Biocinetica ed estrapolazione in vitro-in vivo della dose

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Current safety testing methods

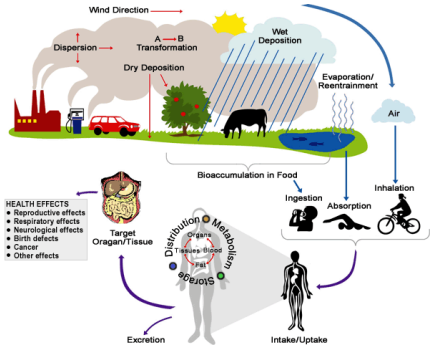
In Vitro

In Vivo

Toxicological profile

vs

Exposure



Risk assessment

The present RA paradigm generally focuses on hazard identification and characterisation as first steps.

There is a demand for changing the basis of RA, giving more focus on

1) modes of action (mechanistic approach)
2) a progressive reduction of tests using laboratory animals
3) exposure driven process

Towards the Tox21 and the EU SC document on New challenges for RA (2013)

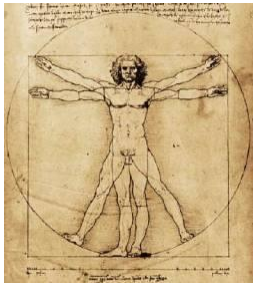
In vitro studies in RA

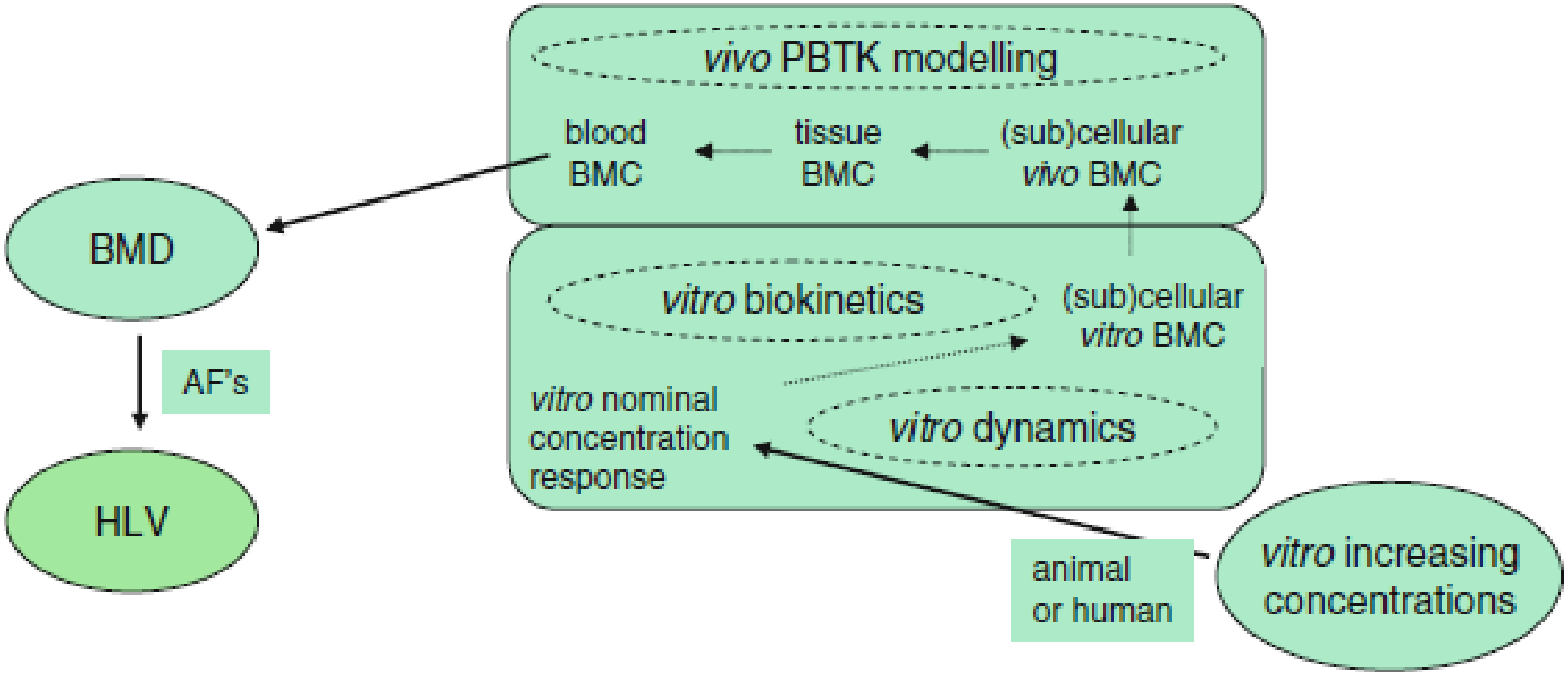
Limited use for risk assessment purposes ⟹ difficulties in carrying out **quantitative *in vitro* to *in vivo* extrapolation**

(QIVIVE) ⟹ translate in vitro effect concentration into human toxicologically equivalent dose

✓Need of translating information from the cell level, to organs and subsequently to organisms and to **distinguish between adaption vs. adversity**, likely identifying **actual *in vitro* markers of adversity** (Blaauboer et al, 2012) or **Key Events in AOPs**

- ✓ Integrated approach: in silico and in vitro **IATA**
- ✓ Lack of information on actual cell exposure ⟹ ***in vitro* biokinetics**
- ✓ Battery KE (TD) + kinetics ⟹ **PBTK models**
- ✓ Integration of human variability in PBTK models ⟹ isoform specific metabolism and or transporters activity





BMC = benchmark concentration
BMD = benchmark dose (external dose)
HLV = human limit value (external dose)

Figure from Adler et al., *Arch Toxicol*, 2011

Kinetics is finally considered the crucial body of information for the design and performance of 'traditional' in vivo toxicological tests, toxicity data interpretation, identification of internal dose…….

Why not to include kinetics in alternative/non animal testing strategy ?

