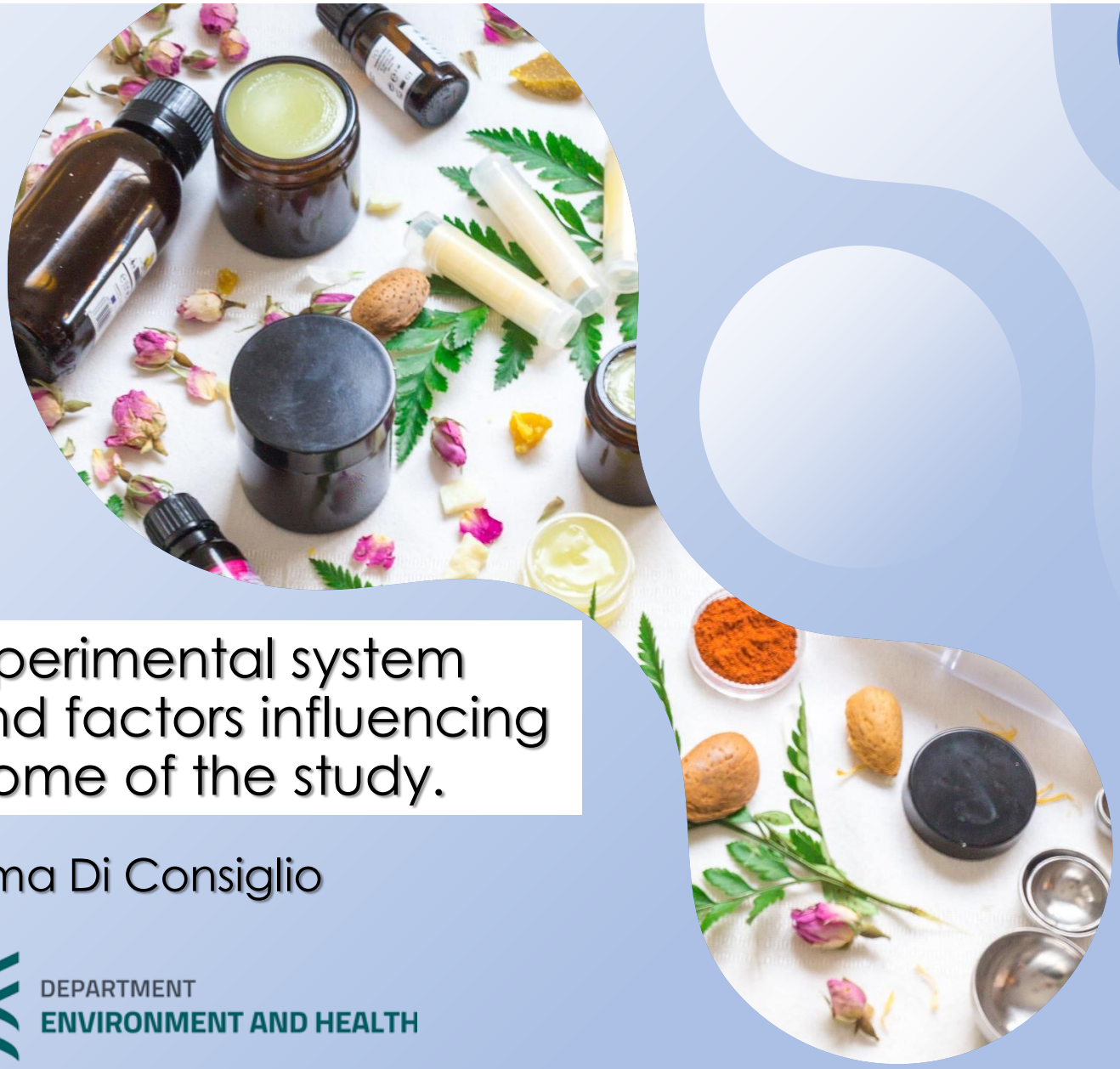
Innovative approaches in cosmetic testing, in compliance with European regulations-
2022, June 22-23 – Genoa, Italy



In vitro experimental system relevance and factors influencing the outcome of the study.

Emma Di Consiglio

ISTITUTO SUPERIORE DI SANITÀ

DEPARTMENT
ENVIRONMENT AND HEALTH

Cosmetic ingredient evaluation: Setting the Scene

Development of New Approach Methodologies (NAMs) by using human cells, with monolayer (co) cultures to more complex cell models (3D or organ-on-chip), together with computational methods (e.g. QIVIVE, PBK),

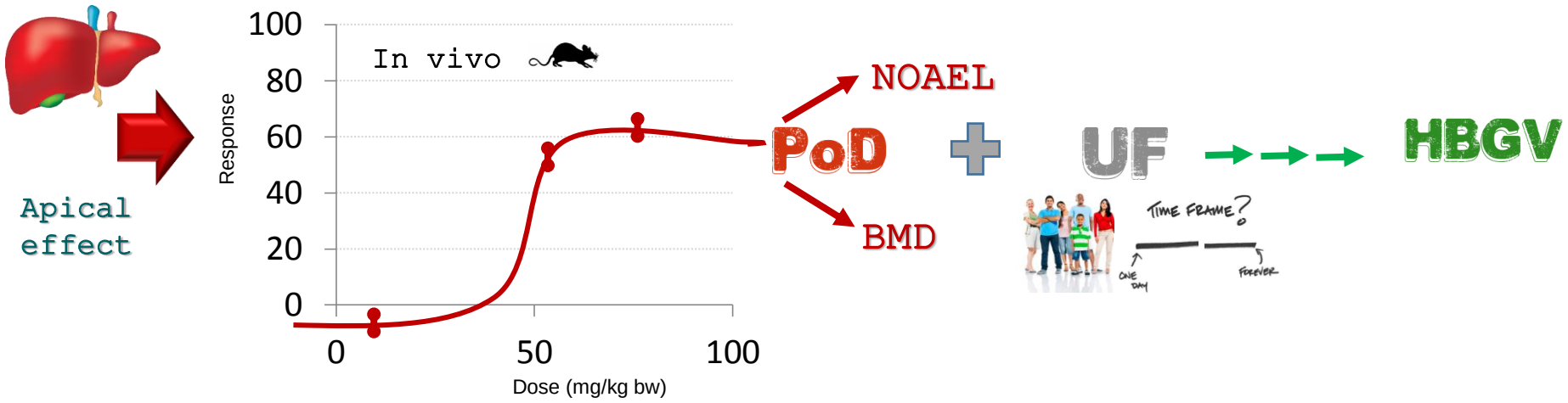
reduction of the number of animals used in different research and regulatory sectors.

- Cosmetic ingredients evaluation by using NAM-based strategies is an essential step to ensure the safety profile of cosmetics (Scientific Committee on Consumer Safety-SCCS).

- A huge challenge to guarantee the absence of risk ONLY based on the current available methods.

