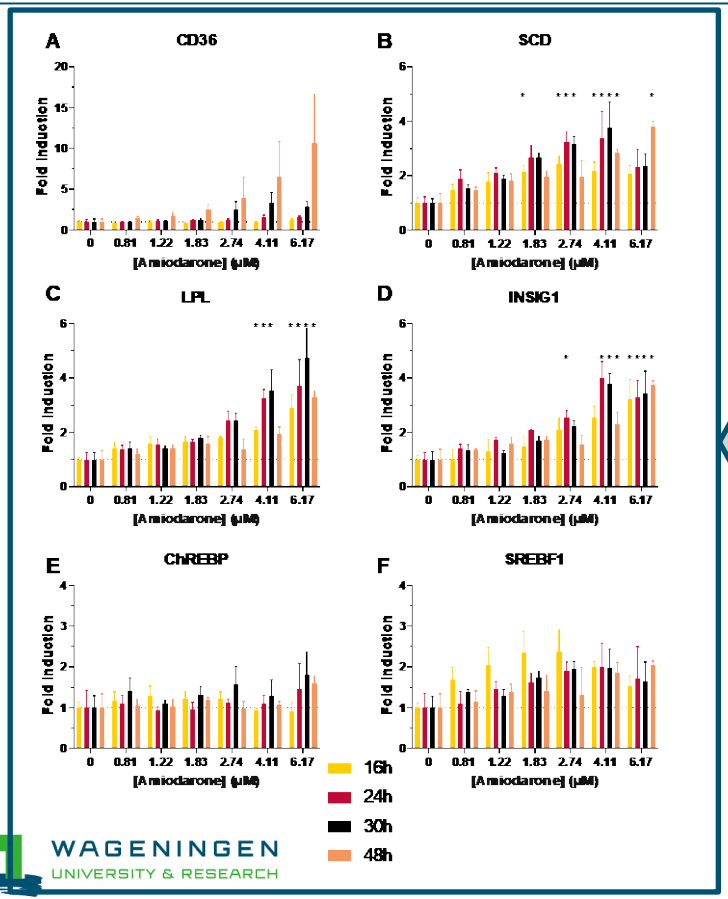
- Lipophilic antiarrhythmic drug with numerous unwanted side effects, including hepatic and neurotoxicity (±3% of patients).
- Setup *in vitro* test battery with HepaRG for liver steatosis qAOP development.

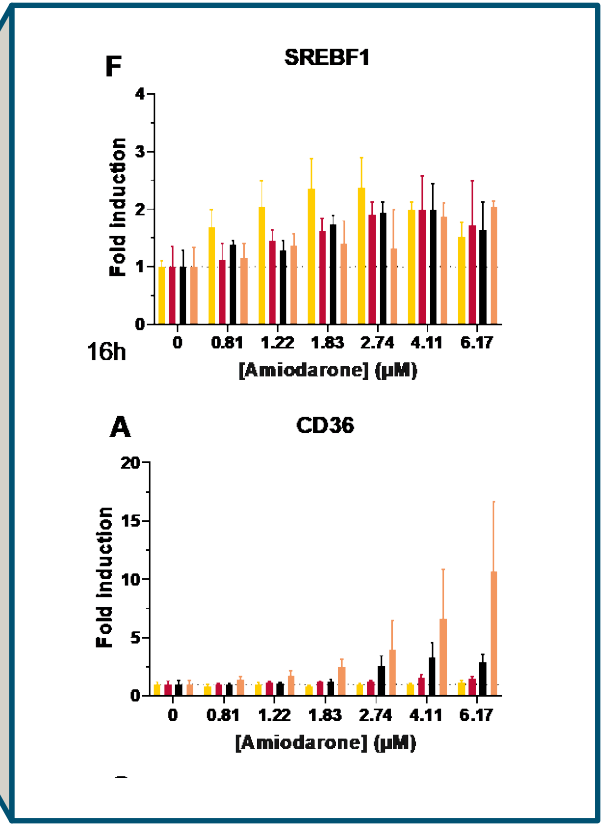






Concentration and time-dependent △gene expression





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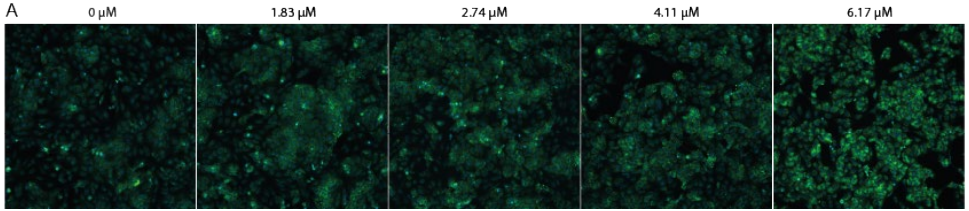
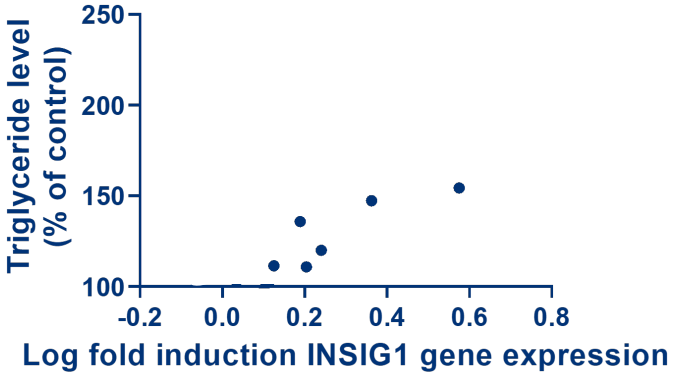
Kasteel et al., manuscript in preparation.