Biokinetics processes have been evoked to explain the in vitro/in vivo differences, but…

***…in vitro* the nominal applied concentration rather than the actual level of cell exposure is usually associated to the observed effects.**

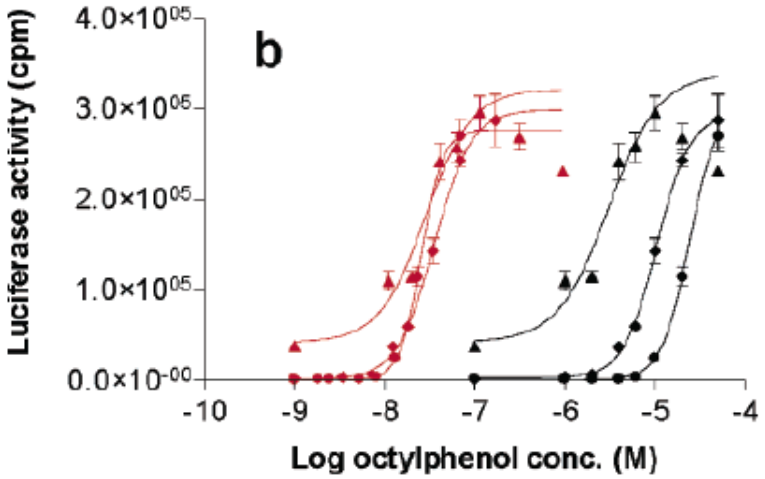
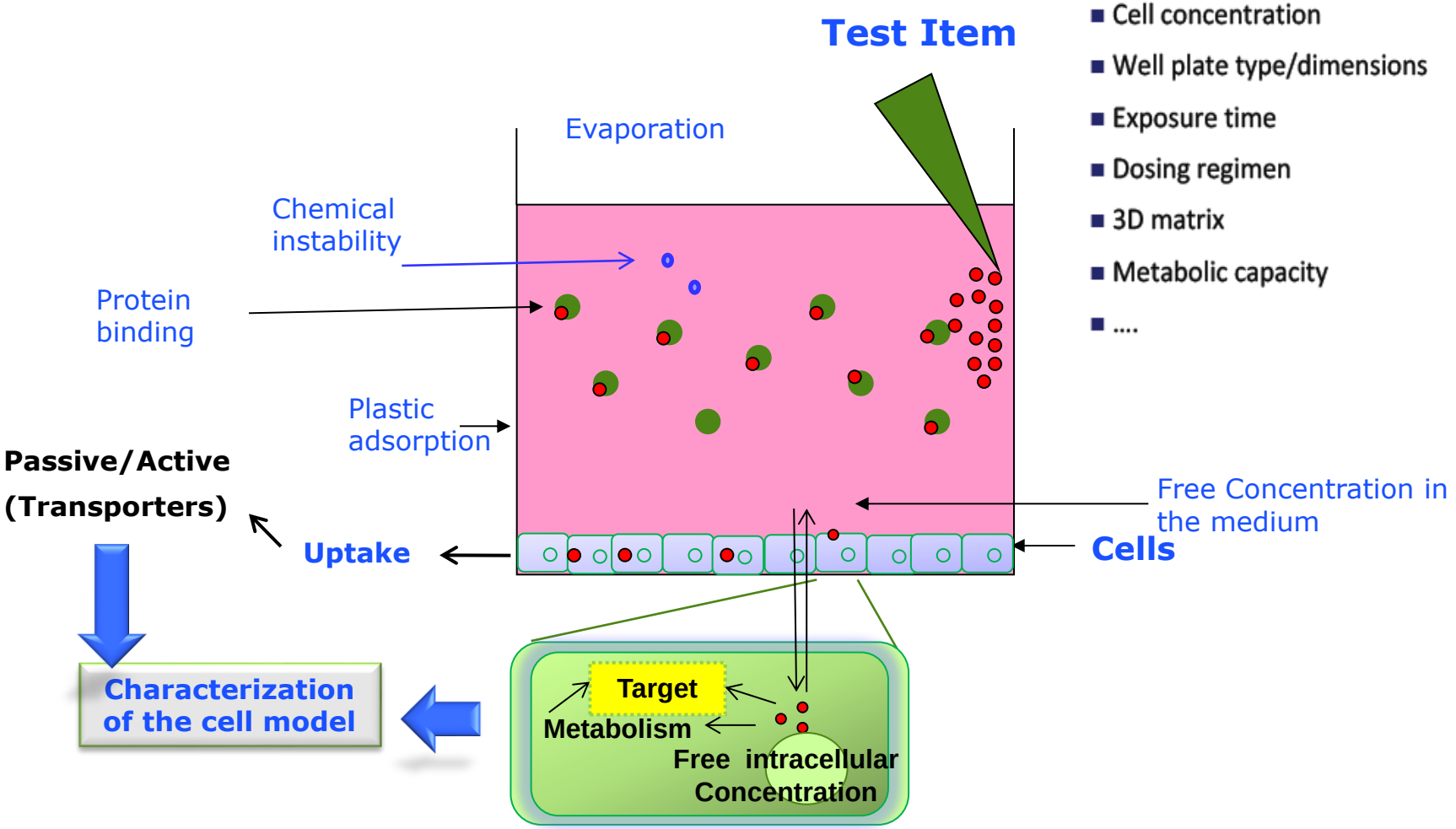


Figure from Heringa et al., *ES&T*, 2004

In vitro biokinetics

Differences between *in vitro* assays:

- Medium composition (e.g. serum)
- Cell concentration
- Well plate type/dimensions
- Exposure time
- Dosing regimen
- 3D matrix
- Metabolic capacity
- ....



Proposed strategy

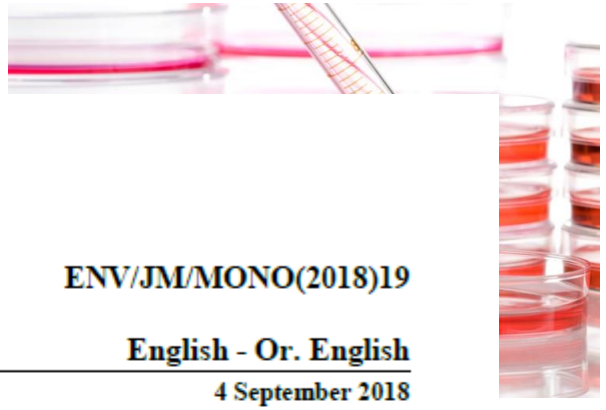
Identification of in vitro relevant exposure parameters and elaboration of a tiered strategy to measure/estimate them

The proposed critical parameters to be measured are:

- Solubility of the compound in the medium (actual testing conditions)
- Stability of the compound over time.
- Adsorption to physical component(s) (plastic and attachment matrices)
- Volatility and cross contamination among wells
- Binding to medium proteins Free *vs* bound concentration over time
- Interaction with cell component(s)
- Metabolic stability

Kramer et al, TIV, 2015

GUIDANCE DOCUMENT ON GOOD *IN VITRO* METHOD PRACTICES (GIVIMP) FOR T IMPLE METH HUMA



Organisation for Economic Co-operation and Development

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4 September 2018

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

Cancels & replaces the same document of 6 August 2018

Guidance Document on Good In Vitro Method Practices (GIVIMP)