Next Generation Risk Assessment NGRA Development New Cosmetic Ingredient

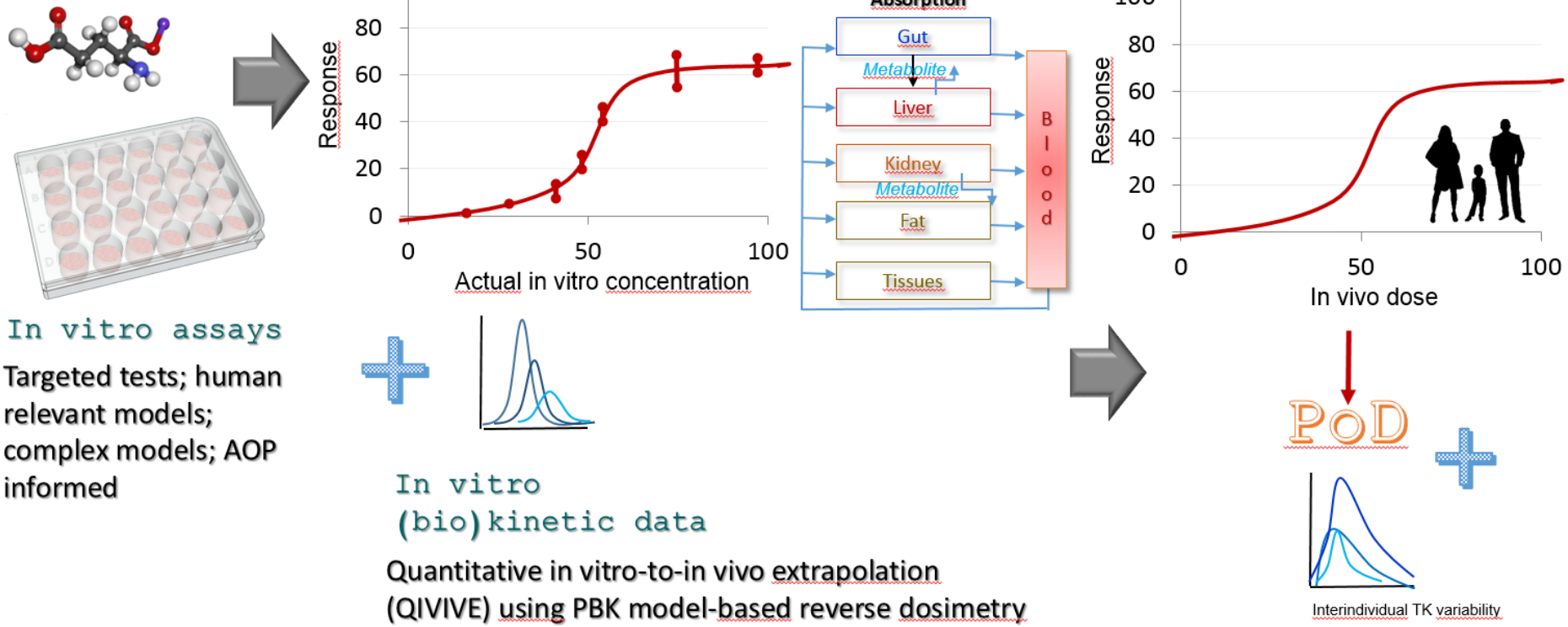
From Classical Toxicology



Modified from:
Ingenbleek et al., 2021;DOI:10.1039/9781839160431-00001;
Levorato et al., 2019 Current Opinion in Food Science

Di Consiglio, et al., Toxicol Lett., (2021);
Vichi, et al., Toxicol Lett., (2021);
Algharably, Di Consiglio, et al., Arch Toxicol., (2021);
Di Consiglio, et al., Reprod Toxicol. (2020)

Next Generation Risk Assessment NGRA Development New Cosmetic Ingredient



In vitro assays

Targeted tests; human relevant models; complex models; AOP informed

In vitro (bio)kinetic data

Quantitative in vitro-to-in vivo extrapolation (QIVIVE) using PBK model-based reverse dosimetry

Modified from:
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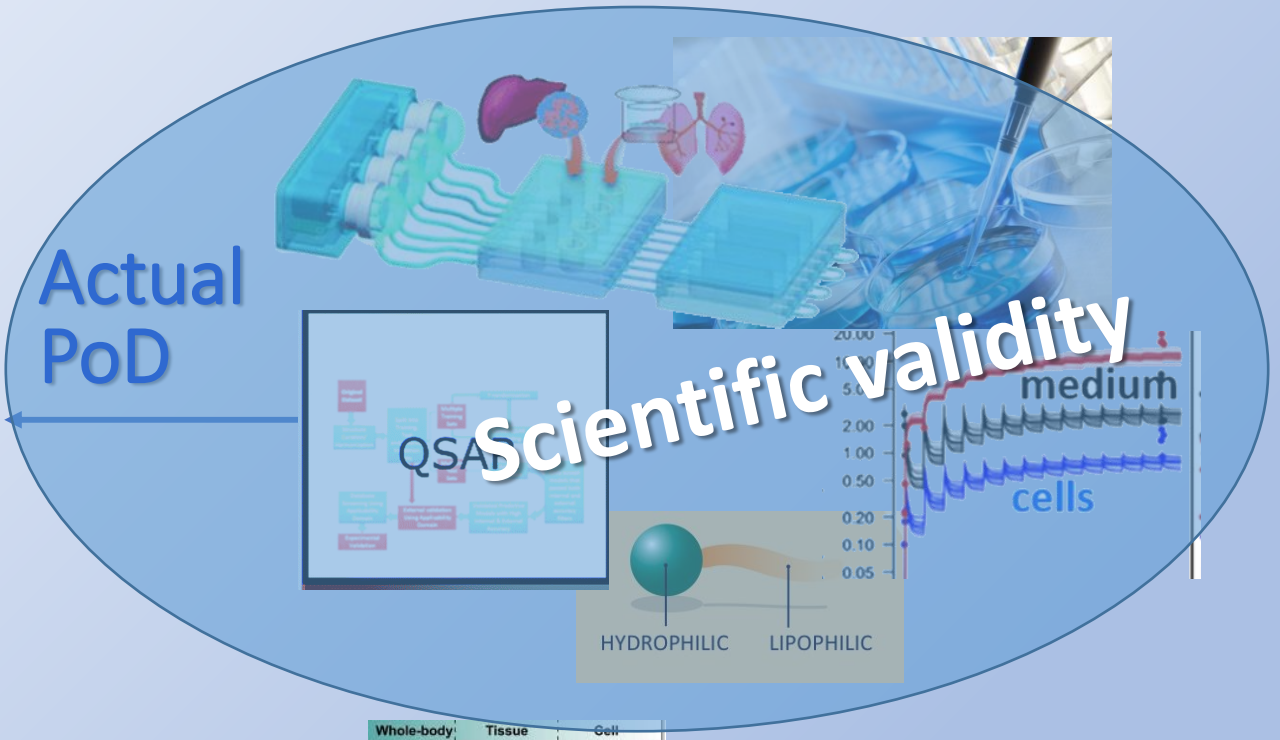
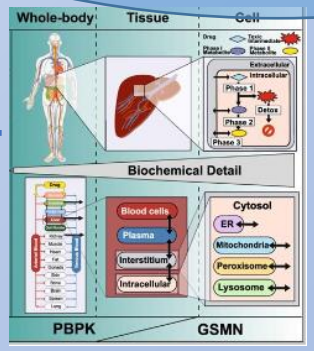
Di Consiglio, et al., Reprod Toxicol. (2020)

Cosmetic ingredient evaluation: Next Generation Risk Assessment



MOS

Exposure



2021- SCCS/1628/21
Modified from Rogiers et al., Toxicology 436 (2020)

Scientific validity of a test method

Evaluating the capacity of a test to protect human health:

how well it correlates with reliable information on activity of chemicals in humans.