…and tryglyceride accumulation

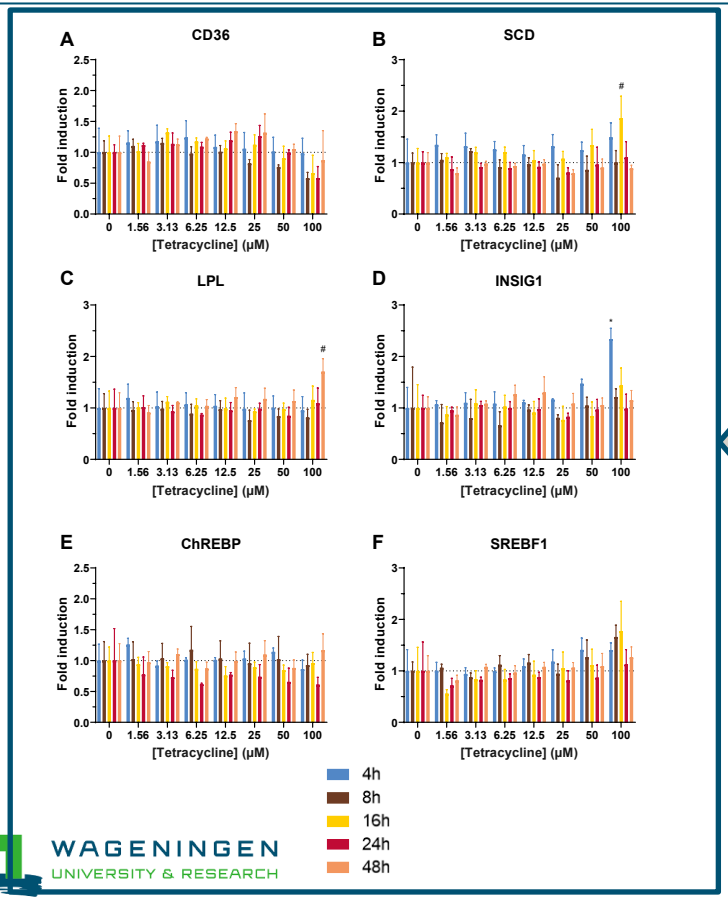


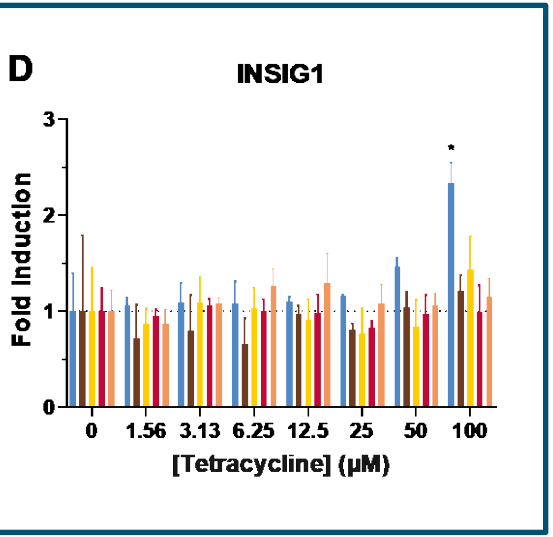
No detectable accumulation before 72h exposure



Response-response relationship for amiodarone using traditional nominal concentrations at 24h exposure

Different concentration-time profiles for tetracycline





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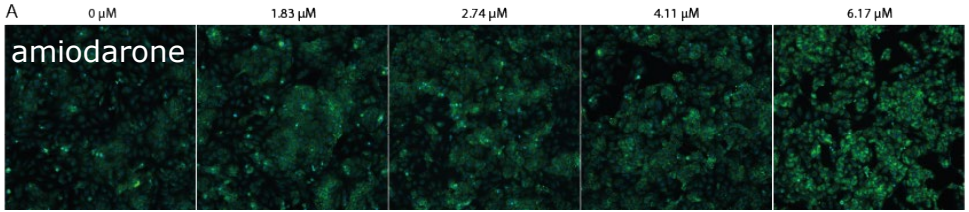
Consequences for response-response modelling



