

Bringing the pieces of the puzzle together: Considering time and biological scale with New Approach Methodologies

ECETOC Workshop - Chronos and Kairos

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CONTEXT



Evolving regulations

EU Cosmetics Regulation (CE 1223/2009)

REACH

TSCA



Societal expectations

Products not tested on animal



Ethical concerns

Animal welfare

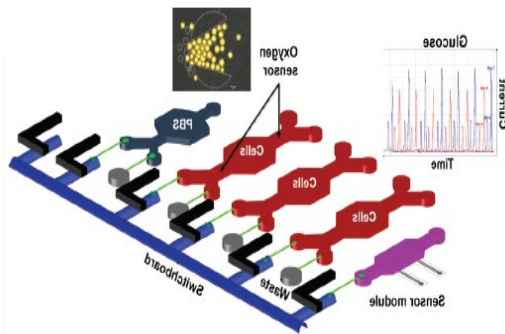
NEW APPROACH METHODOLOGIES

“**NAMs**: include in silico approaches, in chemico, in vitro assays used for investigating the exposure of chemicals, toxicokinetic, toxicodynamic knowledge for substances” *From “New Approach Methodologies in Regulatory Science Proceedings of a scientific workshop Helsinki, 19–20 April 2016”*
https://echa.europa.eu/documents/10162/22816069/scientific_ws_proceedings_en.pdf

“**NAMs**: any technology, methodology, approach (including computational/in silico models like QSARs, or combination thereof that can be used to provide information on chemical **hazard** and **risk assessment** that avoids the use of intact animals.” *US EPA*



In chemico



In vitro

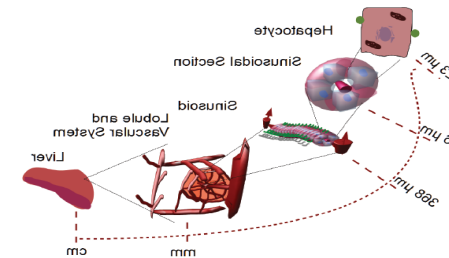
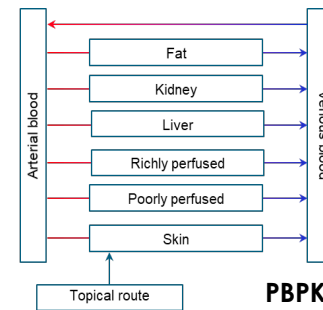
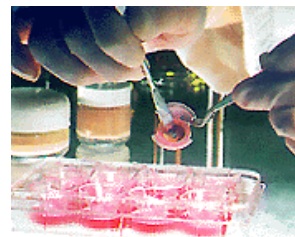
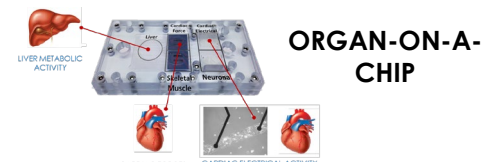
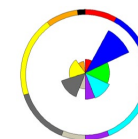


Figure 4-32 Multi-scale system from single hepatocytes to organ level. Single hepatocytes are coupled to liver capillaries (sinusoids) which are coupled to micro-channels called lobules. These lobules are considered to be the smallest functional micro-structure in the liver.