≠ piece of the evidence → ≠ NAMs

- ✓ scientifically valid;
- ✓ properly developed and adequately described;
- ✓ "fit-for-purpose" performance;
- ✓ adequate scientific quality standards: standardization, relevance, specificity, sensibility

2018- Guidance Document on Good In Vitro Method Practices (GIVIMP), OECD Series on Testing and Assessment, No. 286, https://doi.org/10.1787/9789264304796-en
2022- Guidance document on Good Cell and Tissue Culture Practice 2.0 (GCCP 2.0)", ALTEX, 39(1), pp. 30–70. https://doi: 10.14573/altex.2111011
2021 - Guidance document on the characterisation, validation and reporting of PBK models for regulatory purposes, OECD Series on Testing and Assessment, No. 331

What are the goals?

BY Improving performing and *reporting**;

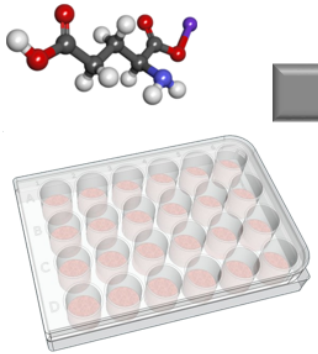
BY Improving implementation of requirements to consider all available information.

Regulatory accepted and **standardised** in vitro methods as internationally recognised OECD TG

Academic data used in regulatory assessments: helping researchers to design, perform and report studies, in order to facilitate regulatory acceptance

**2014, Guidance Document For Describing Non-guideline In Vitro Test Methods OECD Series on Testing and Assessment, No. 211*

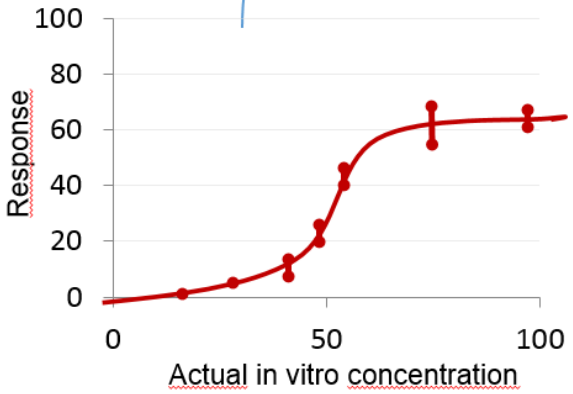
QIVIVE

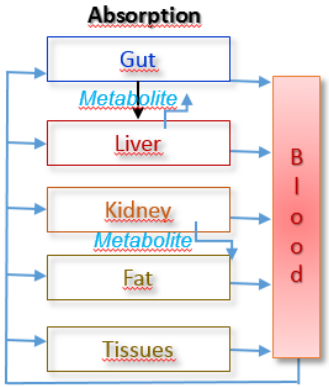


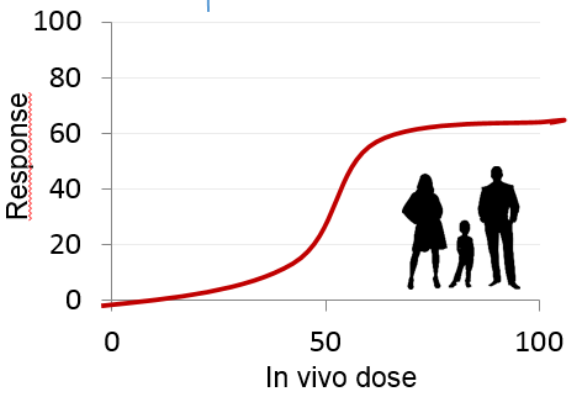
In vitro assays

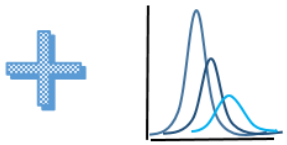
Targeted tests; human relevant models; complex models; AOP informed

Uncertainties





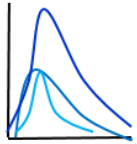




In vitro (bio)kinetic data

Quantitative in vitro-to-in vivo extrapolation (QIVIVE) using PBK model-based reverse dosimetry

PoD



Interindividual TK variability

QIVIVE

In vitro
(bio)kinetic data

Quantitative in vitro-to-in vivo extrapolation (QIVIVE) using PBK model-based reverse dosimetry

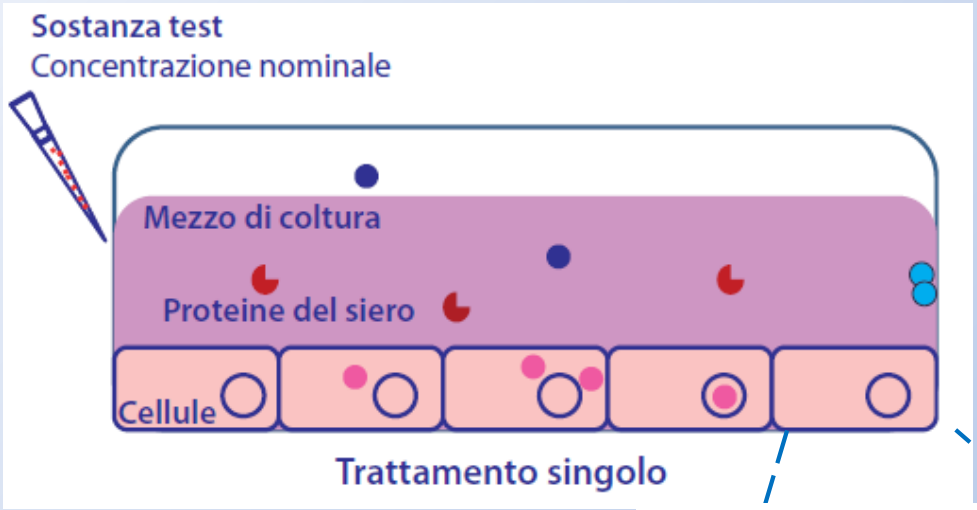
Broadly defined as a **quantitative or qualitative transposition of in vitro experimental data** to predict in vivo effects, refer to :

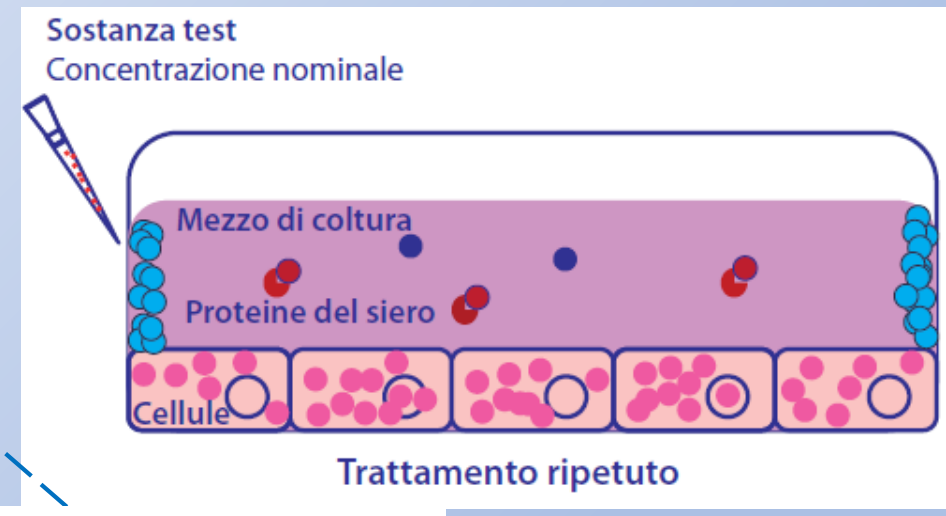
1. The **estimation** of the **in vivo ADME**, based on parameters measured in vitro, often used for constructing PBPK models;
2. The process of converting an **in vitro concentration** associated **with bioactivity** to an external exposure level (i.e. **reverse dosimetry**) → use of PBPK model to determine an exposure level that leads to a tissue plasma concentration = to the in vitro concentration.