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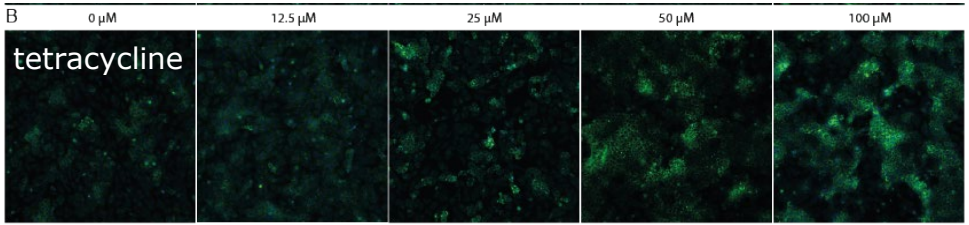
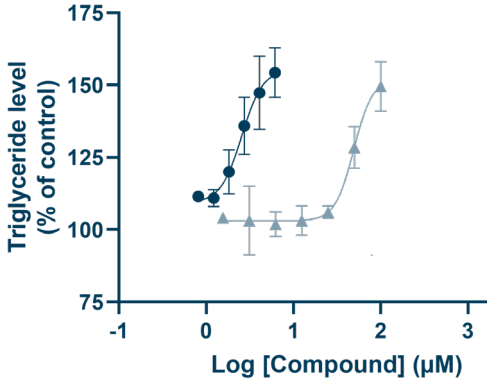
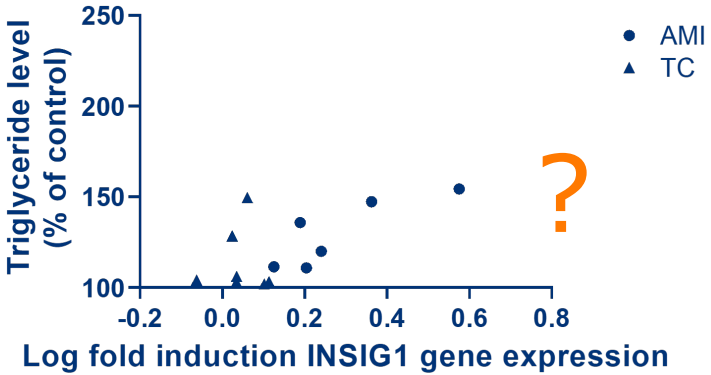


Max. accumulation at 48h exposure

Response-response relationship using traditional nominal concentrations at 24h exposure





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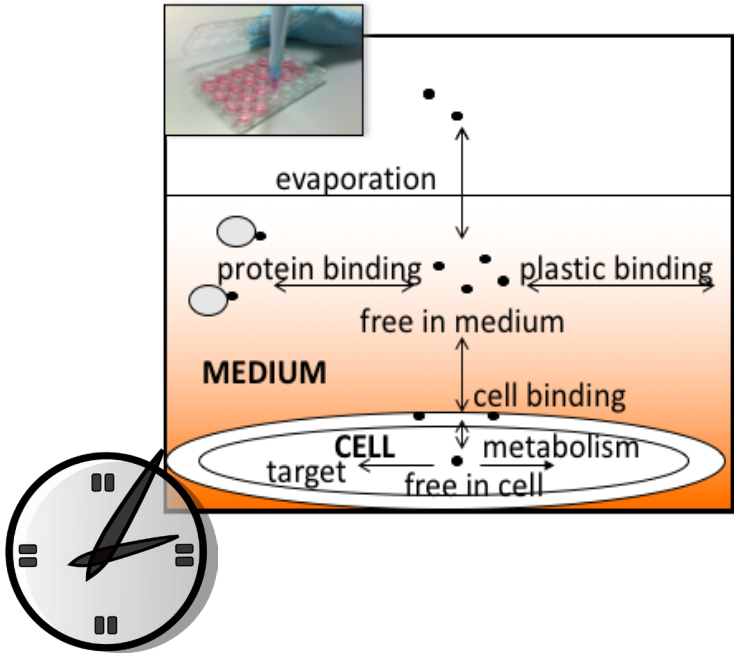
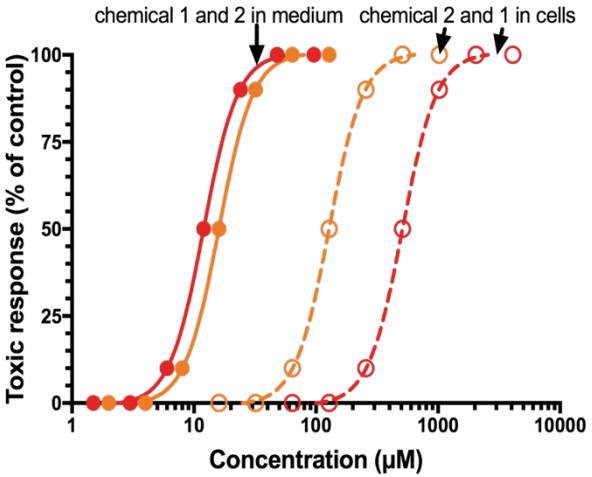
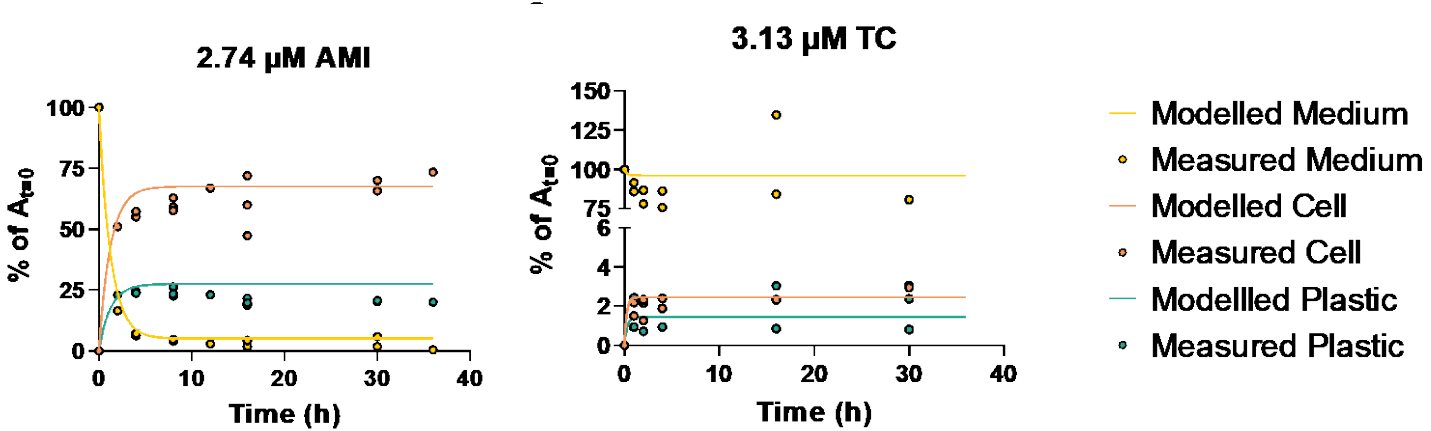
Role of *in vitro* distribution kinetics in AOP development