TOLERANCE

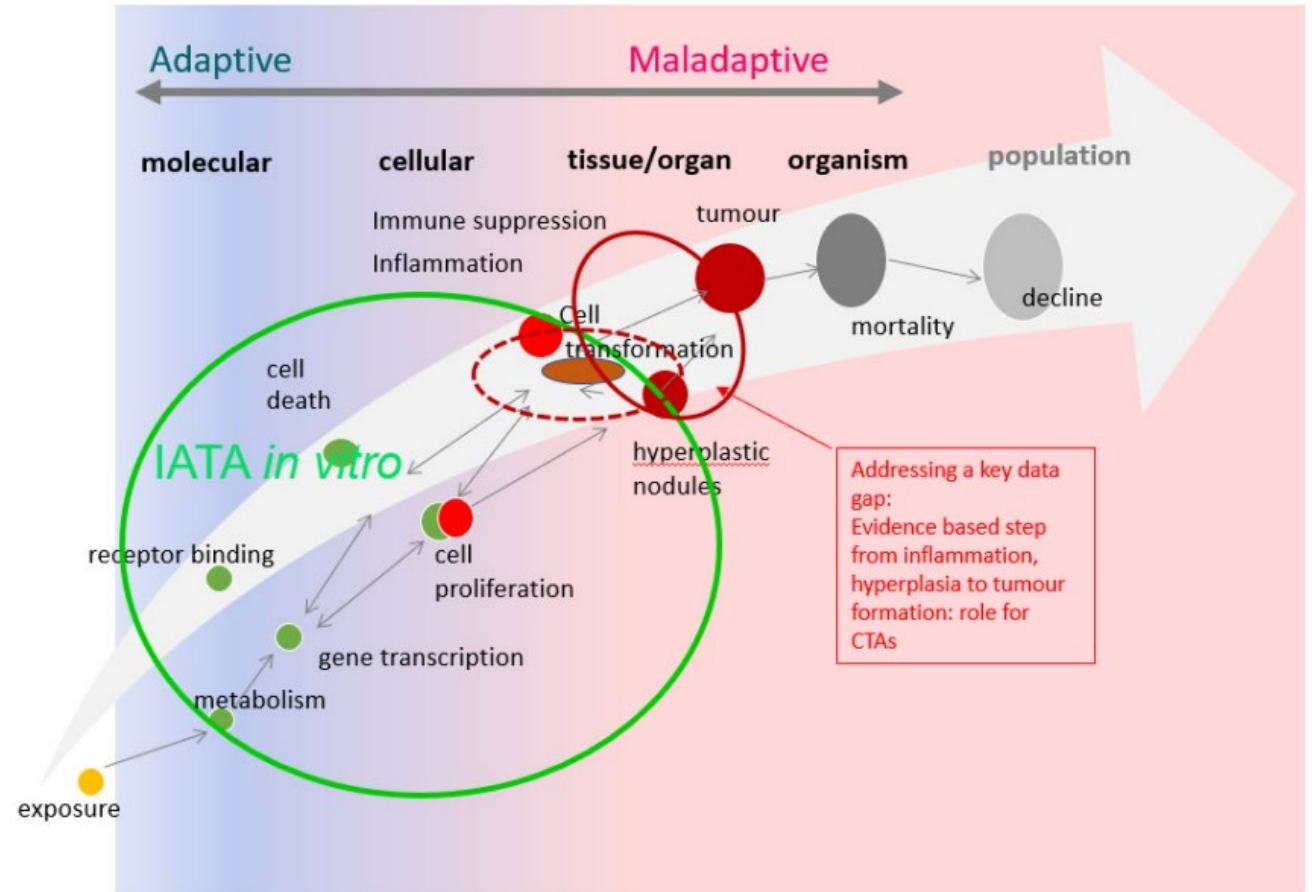
TOXICOGENOMICS



CELL TRANSFORMATION ASSAYS AND CARCINOGENESIS

Carcinogenesis: a multi-step process.

Cell transformation assays as part of an IATA for (non-genotoxic) carcinogenesis: *Jacobs et al., 2020; Arch Toxicol 94:2899-923*