Series on Testing and Assessment No. 286

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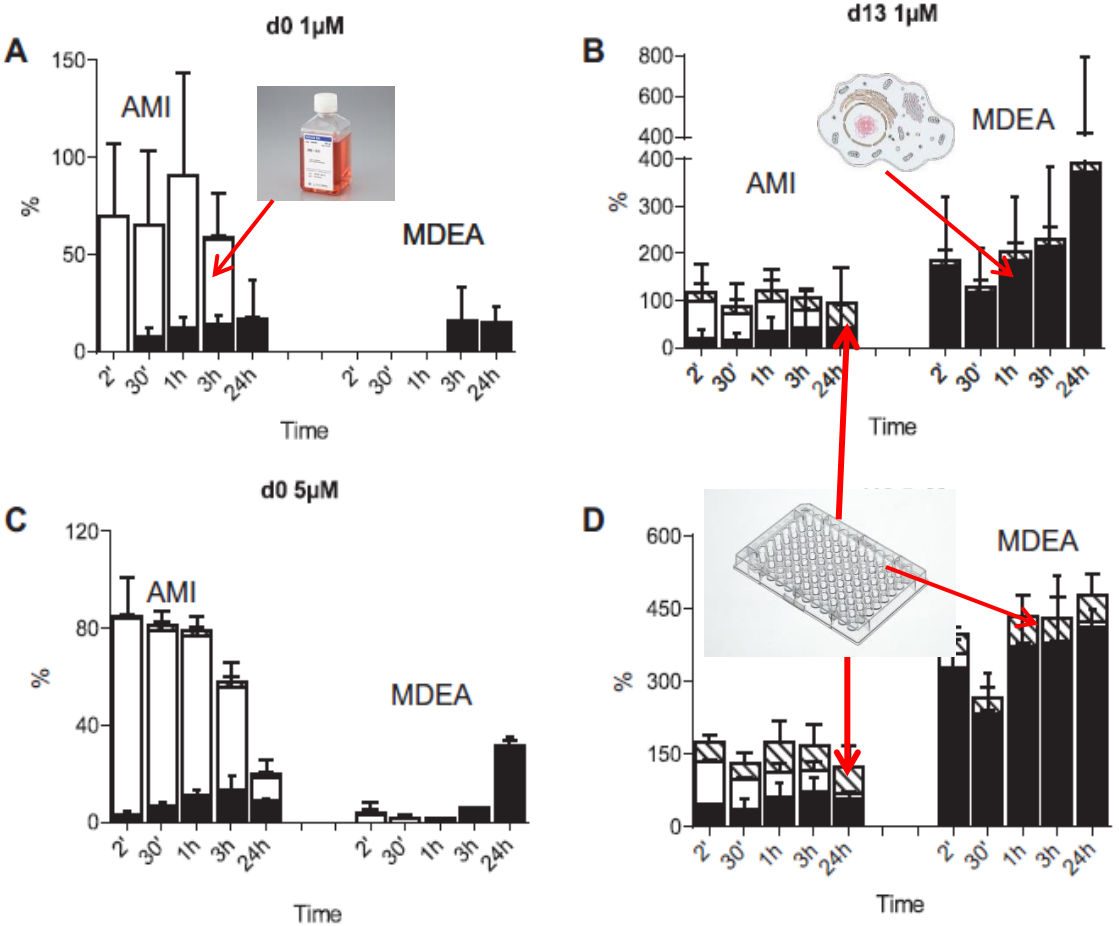
Adsorption to plastics and attachment to matrices

Dependent on:

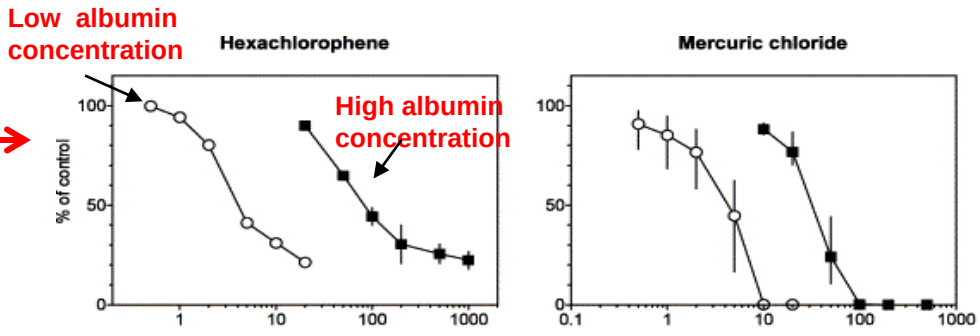
- ✓ Lipophilicity : $LogD_{7.4}$>2.5 (e.g. amiodarone, CsA, Chlorpromazine) up to 70% plastic bound. Negligible binding for Ibuprofen, cisplatin, adefovir
- ✓ Time : increase with time of treatment
- ✓ Dose: increase with dose up to a plateau
- ✓ Serum competes with plastics
- ✓ Possibility of sequestration by Collagen; lower by Gelltrex



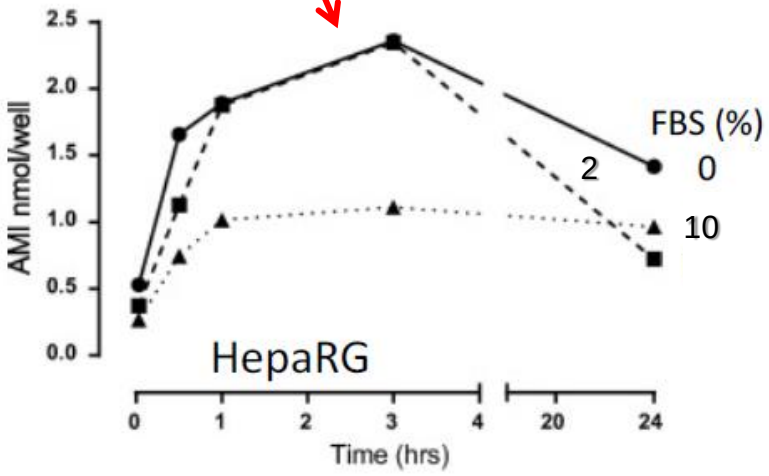
Binding to protein in the medium

Cytotoxicity depends on protein binding in the medium

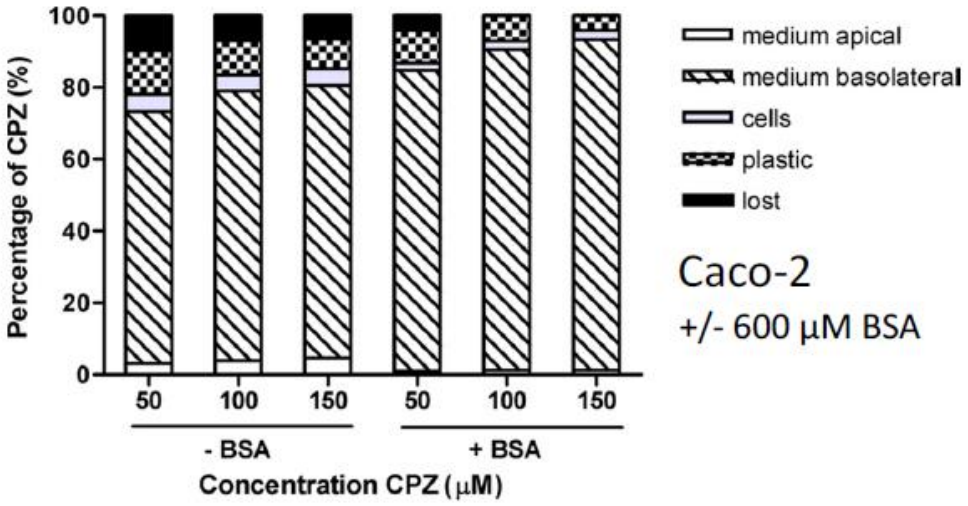
Cell uptake is reduced by protein binding in the medium



Gülden M. et al. Toxicol. Letters 2003, 137, 159-168.