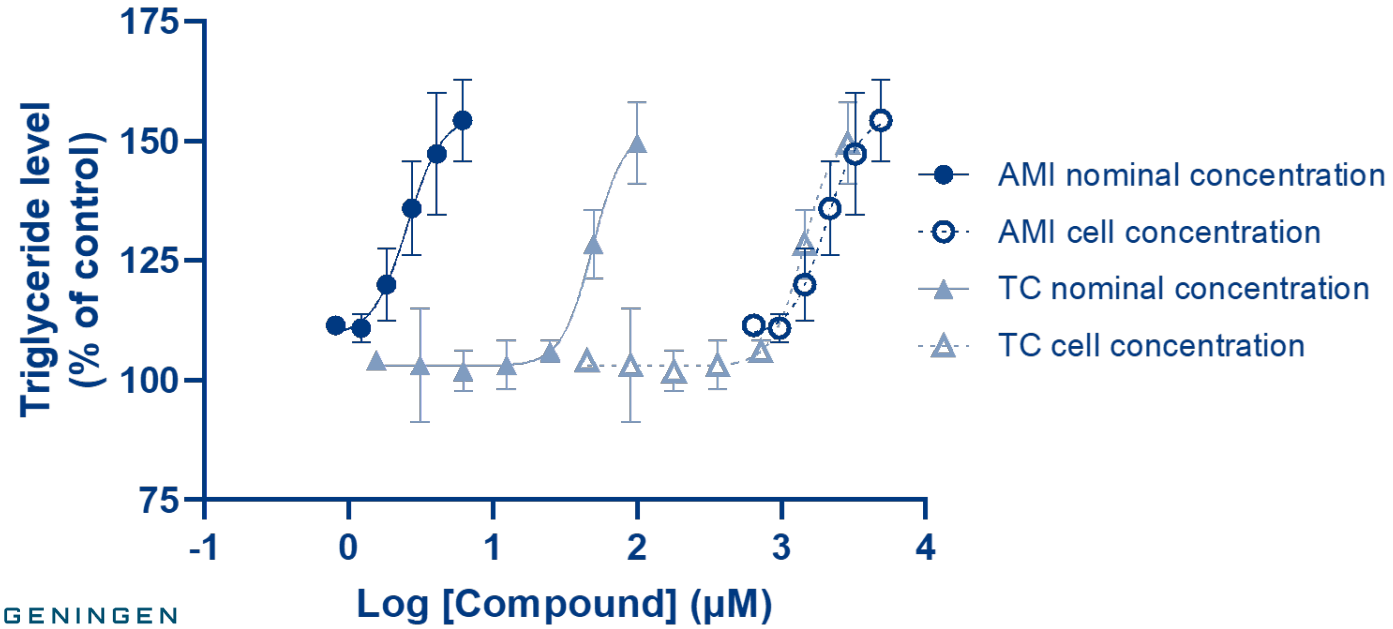
Role of *in vitro* distribution kinetics in AOP development



- Using a compartmental model, concentrations in plastic, medium and cells were simulated.
- It takes 6h for 60% of amiodarone to accumulate in HepaRG whereas 2% of dose of tetracycline is instantaneously associated with cells.

Role of *in vitro* distribution kinetics in AOP development

Use concentration-effect relationships based on cell-associated concentrations at exposure time point leading to lowest effect concentrations.