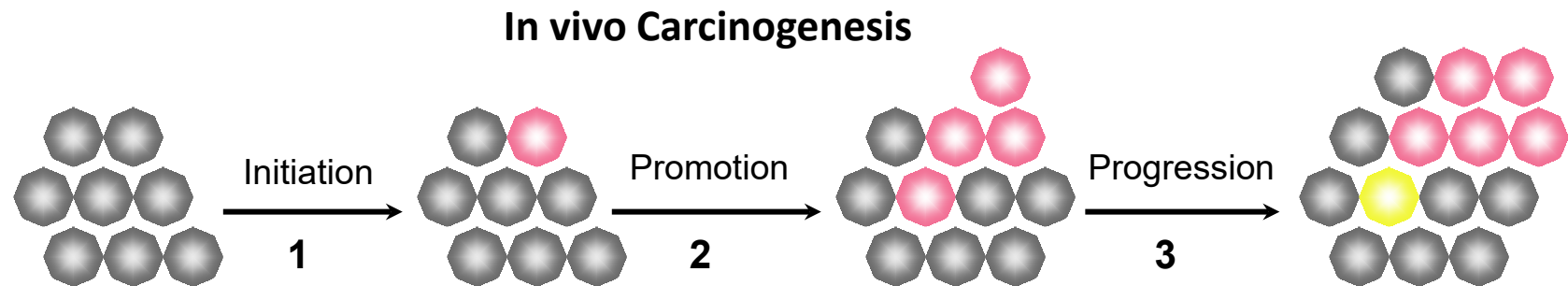


From Jacobs et al., 2020 Archives of Toxicology 94:2899–2923

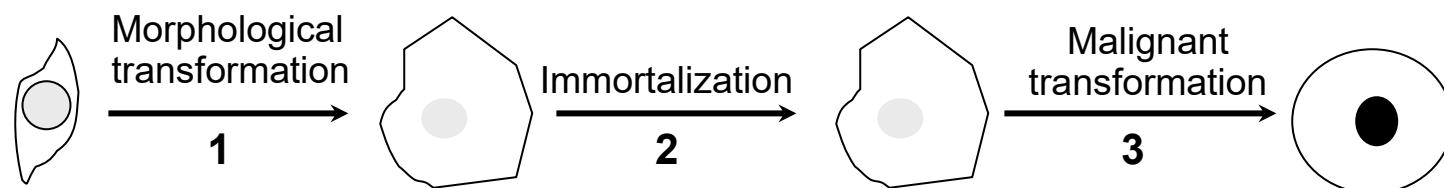
CELL TRANSFORMATION ASSAYS AND CARCINOGENESIS

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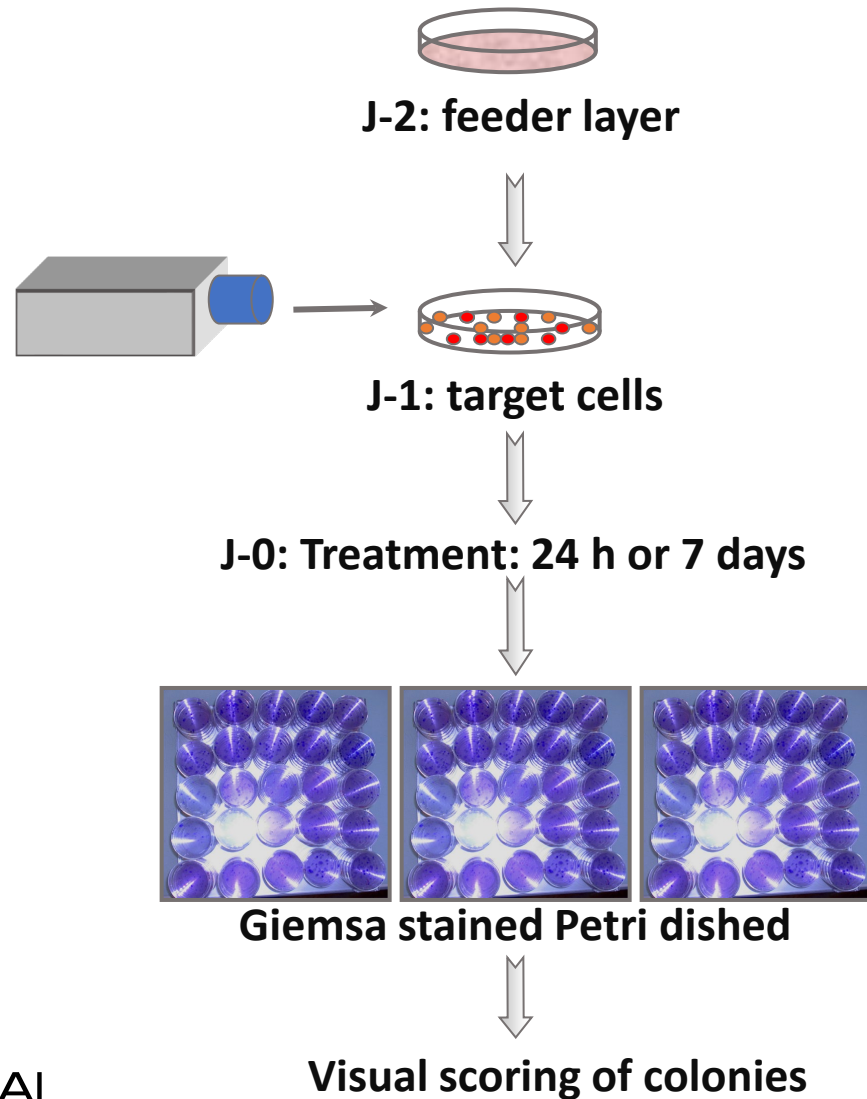