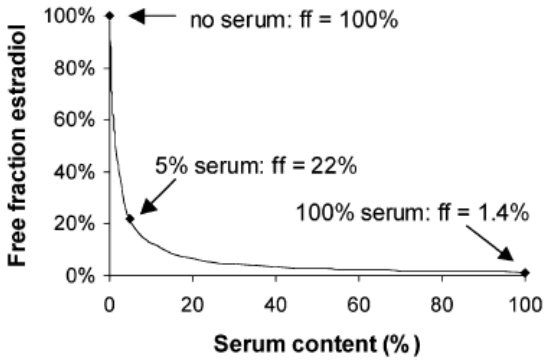


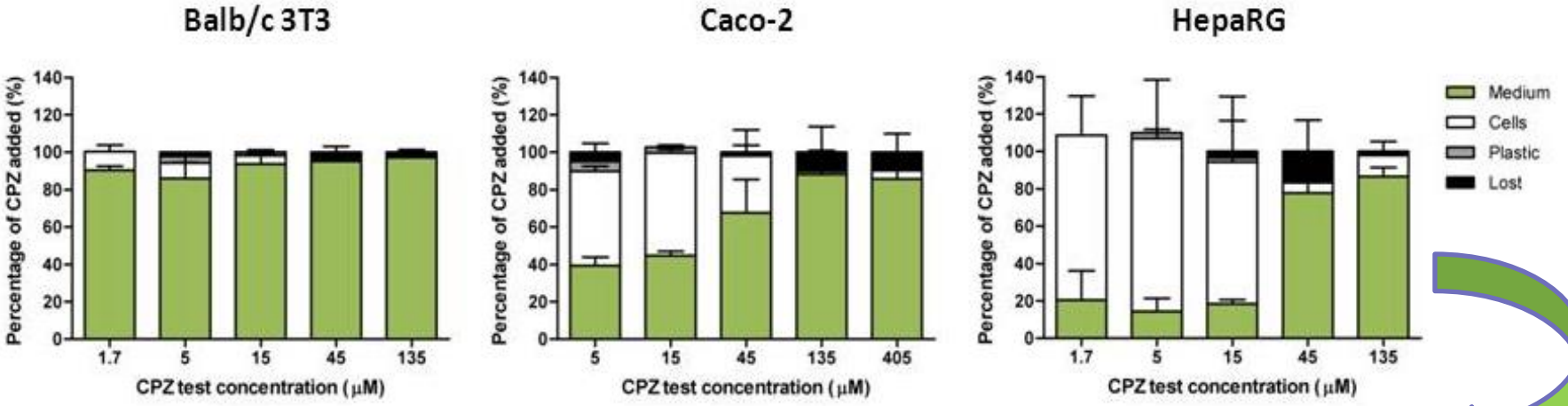
Pomponio et al. (2015) Toxicol. In Vitro 30: 36



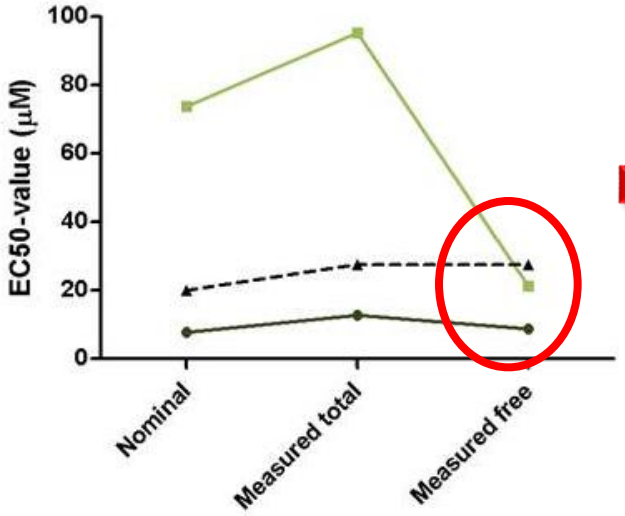
Broeders et al. (2012) Chem. Res. Tox. 25: 1442

Measuring free concentration





Cell death→ CPZ in the medium



Sensitivity ranking of the 3 cell systems to Chlorpromazine is dependent on the dose metric used

Broeders et al, *Toxicol In Vitro*. 2013

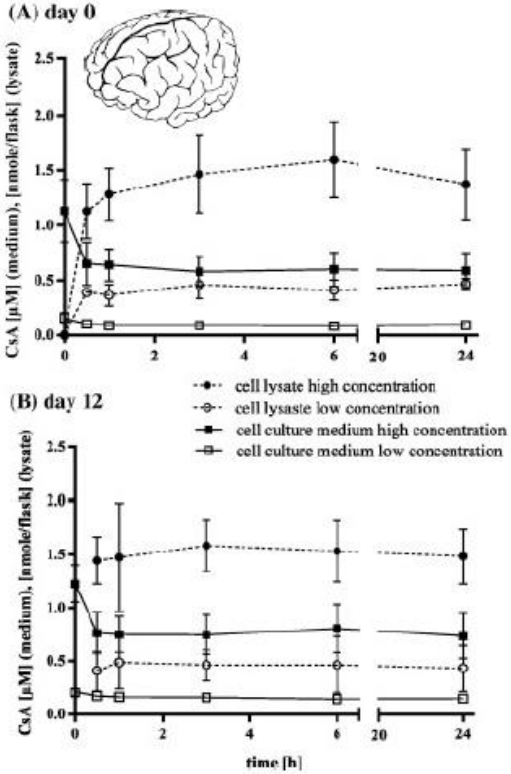
Accumulation in cells

It depends on:

- ✓ the cell type
- ✓ Dose
- ✓ time

→ **Transporter activity** and **Metabolic competence**

CsA



Low intracellular CsA uptake, steady state reached within 30 min. maintained for 14 days

no biotransformation or bioaccumulation observed

Cell membranes absorption dominated by lipophilicity→ CsA not fully available for target

Chemical equilibrium between aqueous (cell culture medium) and organic compartments (cell membrane, myelin and plastic).