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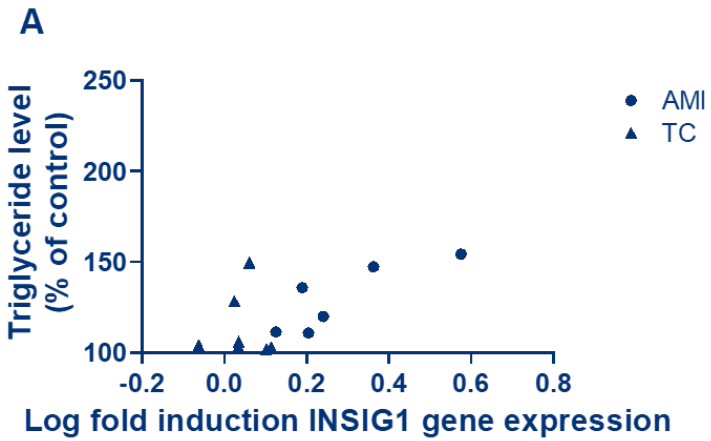
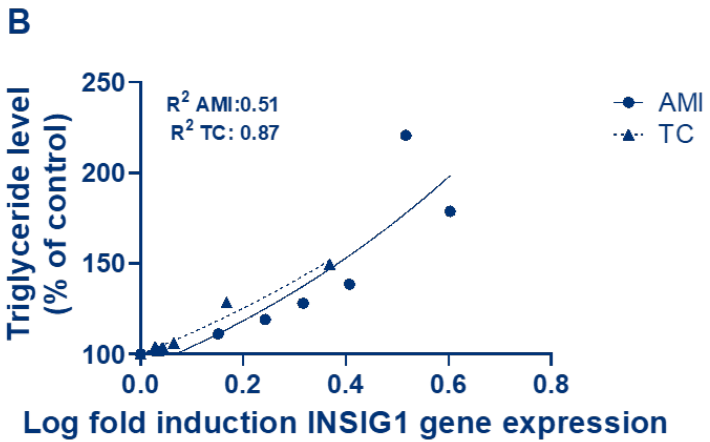
Role of *in vitro* distribution kinetics in AOP development

Standard: readout @24h nominal exposure



Suggested: readout @Tmax C_{cell} exposure



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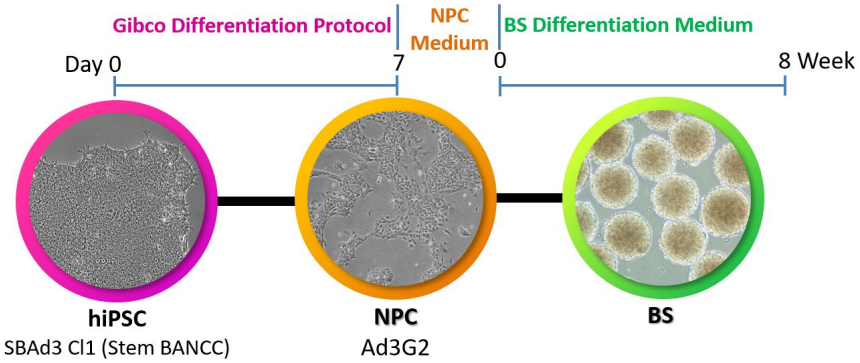
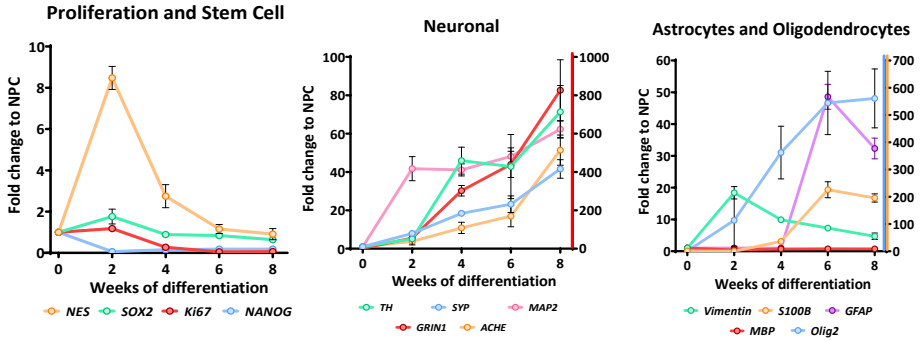
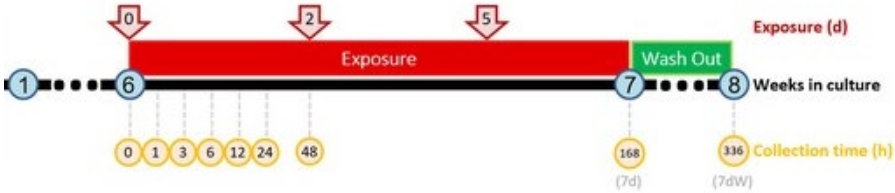
Amiodarone Toxicity in BrainSpheres





- hiPSCs-derived 3D model BrainSpheres
- Acute (48 h) vs. repeat (7 days) exposure to similar concentrations of amiodarone