In vitro Syrian Hamster Embryo cell transformation (OECD GD214)



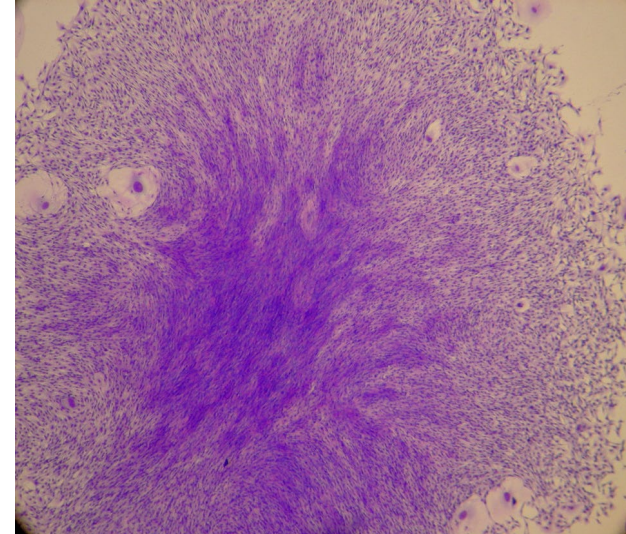
THE SYRIAN HAMSTER EMBRYO ASSAY

OECD Series on Testing & Assessment No. 214, 2015

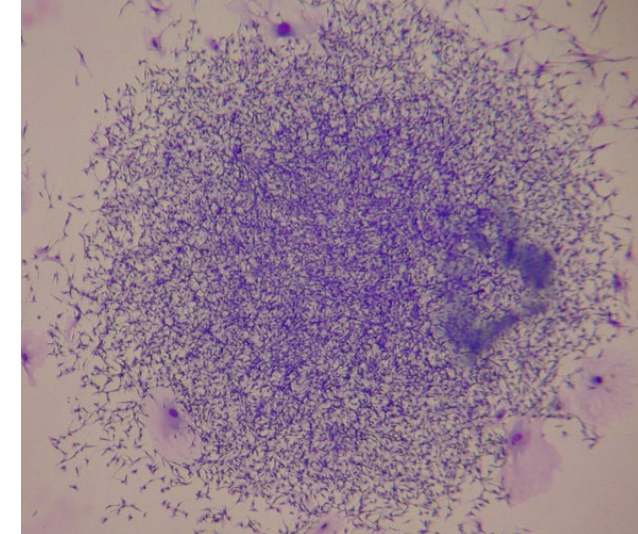


Sensitivity	Specificity	concordance
86%	85%	85%

LeBoeuf et al., The pH 6.7 Syrian hamster embryo cell transformation assay for assessing the carcinogenic potential of chemicals, Mutation Research 356 (1996) 85 - 127