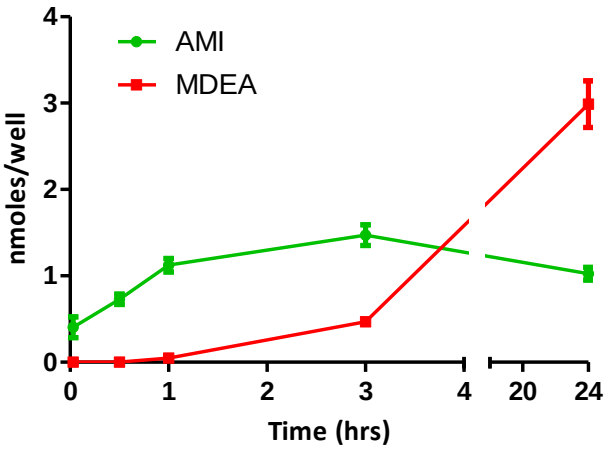
Bellwon et al, TIV, 2015a
Bellwon et al, TIV, 2015b
Wilmes A., et al. Journal of Proteomics , 2013

Accumulation in cells : parent vs metabolite

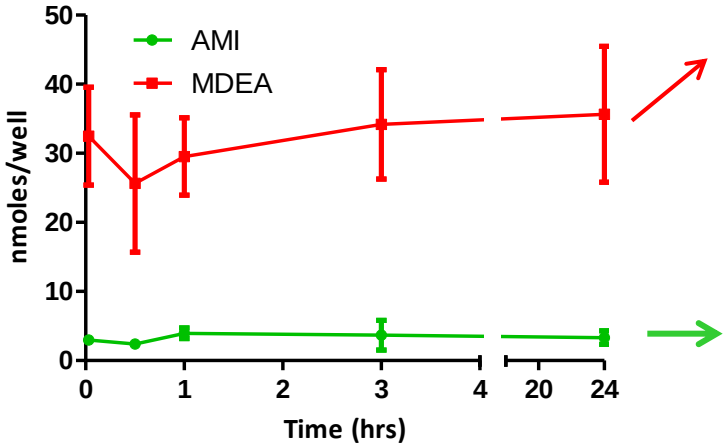
HepaRG

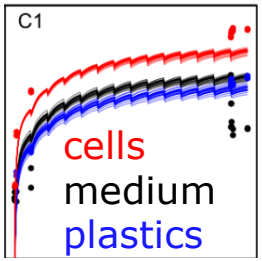
Amiodarone LD-HD: 1-5 µM

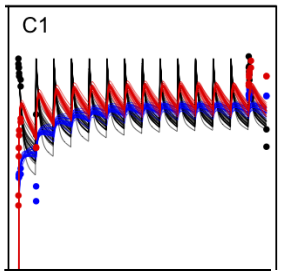
D0/HD



D13/HD





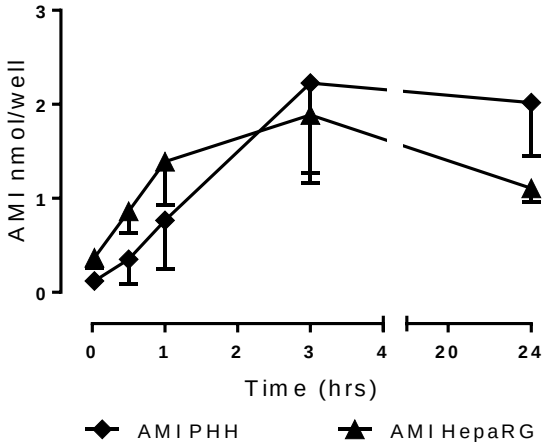


MB (%)		
Tempo	**D0**	**D13 (~90%MDEA)**
2 min	80,028	605,46
30 min	76,187	432,60
1 h	77,603	618,90
3 h	54,785	624,86
24 h	52,477	640,50

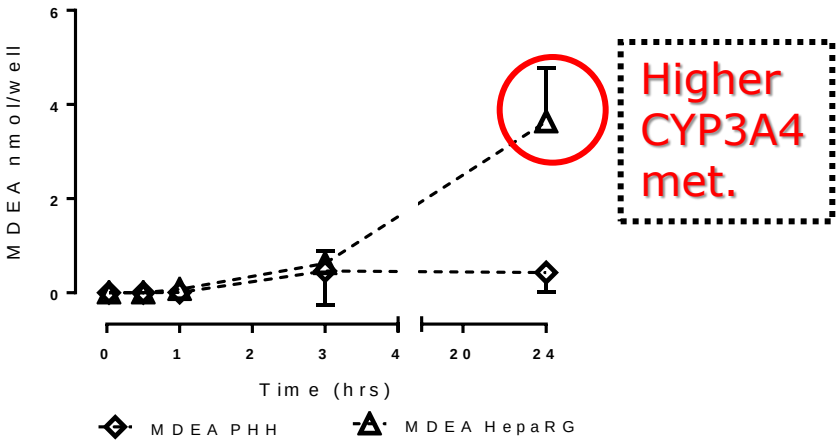
D13 HD → bioaccumulation of the toxic metabolite

Pomponio et al, TIV 2015a

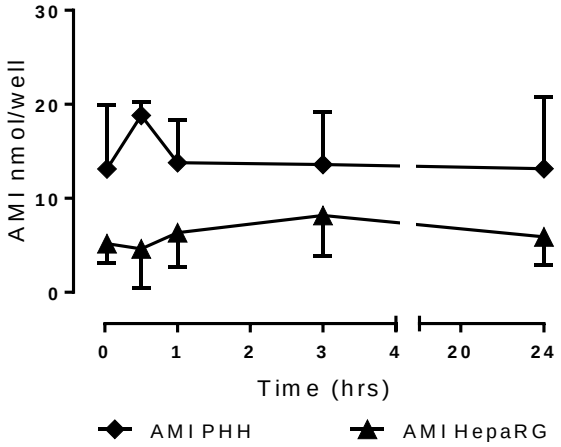
A comparison between HepaRG and PHH



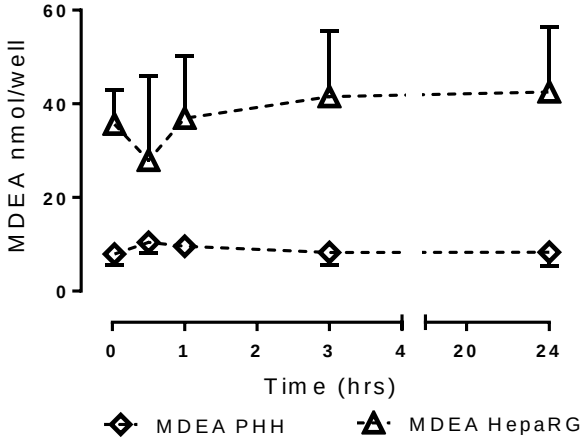
d0



Higher CYP3A4 met.