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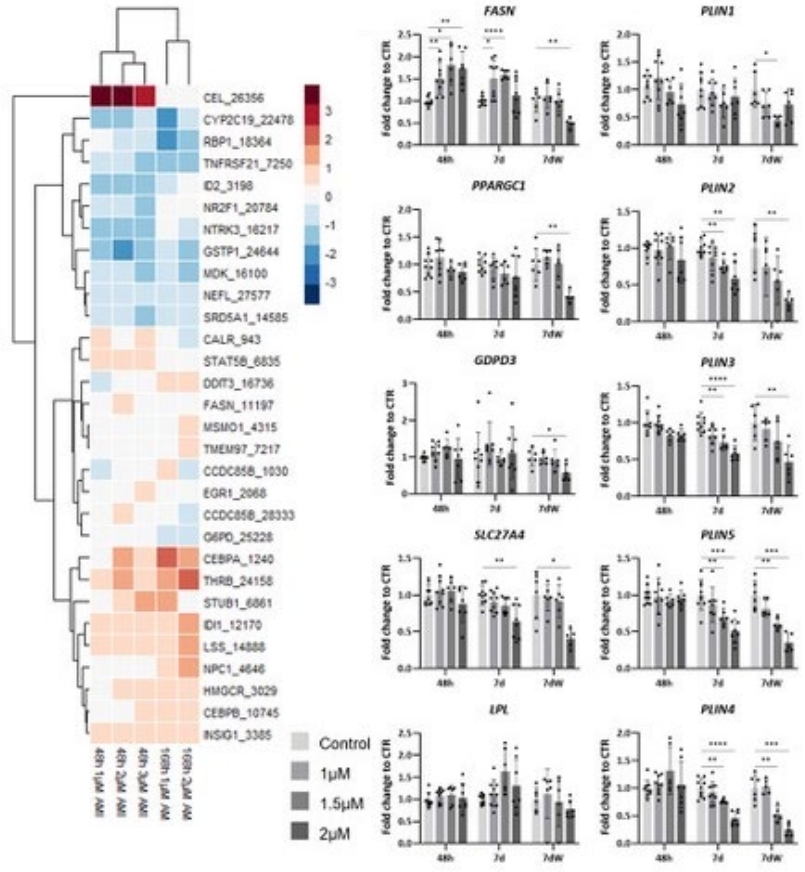
Nunes et al. (in press) Frontiers in Pharmacol.

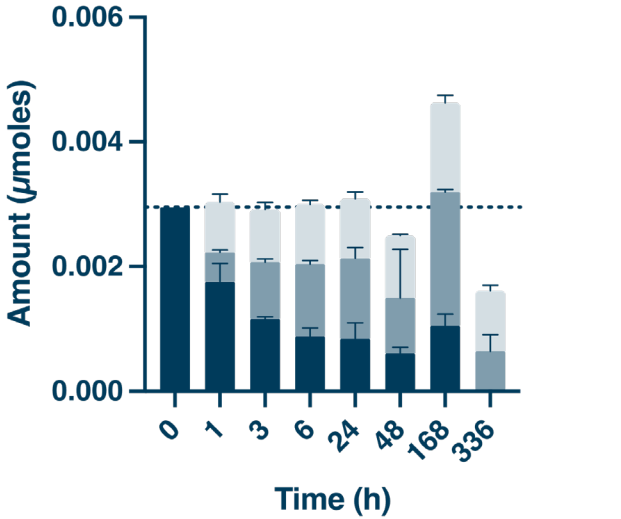
Gene Expression Changes in BrainSpheres

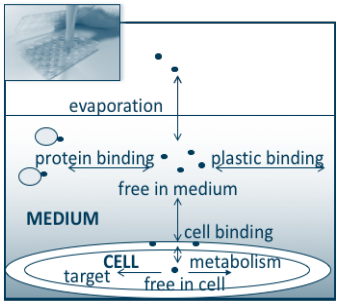
- Neurotoxicity assessed using transcriptomics and immunohistochemistry for cell type-specific markers.
- Time and concentration-dependent gene expression changes observed.
- Lipid metabolism genes induced at lowest exposure times and concentrations, followed by differentiation and neuronal function genes.



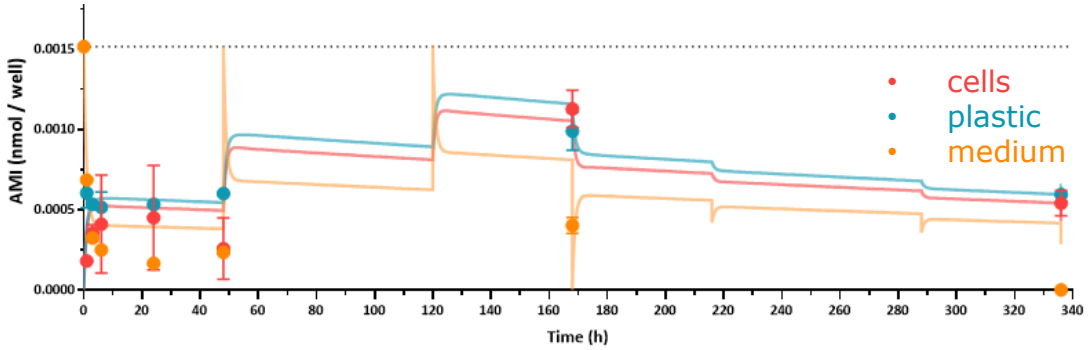
Role of *In Vitro* Kinetics in Amiodarone Toxicity

- Amiodarone accumulation in cells after repeat dosing explains increased toxicity with repeat dose.
- 30%+ amiodarone in cells after 6h exposure
- Refreshing medium does not remove chemical from cells and plastic, leading to accumulation with repeat dosing.