Normal colony



Transformed colony

THE SYRIAN HAMSTER EMBRYO ASSAY

Challenges

Biological model: rodent embryonic stem cells

Incubation time: 24h, 7 days

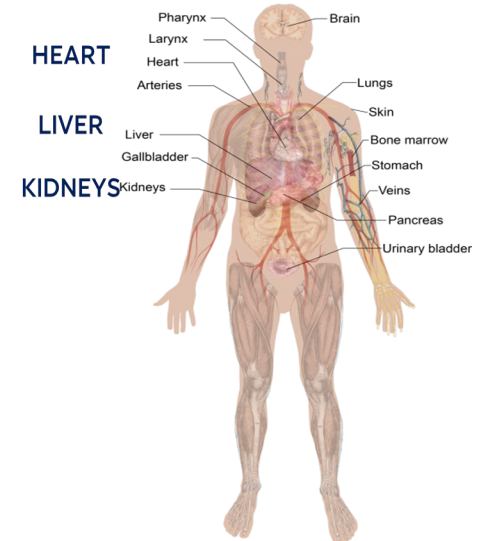
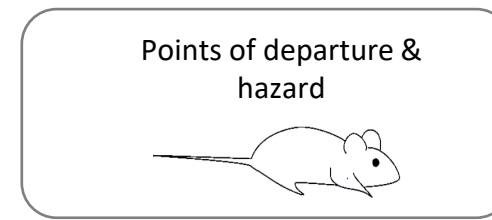
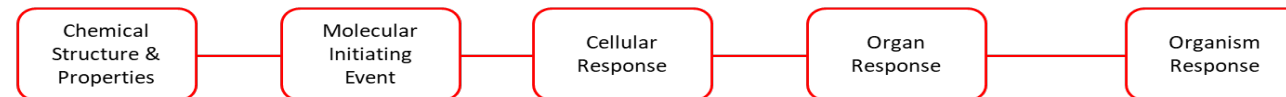
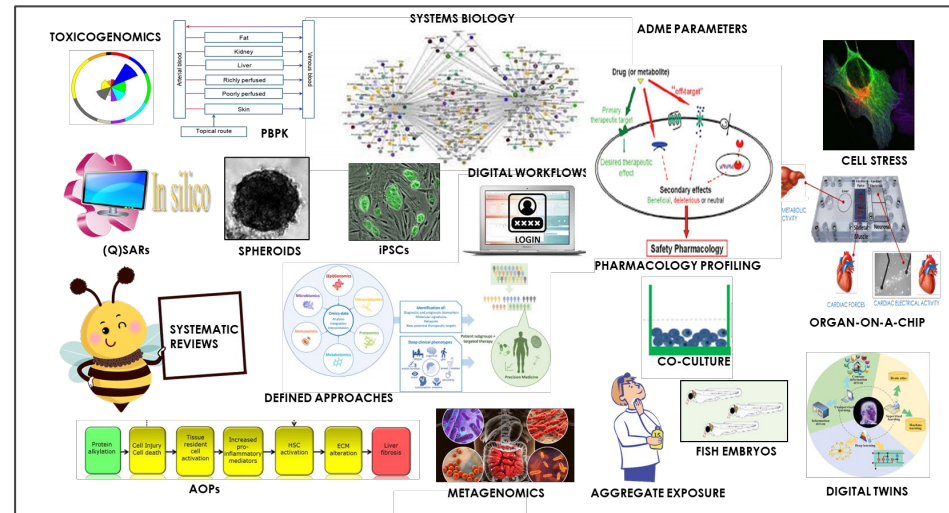
- Interplay between damage/(accurate) repair?
- Balance proliferation/cell death?
- Role of kinetics?