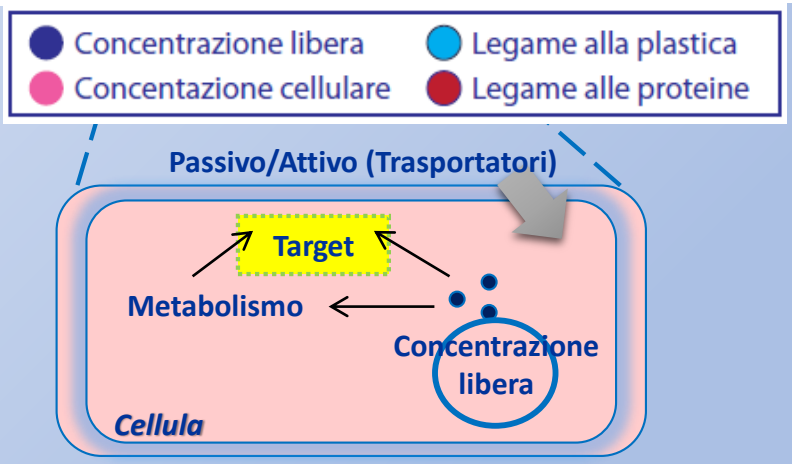
The predicted exposure level can then be compared with the actual or estimated human exposures to estimate potential health risks

One of the key barriers for the acceptance of in vitro toxicity testing data ? Failure to relate the nominal concentration to a relevant in vivo exposure level.