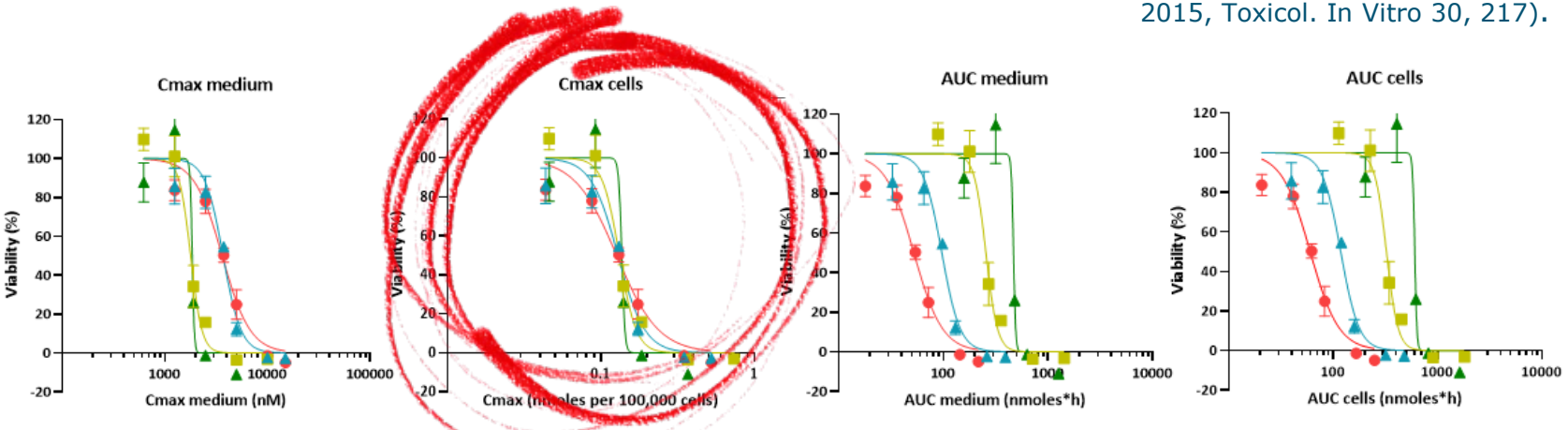




Role of *In Vitro* Kinetics in Amiodarone Toxicity



- *In vitro* kinetic modelling simulates cell-associated concentrations over time in BrainSpheres to assess appropriate dose metric for POD in QIVIVE.
- Modelling indicates lower EC in BrainSpheres than HepaRG and rat brain aggregates (Kramer et al., 2015, Toxicol. In Vitro 30, 217).



From Internal to External Dose

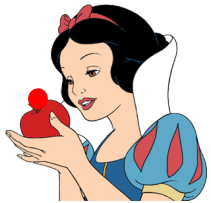
Toxicokinetics

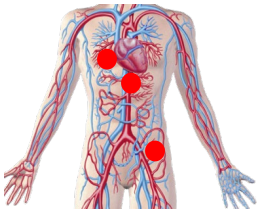
External dose | Internal dose | Target dose | Interaction with target | Effect on tissue | Effect on organism(s)

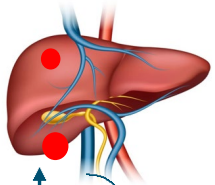
oral dose → C_{plasma} → C_{tissue} → BED