NAMs AND REPEATED DOSE SYSTEMIC TOXICITY

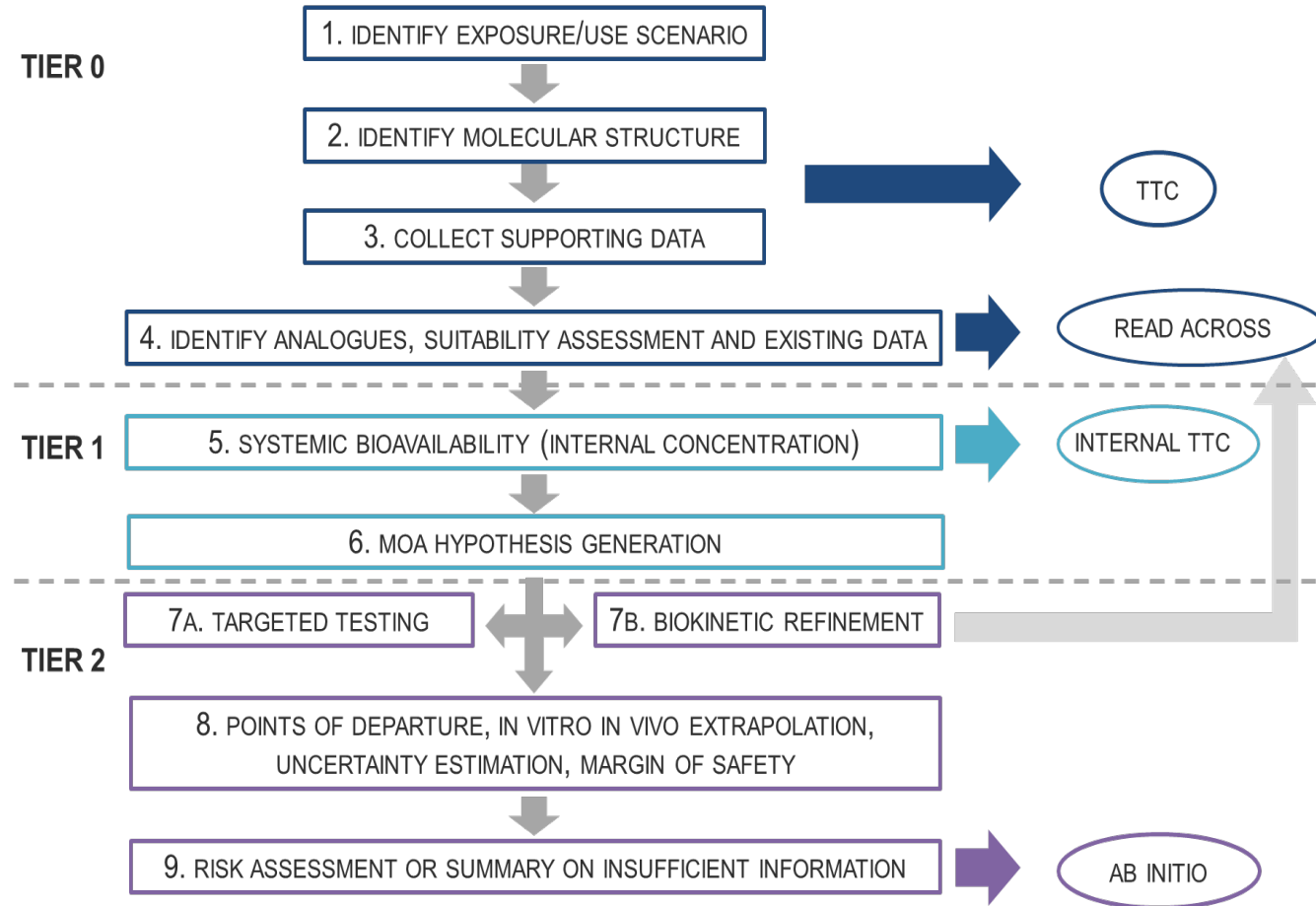
Banned in EU Cosmetic regulation EC 1233/2009

Repeated-dose systemic toxicity: determine the fate of chemicals after they reach the systemic circulation

GHS definition "specific target organ/systemic toxicity arising from a repeated exposure" (OECD TGs -non-exhaustive-TGs 407, 408, 410, 411, 412, 413, 422, 452, 453)

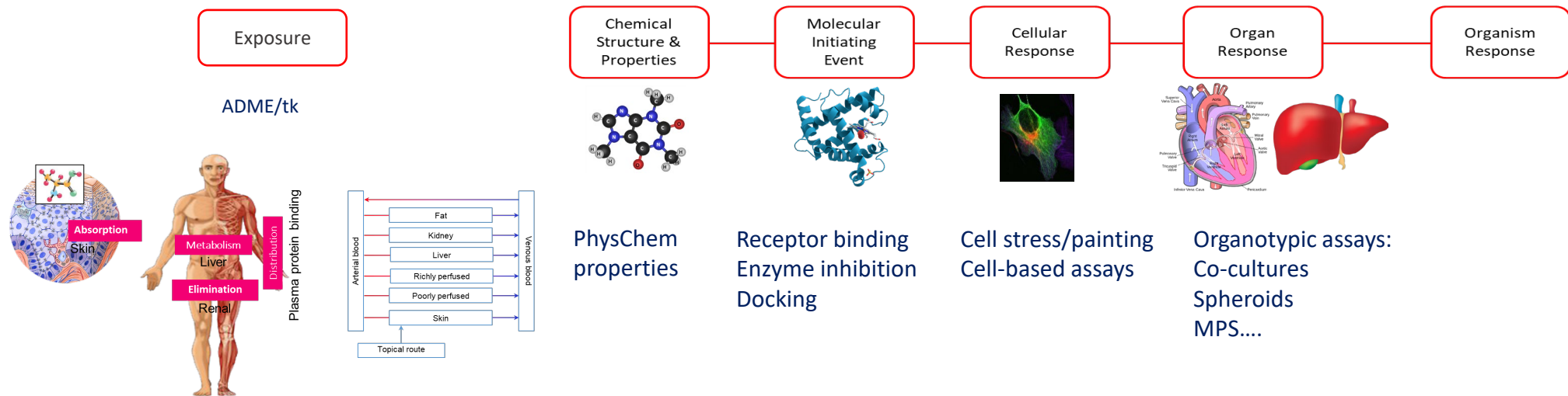


A NEXT GENERATION FRAMEWORK



Berggren et al. Computational Toxicology. 4. P31-44. (2017)
OECD IATA Case Studies Project, Series on Testing & Assessment No. 275.
ENV/JM/MONO(2017)27