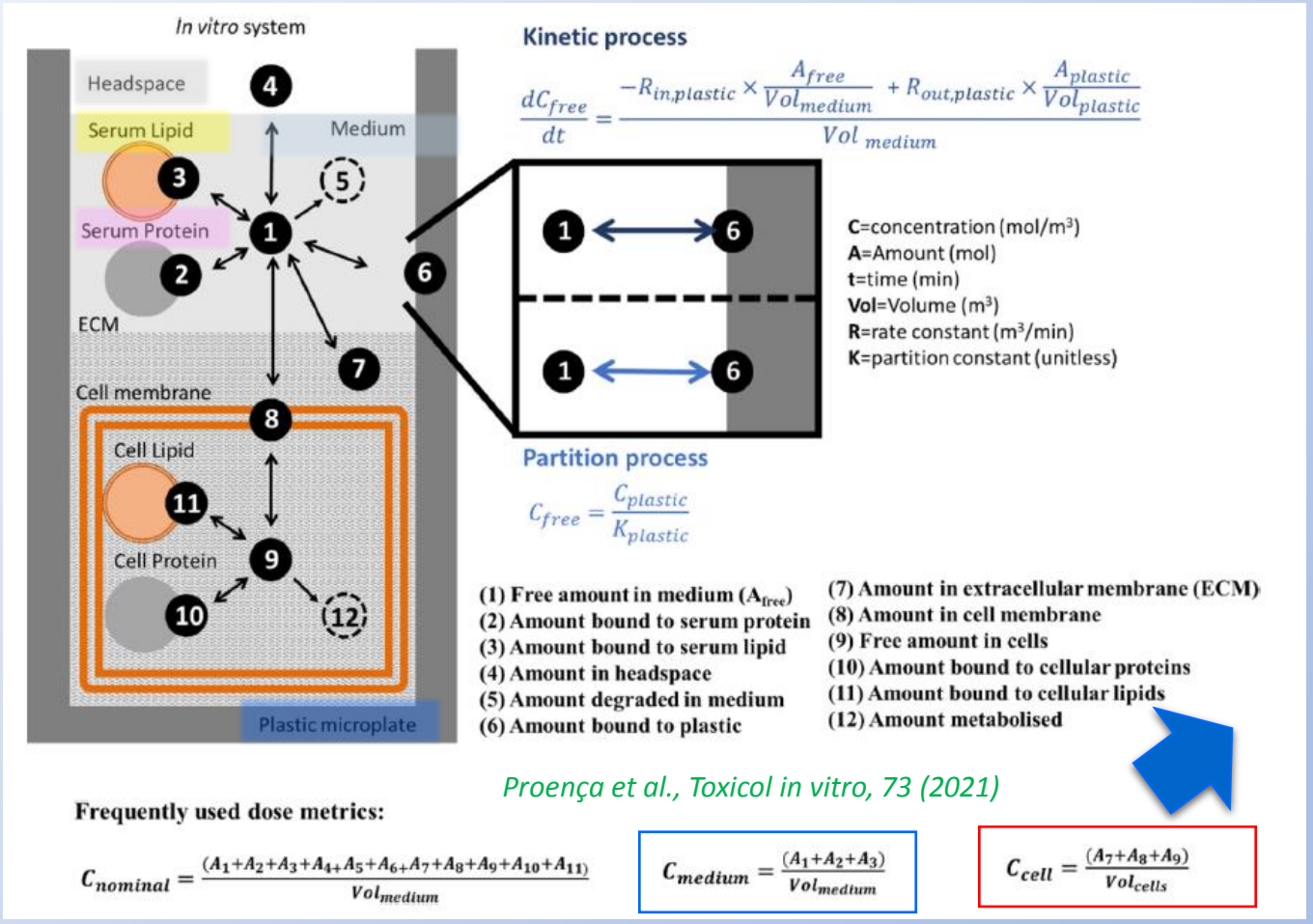
In vitro system complexity: Biokinetics



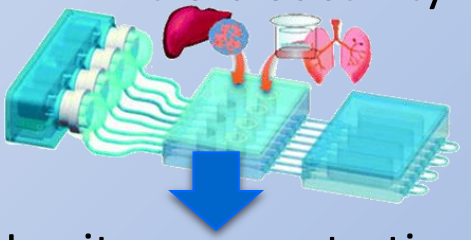




Biokinetic factors influencing the outcome of the study

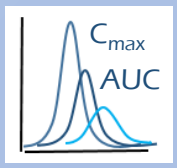


In vitro bioactivity

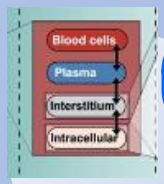


In vitro concentration
+
kinetic factors

In vivo
plasma or
tissue
concentration



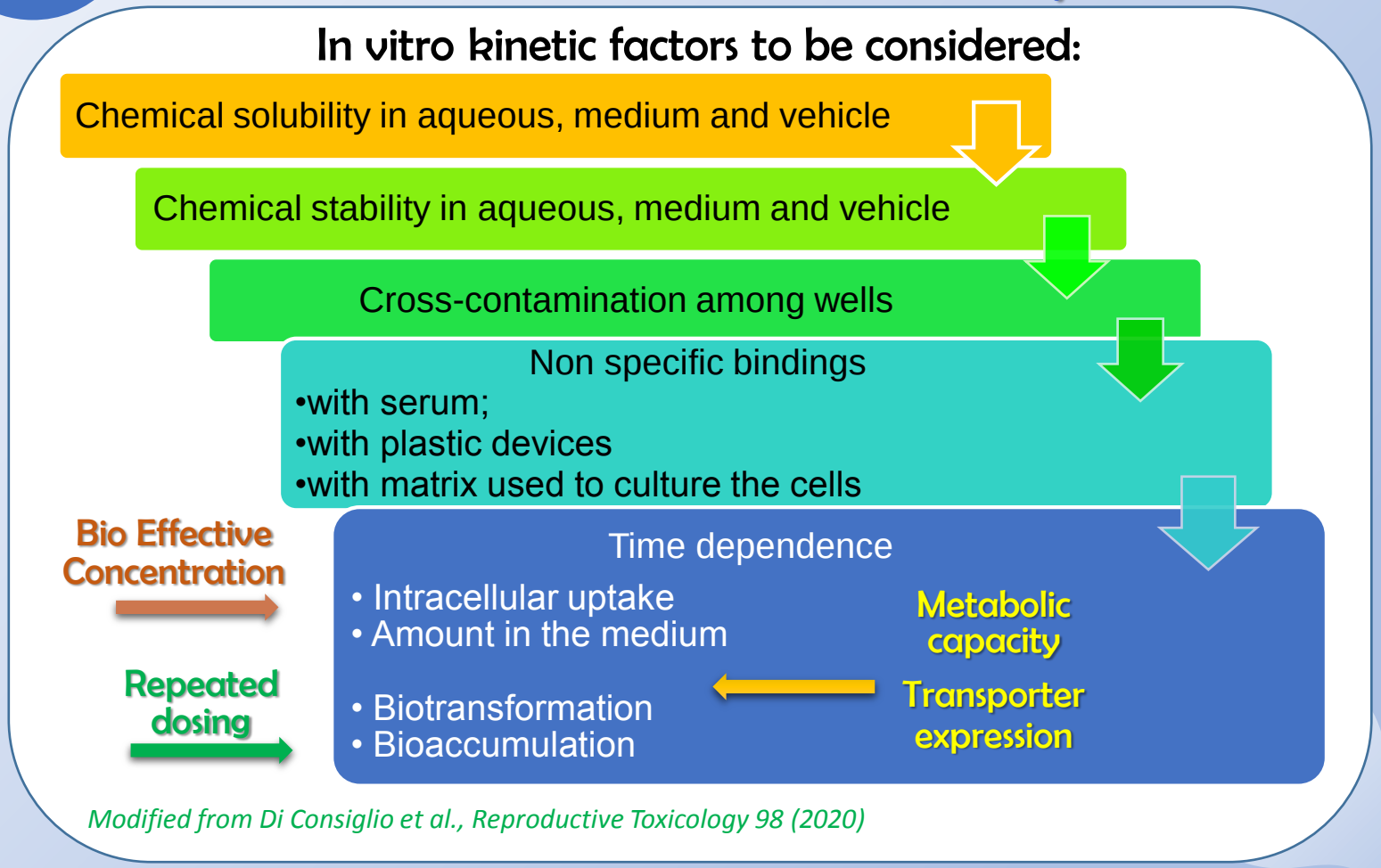
PBK



External
dose

In vitro biokinetics:

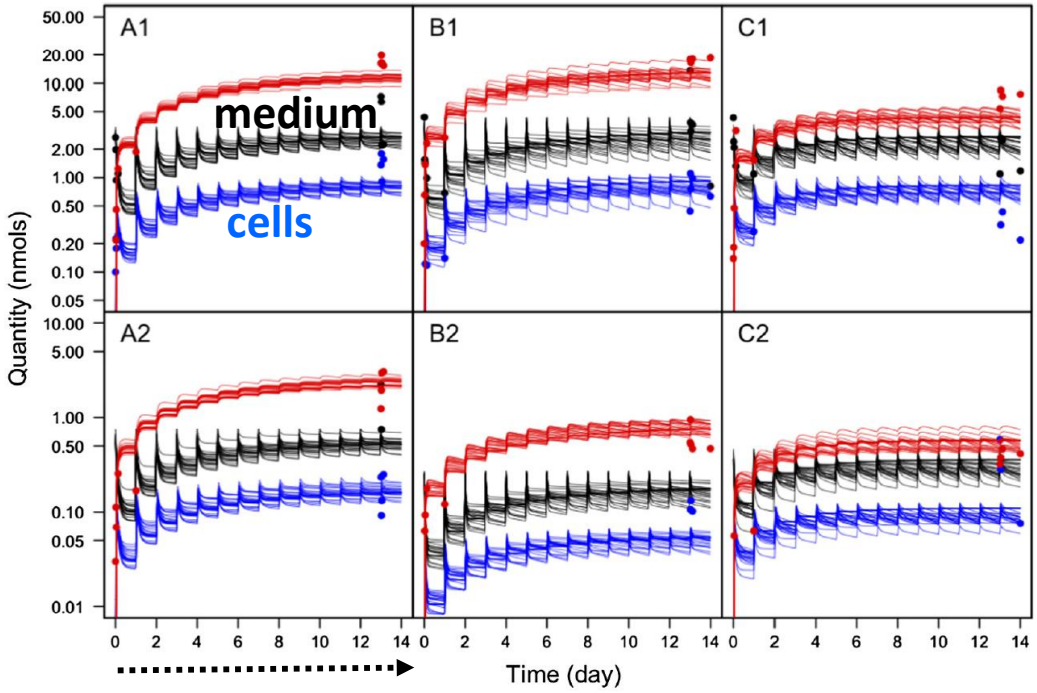
Step-wise procedure for determination of chemical cellular bioavailability.



Modified from Di Consiglio et al., Reproductive Toxicology 98 (2020)

Biokinetics "FEASIBLE" after Repeated dosing

Lipophilic substance: (log $P_{O/W}$ 7.57); high protein binding (96–99%); extensive tissue distribution