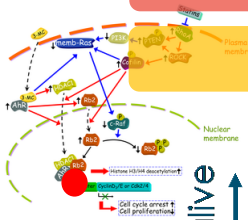
Toxicodynamics

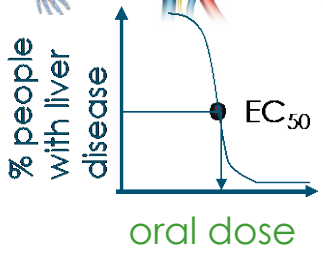
Adverse Outcome Pathway

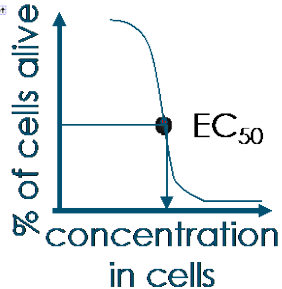


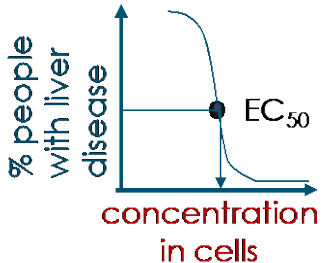












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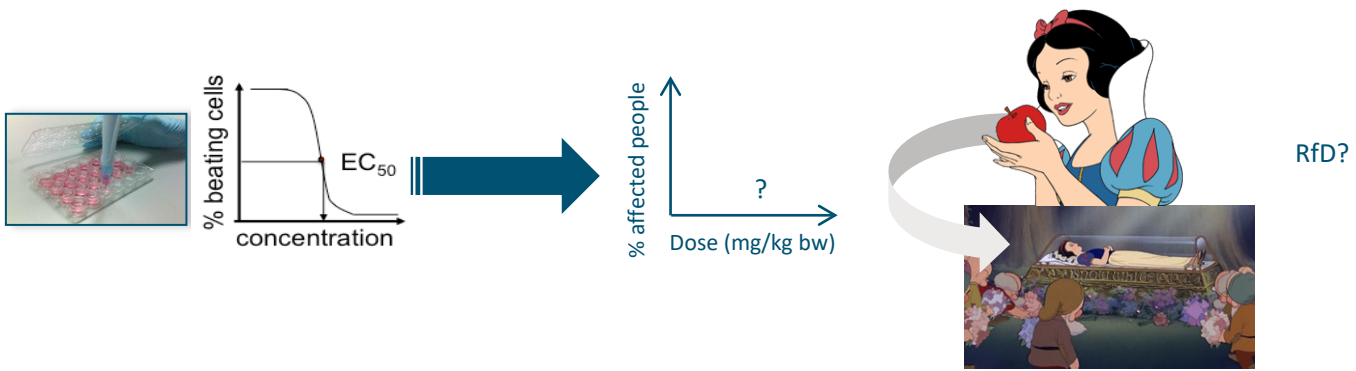
Quantitative In Vitro-In Vivo Extrapolation (QIVIVE)

def. The estimation of chemical exposures producing target tissue exposures in the species of interest equivalent to those associated with effects in *in vitro* toxicity tests.

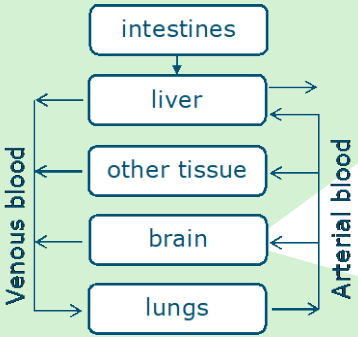


Physiologically Based Kinetic (PBK) Models

Series of mass balance differential equations
simulating transport of chemical between tissues.

Input parameters
External/internal exposures
Organism properties
Chemical properties

Absorption (P_{app})
Distribution (P_t, fu, P_{app})
Metabolism (V_{max}, Km, CL_{int})
Excretion (P_{ba}, Km, V_{max})

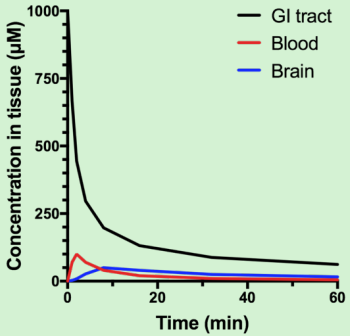


$$\frac{dA_{brain}}{dt} = Q_{brain}\,(l/h) * C_{arterial\ blood}\,(mg/l) - Q_{brain}\,(l/h) C_{venous\ blood\ in\ brain}\,(mg/l))$$

$$A_{brain} = \int_0^t dA_{brain}$$

$$C_{brain} = A_{brain}/V_{brain}$$

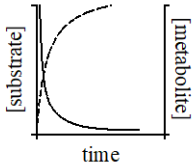
$$C_{venous\ blood\ in\ brain} = C_{brain}/P_{brain:blood}$$

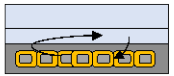


Quantitative *In Vitro* to *In Vivo* Extrapolation

In vitro toxicokinetics assay

Biotransformation kinetics in rat hepatocytes



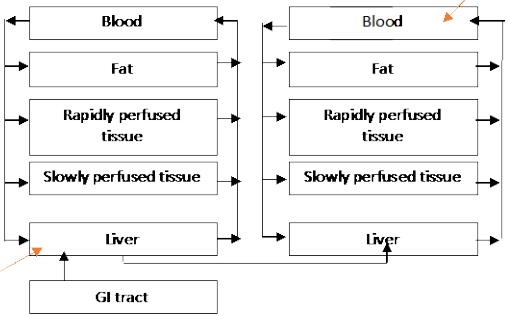


$C_t = C_0 \cdot e^{kt}$

CLint (uL/min/# hepatocytes) = k (min^{-1}) x (volume of medium (uL) / # hepatocytes)

in vivo CLint (uL/min) = CLint (uL/min/# hepatocytes) x (# hepatocytes/g liver) x (g liver)

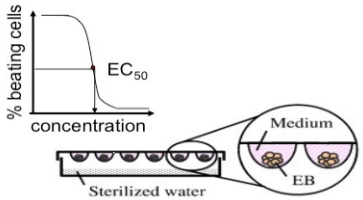
Input: BMC values metabolite alkoxyacetic acid



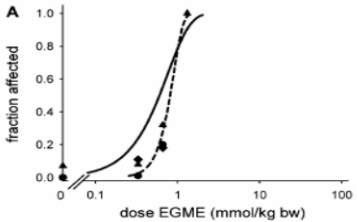
Output: predicted oral embryotoxic dose parent glycol ethers

In vitro toxicodynamics assay

Mouse embryonic stem cell test



Evaluation of IVIVE model with *in vivo* embryotoxic doses



Louisse et al. (2010) Tox. Sci. 118, 470

This is the last slide!

Thank you for
your attention!

Questions?