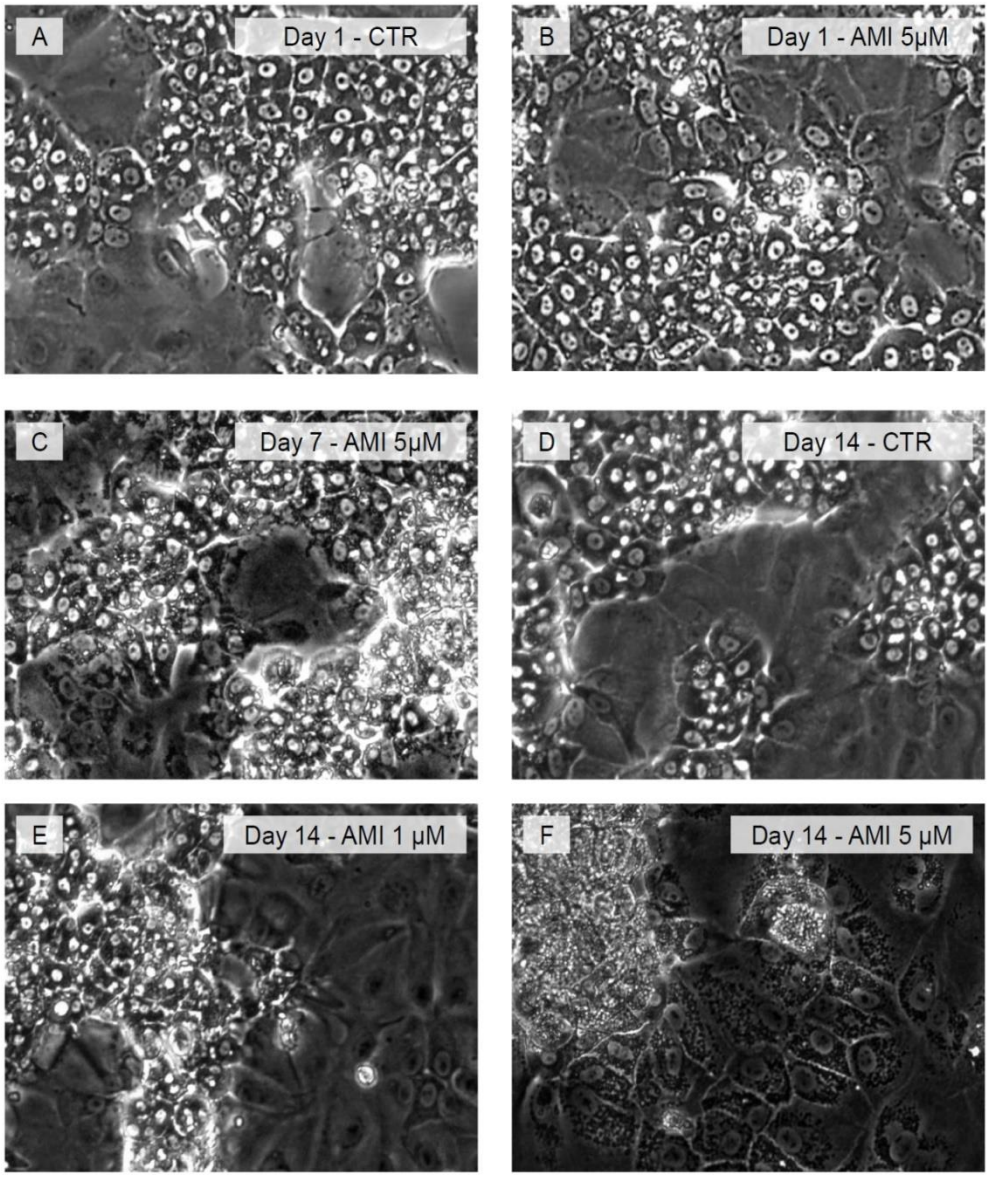


d13



In **HepaRG CYP3A4** activity in control cultures after **1, 3 and 14 days was 18, 20 and 30 pmoles/min/mg protein**, respectively. **Lower in selected PHH**.

Amiodarone-induced phosholipidosis



Accumulation of intracyto-plasmic clear vesicles (after 2–3 d) in HepaRG hepatocytes treated with 5 μM AMI (actual conc. 6 μM).

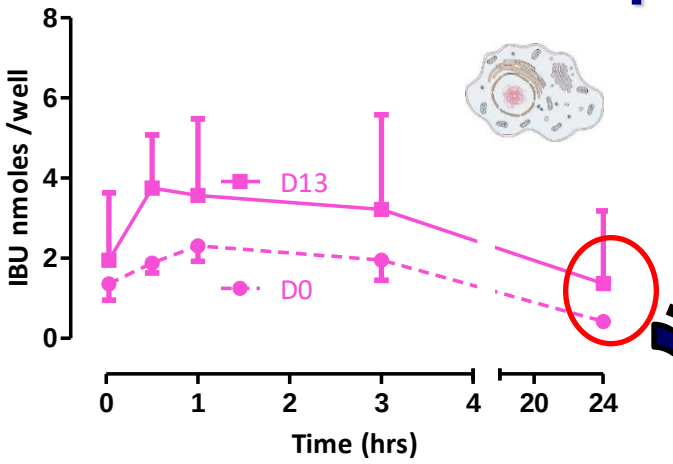
Vesicles contained lamellar bodies, a hallmark of phospholipidosis (Anthérieu et al., 2011).

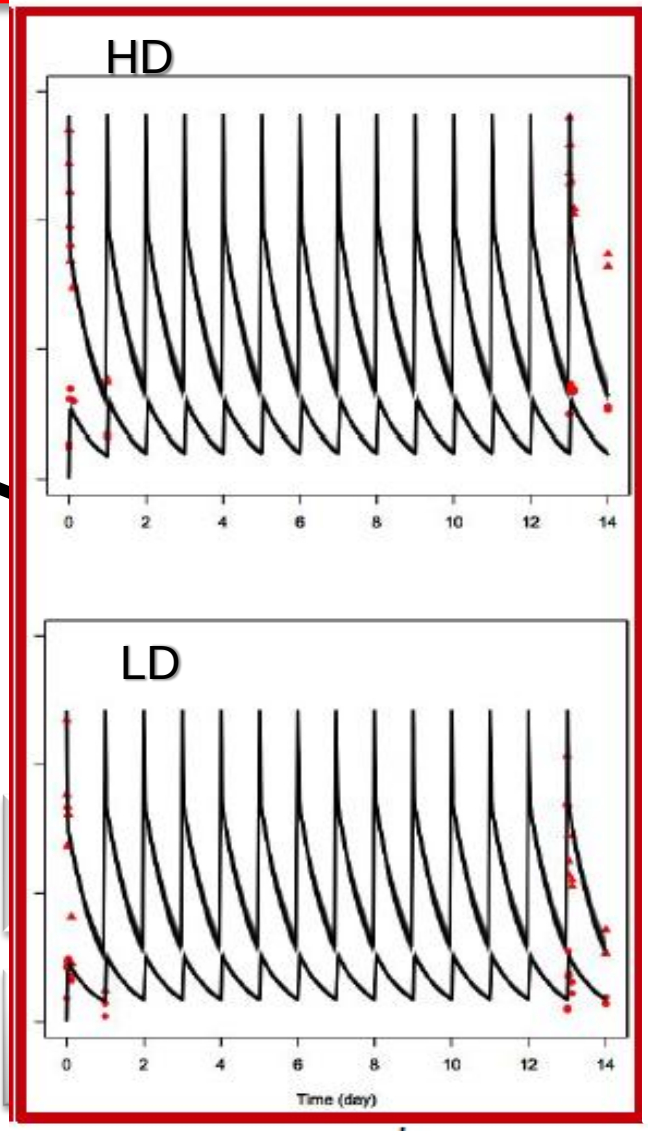
The most plausible explanation for intracellular AMI and MDEA accumulation was their ability to bind to phospholipids inducing phospholipidosis.