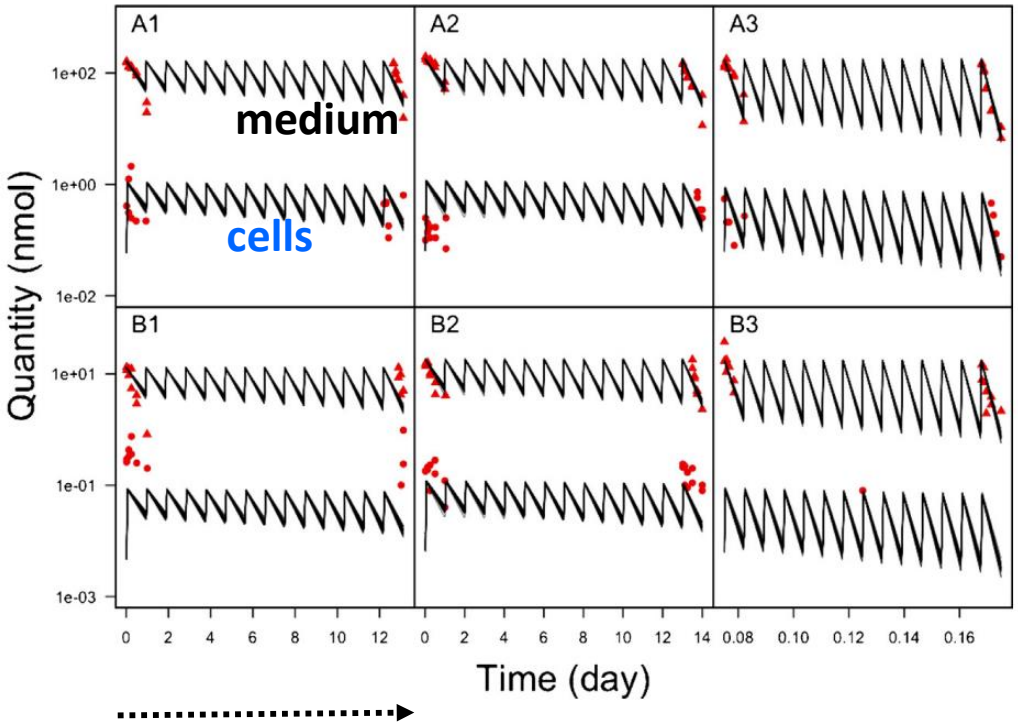


Repeated dosing

Pomponio et al., Toxicology in Vitro 30 (2015) 36–51
Algharably, et al., Archives of Toxicology, 93, (2019) 615-621.

Biokinetics "FEASIBLE" after Repeated dosing

Hydrophilic substance:
log $P_{O/W}$ = 2.23;
a nonprotein-bound fraction of 0.01



Repeated dosing

Truisi et al., Toxicology Letters 233 (2015) 172–186
Mielke, et al. Arch Toxicol, 91 (2017), 1663-1670.

Protein binding: protein content in the media → key for the dose/concentration causing a toxic effect

QIVIVE : Quantitative In vitro to in vivo extrapolation Case-study 1

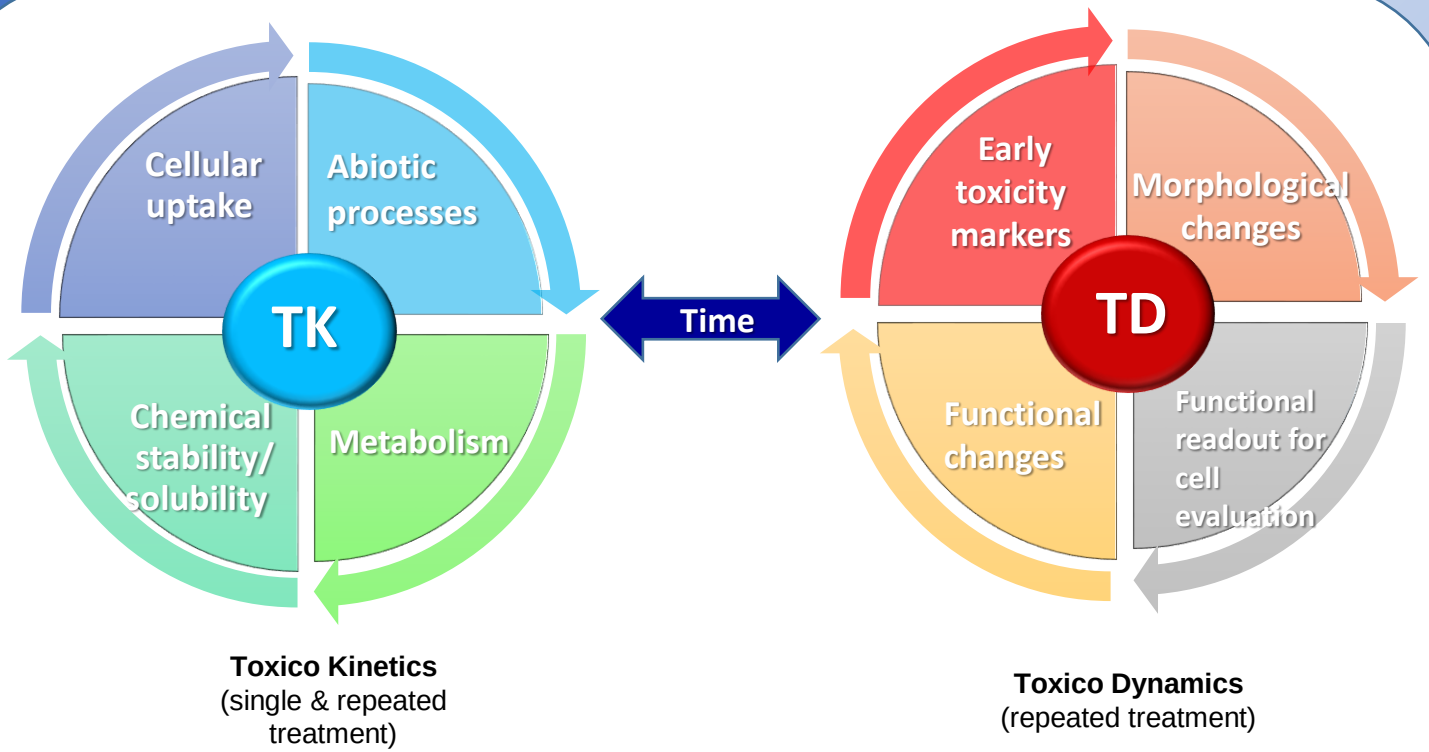
- Modelling the in vivo doses starting from the in vitro actual measured concentrations in the medium and cells;
- Conditions used in the in vitro study (protein-free in vitro culture medium) : Set the protein binding at 0% for the modelling;
- ≠ conditions → in vitro: the culture medium could not bind the substance, in vivo the substance may have high affinity for plasma proteins;
- Higher in vitro free fraction than in the blood in vivo → ≠ organ/blood partition coefficient.

Protein binding →

In vitro conditions must be considered and compared to the in vivo situation, particularly, for protein binding.

Algharably, Di Consiglio et al., (2022, in press)
In Vitro–In Vivo Extrapolation by PBPK modeling: Experience With Three Case Studies and Lessons Learned. Front.Toxicol. 4:885843.doi: 10.3389/ftox.2022.88584

Kinetics *vs* Dynamics



Modified from Di Consiglio et al., Reproductive Toxicology 98 (2020)

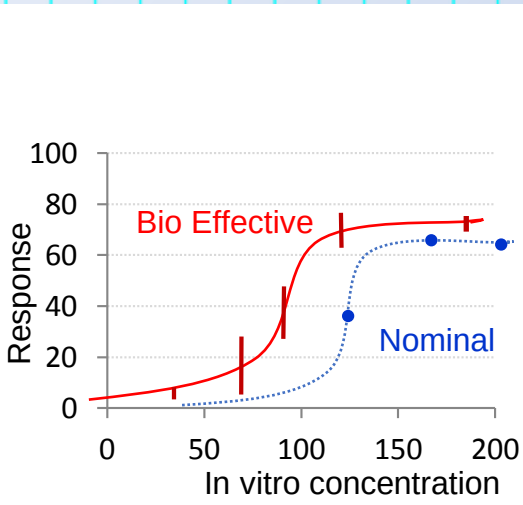
Nominal vs actual concentrations

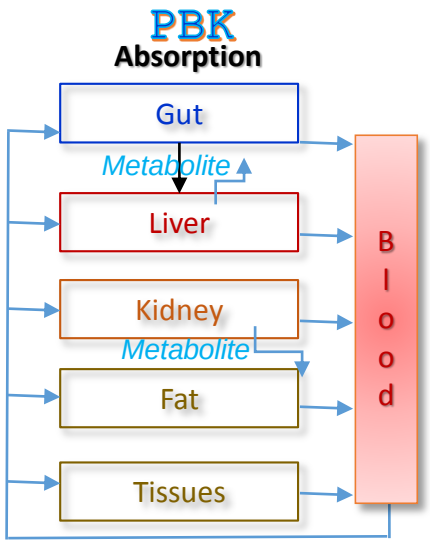
QIVIVE for the relevant adverse effects Case-study 2