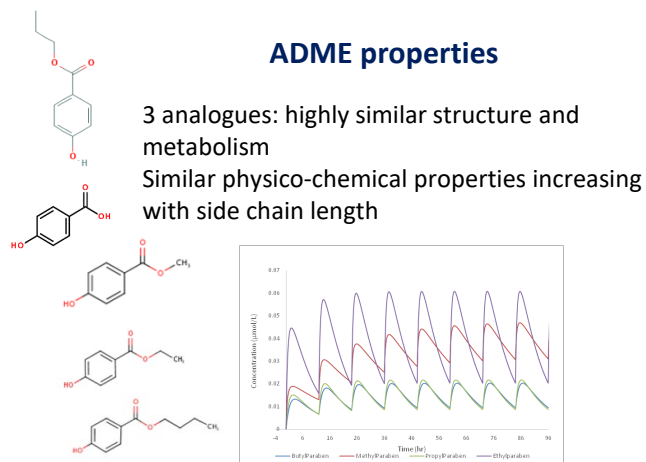
NAMs AND REPEATED DOSE SYSTEMIC TOXICITY



Eg: Propylparaben use in cosmetics at 0,18%

Read-across for hypothetical gap for reproductive toxicity

Ouédraogo et al., 2022 – Reg. Tox. Pharmacol



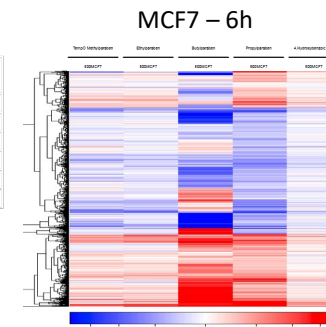
Nuclear receptors binding, docking

US EPA ER model

	AC10.median	Calculated Scaling (potency) Factor*
17beta-Estradiol	-3.07	-16.6
Butylparaben	0.18	1.0
Propylparaben	0.50	0.4
Ethylparaben	0.95	0.2
Methylparaben	1.41	0.1

* calculated as 1/(AC10 of individual paraben/AC10 of BP)

Cell stress, endocrine bioactivity, transcriptomics



NAMs AND REPEATED DOSE SYSTEMIC TOXICITY

Challenges

- **Large number of modes of action**, many of which are still undefined
- **Relevance of late vs early responses**
- **Interplay between them** and different cell types
- **Temporal aspects**, including the relationship between perturbation and repair
- **(Toxico)kinetic and metabolic considerations**
- **Human relevance**: from legacy animal data to human-based models

CHALLENGES AND PERSPECTIVES

Biological model characterization

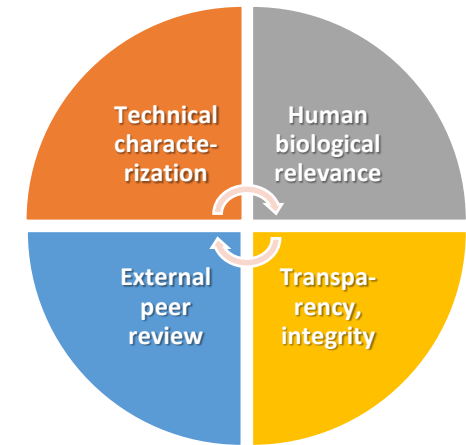
Pragmatism with complexity

Relevant **incubation time**

Relevant **biological level**

Metabolism and kinetics

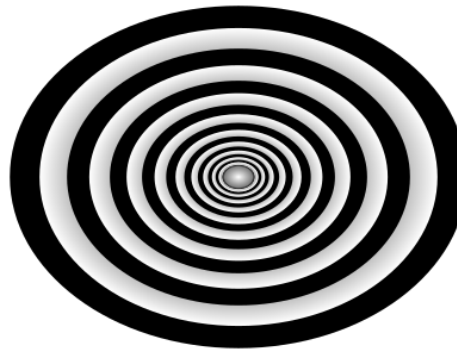
Data Integration



Adapted from van der Zalm et al., 2022



THANK YOU



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