At similar cell exposure no similar effect seen in PHH: lower metabolism

Ibuprofen: a hydrophilic highly metabolised drug

PRH: 10 and 100 µM

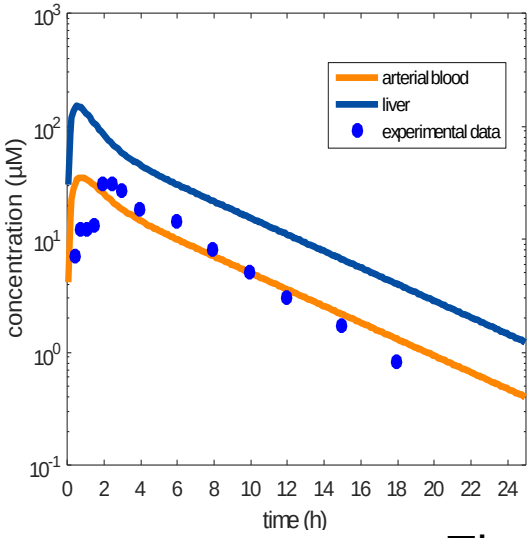




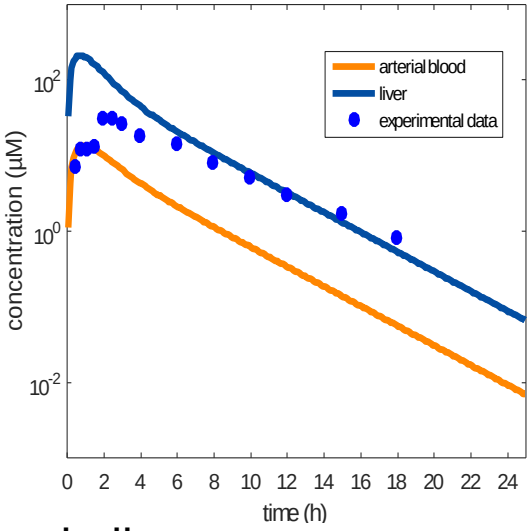
Truisi et al, Tox Lett, 2015

From in vitro to in vivo : modelling data

We used a PBPK human model to simulating the concentration time profile of Ibuprofen in blood and tissues of an adult male (data from an elderly volunteer after oral intake of a single dose of 600 mg ibuprofen Greenblatt et al. 1984).



What is wrong?



The clearance values were similar.

Hepatic partition coefficient calculated by the algorithm of Schmitt (2008): **3.01**

hepatic partition coefficient determined by experimental *in vitro* data (cell lysate/ supernatant): **11.1** (Truisi et al. 2015)

Mielke et al. Arch Toxicol., 2018

A plausible explanation might by a difference in the protein binding and hence the fraction of unbound Ibuprofen.

Differences in protein content (specifically albumin; the binding protein for ibuprofen) *in vivo* vs *in vitro*
in vitro : lower protein content (serum-free culture medium used).

In vivo : 99% of protein binding was reported

Liver:blood partition coefficient influenced by unbound fraction (fu) :

fu = 0.01 the ratio was 3 (e.g. in vivo situation)
fu = 0.035 the ratio was 10
fu = 1 the ratio was 296.

The higher the free conc. in the blood (medium)

the higher the conc. in the tissue/cell

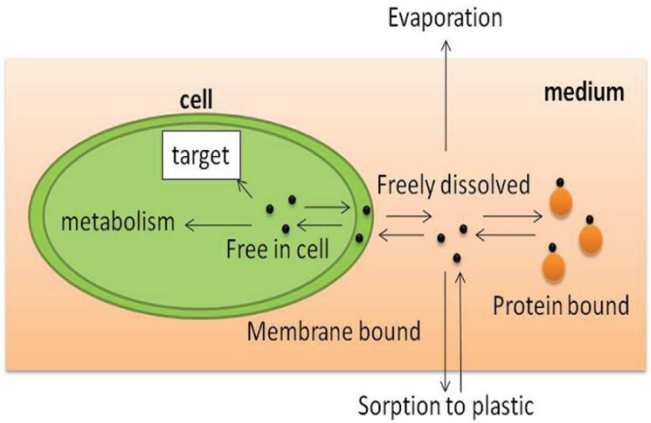
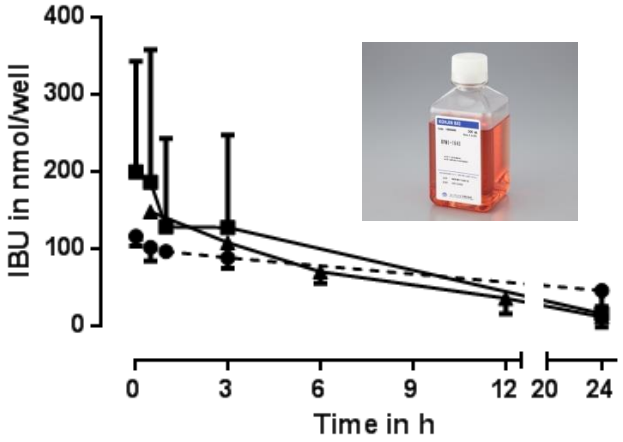


Figure from Kramer et al. *Chem Res in Toxicol*, 2012

100 μM IBU in three cell types



Following the kinetic only in the supernatant, no clear conclusions can be drawn on the kinetic profile of a substance and on specie differences.