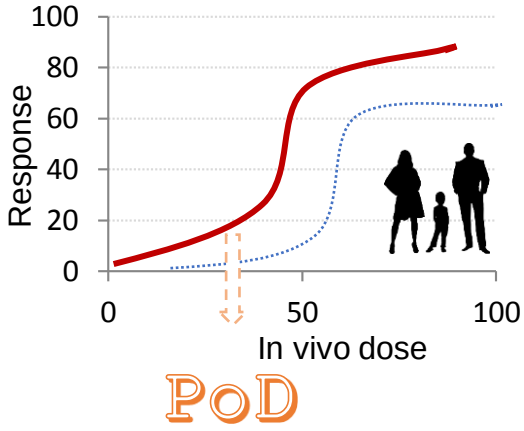
In vitro measured intracellular concentration→

Bio Effective Concentration ⟶ Tissue/organ concentration

Appropriate QIVIVE







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In Vitro–In Vivo Extrapolation by PBPK modeling: Experience With Three Case Studies and Lessons Learned. Front.Toxicol. 4:885843.doi: 10.3389/ftox.2022.88584

Nominal vs actual concentrations

QIVIVE for the relevant adverse effects Case-study 2

In vitro measured intracellular concentration→

Bio Effective Concentration ⟶ Tissue/organ concentration

Appropriate QIVIVE

PBK

Absorption

Gut

100

QIVIVE dose (mg)		Doses in patients with neurological side effects (mg)
BMDU based on measured in vitro intracellular $AUC_{0\text{-}24}$	593	400–500
BMDU based on nominal concentration	10833.3	

0 50 100 150 200

In vitro concentra

In vivo dose

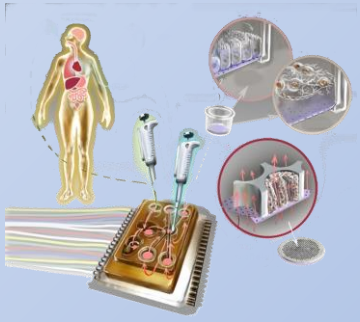
Underestimation of potency

PoD

IMP. to estimate the actual PoD:
Phys-Chem properties; kinetic parameters
e.g. protein binding; metabolism

Algharably, Di Consiglio et al., (2022, in press)
In Vitro–In Vivo Extrapolation by PBPK modeling: Experience With Three Case Studies and Lessons Learned. Front.Toxicol. 4:885843.doi: 10.3389/ftox.2022.88584

Predictive models: Improving cell models



Complex cell models: e.g. *human* induced pluripotent stem cells (hiPSCs) and their differentiated derivatives, cultured in *3D* to improve the level of physiological complexity; *organoids* in *microfluidic devices*; engineered tissues or *multiorgan systems*

In vitro assays should be associated to **HUMAN relevant** key events, in **HUMAN relevant** cellular models

Metabolic competence and transporter expression of the cell models → relevance to the human in vivo situation

Metabolic capacity

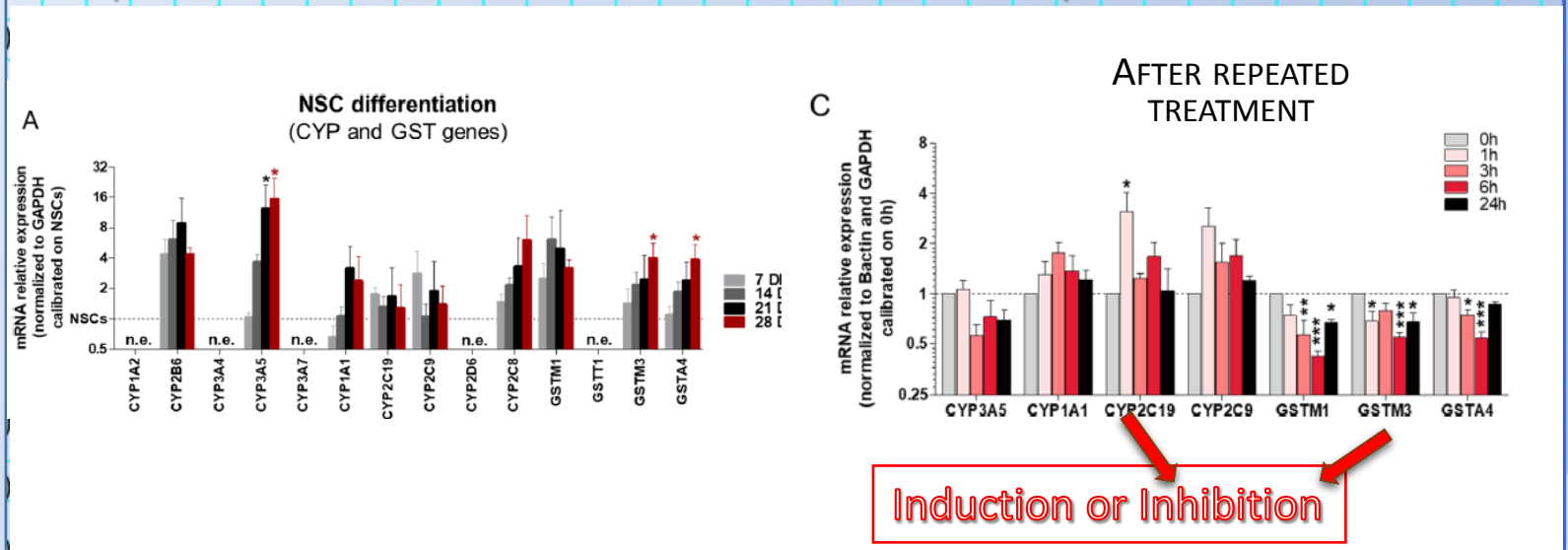
Transporter expression

Importance to consider the BIOTRANSFORMATION and TRANSPORT

Biotransformation and transport capacity of the test system used.

Case-study 3

- Are the elements of concern expressed?
- Is it possible to predict systemic exposure to both the parent molecule and relevant metabolites for a correct NGRA?
- Are the right test substances (i.e. and metabolite) tested in vitro? Is it possible to calculate in vitro clearance to be extrapolated to the in vivo



IMP. to focus the risk assessment on both the parental substance and its "MAJOR" metabolite(s)→ clearance calculation + time component

Di Consiglio et al., Reproductive Toxicology 98 (2020); Di Consiglio, Pistollato; Pino et al. (in preparation)

Importance to consider the BIOTRANSFORMATION and TRANSPORT

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The case of systemic exposure to phenoxyethanol and its major metabolite phenoxyacetic acid in body lotions

Di Consiglio et al., Reproductive Toxicology 98 (2020); Di Consiglio, Pistollato; Pino et al. (in preparation)

Importance to consider the BIOTRANSFORMATION and TRANSPORT

Biotransformation and transport capacity of the test system used.

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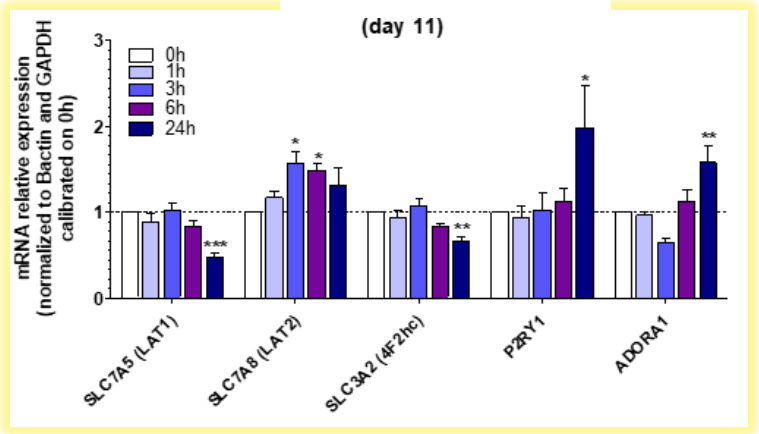
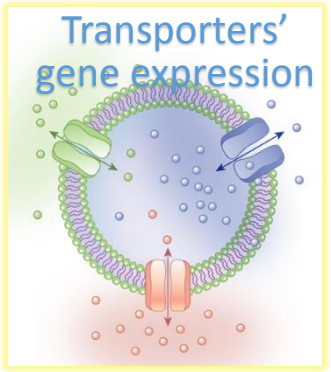
AFTER REPEATED TREATMENT





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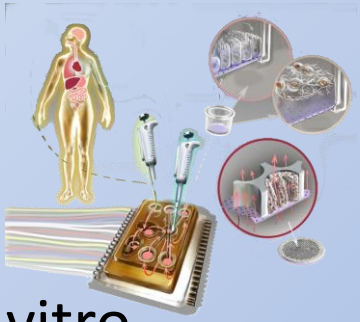
Predictive models: Improving cell models



Organ-on-Chip (OoC): among new non-animal in vitro technologies → *considerable interest* within the scientific community:

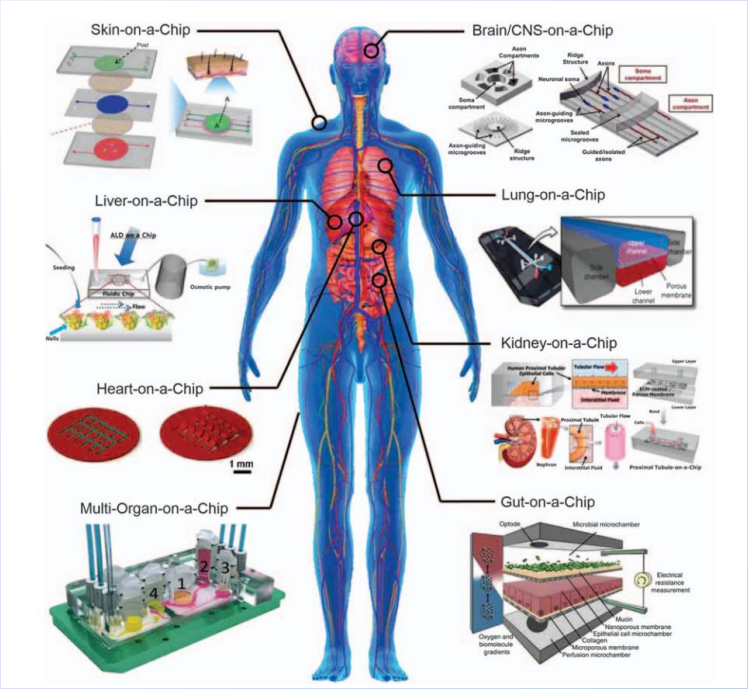
They recreate *body physiology capacities* to transform science, in the field of biomedical research, drug development, consumers safety and personalised medicine.

Full qualification process→ necessary to demonstrate the relevance, promoting implementation both by end users and regulators.

Qualification **for contexts instead of a full validation: in order to support decision-making in regulatory setting.**

Organ-on-chip (OoC)

ongoing /



Microfluidic microphysiological systems (MPS)

- **Context of use**: circumstances under which the MPS is applicable + limitations (e.g. applicability of the test method for a particular class of compounds);
- **Stepwise approach** (start simple…) taking into account both safety and efficacy testing;
- **Reliability**; reproducibly over time;
- **Relevance**, how the test method correctly measures or predicts the biological effect;
- **Qualification** implies the availability of defined test methodology including aspects related to in vitro to in vivo extrapolation (**QIVIVE**),e.g. **biokinetics**;
- **Standardization**: to allow the regulatory acceptance of OoC.

EURL ECVAM

Analysed the state of the art of standards in OoC, describing the technical and biological aspects of OoC, focusing on standardisation needs and opportunities.

Preliminary
Assessment
REgulatory
RElevance

Piergiovanni et al., Lab Chip, 2021

Standardization

Universal approach to validation or harmonization : probably unrealistic (too long, might not be possible) → application of OoC devices in the short/medium term primarily *target specific* and *well-defined contexts of use*.

Standardization by verifying:

- ✓ *reproducibility* and *robustness* of results intra- and inter-lab;
- ✓ *consistency* or *compatibility* of cell/tissue types and sources;
- ✓ *producer compatibility* among chips or modules also in interconnection into a multi-organ system.

Standardization will aid in the concrete incorporation of OoC-based studies into regulatory workflows and decision-making contexts: Ex. EURL ECVAM, liver and brain 3D models for DNT applications →reliability and relevance of OoC for its intended purpose

Regulatory requirements in NGRA (NAMs+Biokinetics)

*Take home message

- For scientific acceptance agreed standards needed for using tools, recording methods or protocols, data analysis and reporting → transparency & consistency;

- Formal validation process not possible?
  Application of new approaches in an accepted fit-for-purpose context.

- **General challenges of in vitro systems & study design:**

a. Key biological and fisiological functions of organ to be represented

b. Stability of the model over time

c. Does the in vitro system have the adequate metabolic/transporter capability?

d. What is/are the relevant substance(s) (parental or metabolites) and at which internal exposure conditions?

e.How does the chemical behave in the in vitro system with respect to protein, lipid and plastic binding and evaporation?

Regulatory requirements in NGRA (NAMs+Biokinetics)

*Take home message

- QIVIVE:

a. What are the internal dose of a chemical that are reached in a certain exposure scenario ?

b. How do these doses relate to in vitro bioeffective concentrations ?

c. Extrapolation of an in vitro biological effect concentration (e.g., BMC10) to an equivalent oral/skin/inhalation dose.

Thank You For Your Attention!

emma.diconsiglio@iss.it

National Representative

Network PARERE Italiano

Special thanks to all the colleagues, who took part to the cited papers and made this dissertation possible.

These slides have been prepared by the expert, on the basis of personal knowledge and expertise, and therefore cannot be considered as an official ISS opinion.