Truisi et al, Tox Lett, 2015

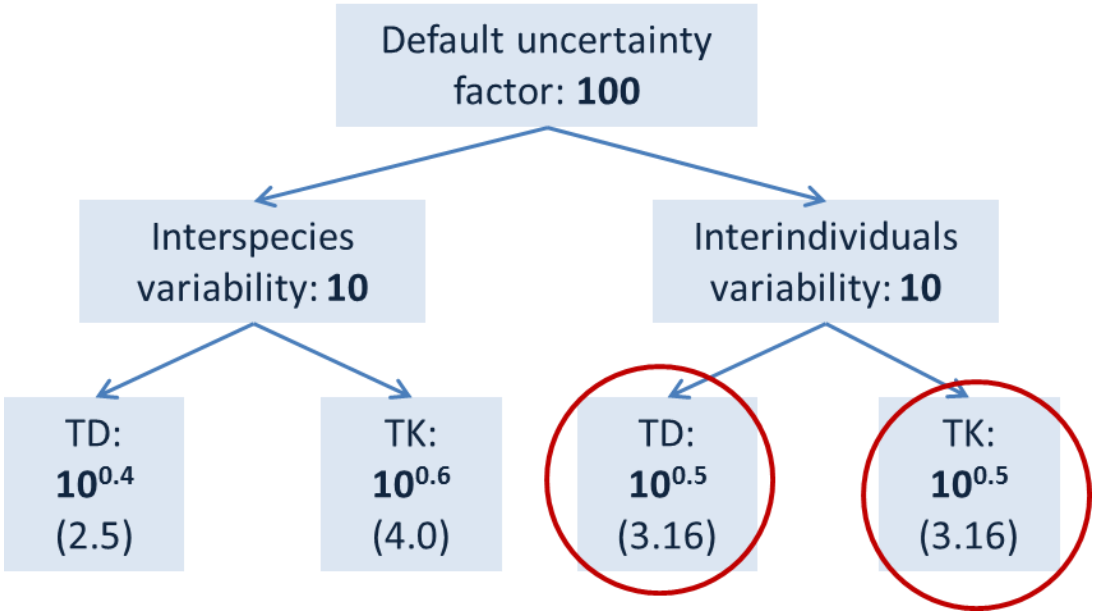
The supernatant could result not to be a perfect surrogate for blood, when highly protein binding chemicals are studied.

Advantage of serum free medium vs the need of correcting for protein (albumin content)

When applying reverse dosimetry (QIVIVE) to reach the same effect *in vivo* as seen *in vitro* it is necessary to know the time course of the concentration in the cells relevant for toxicity and not only the concentration time course in the supernatant.

Mielke et al. Arch Toxicol., 2018

Reducing uncertainties by re-evaluating of the uncertainty factors related to inter-individual variability in current non-cancer risk assessment strategy.



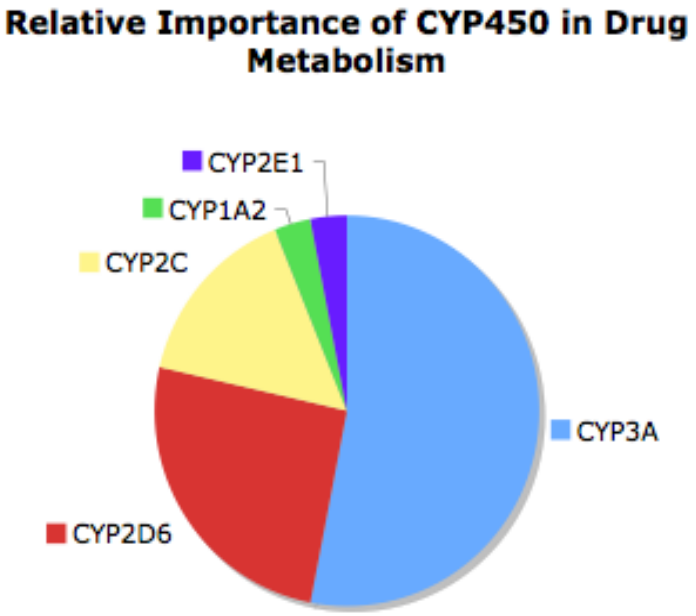
Such subdivision was proposed in order to allow for the replacement of **default uncertainty factor** with **chemical-specific factors**.

The approach followed is to collect data analysing human variability for both the kinetic and the dynamic aspects that can be used to propose **pathway-related default uncertainty factors**.

CYP3A4 is the major human cytochrome P450 isoform responsible for the metabolism of more than 50% of known xenobiotics.

Variability and inter-ethnic differences in CYP3A4 metabolism:

- **ELS of TK data for 15 CYP3A4 probe substrates** to collect parameters reflecting acute (Cmax, oral route) and chronic exposure (clearance and AUC, oral and intravenous route).



All data were **extracted** in a structured database and **meta-analyses** were performed using a hierarchical Bayesian model to derive parameter, route and ethnic-specific variability distributions for CYP3A4 metabolism.

Darney et al. Computational Toxicol, 2019

Meta-analysis and calculation of CV for inter-individual variability in healthy adults:

- **lower inter-individual variability for the IV route compared with the oral route** for both chronic and acute oral exposure,
- all values within the default TK UF

❖ **Oral: 2.5-3.0 (UF_{95} and $UF_{97.5}$, 10 compounds)**
❖ **I.V. : 1.7-1.8 (UF_{95} and $UF_{97.5}$, 2 compounds)**

The biological basis for this difference:

- CYP3A4 is expressed in the **liver** and the **intestine**
- the oral route reflects CYP3A4-metabolism in the two organs
- IV exposure reflects only CYP3A4-metabolism in the liver

Healthy European, East Asian and Middle East and North American adults showed generally similar CYP3A4-related UFs ⇒ **limited interethnic differences.**

- ✓ **Inter-individual variability** for the oral route for healthy adults averaged 51% (AUC).
- ✓ Overall, the **CYP3A4 related UF** for healthy adults were below the default kinetic factor (3.16) for at least 97.5% of healthy adults.
- ✓ The **ethnic differences** are generally limited, but data gaps were identified for specific ethnic groups.

These distributions allow to:

1. apply CYP3A4-related UFs in the risk assessment process for compounds for which *in vitro* CYP3A4 metabolism evidence are available

2. integrate CYP3A4-related variability distributions with *in vitro* metabolism data into physiologically based kinetic (PBK) models for quantitative *in vitro in vivo* extrapolation (QIVIVE)

3. estimate UFs in the risk assessment process using variability distributions on metabolism.

Take Home message

- ✓ Uso di strategie di testing in vitro su **modelli cellulari umani** caratterizzati per la loro capacità metabolica e la presenza di trasportatori
- ✓ Misura della biocinetica in vitro per estrapolare **concentrazioni efficaci in vitro a dosi rilevanti per l'uomo**.
- ✓ Necessità di metodi analitici sufficientemente sensibili
- ✓ Integrazione attraverso modelli computazionali tenendo conto di parametri legati a differenze sperimentali in vitro/in vivo (es: legame alle proteine).
- ✓ Dati biocinetici come input per **modelli PBPK**, uno strumento importante con cui migliorare l'estrapolazione quantitativa in vitro in vivo (QIVIVE) e caratterizzare la **relazione dose-risposta** come base per comparare i possibili rischi associati ad una specifica esposizione.
- ✓ Integrazione dei dati di **variabilità individuale** (attraverso isoform-specific metabolism e UF relativi a specifici pathway)

GRAZIE!

A voi per l'attenzione

Un ringraziamento speciale a:

- ✓The PredictIV team
- ✓Al consorzio del progetto EFSA 2016-2020
- ✓Alle colleghe del reparto : Franca M. Buratti, Emma Di Consiglio, Nicoletta Santori, Laura Turco, Susanna Vichi e a tutti coloro che hanno collaborato