New methods: miniorgans

C. Rovida

CAAT-Europe, University of Konstanz

16 maggio 2018

Parma Summer School 2018

Emerging Risks for Food Safety and Public Perception

ecopa

www.ecopa.eu

1981

2010

Universität Konstanz

JOHNS HOPKINS BLOOMBERG SCHOOL of PUBLIC HEALTH

2012

Stakeholder Platform

Transatlantic Hub

CAAT EU Policy Program

JOHNS HOPKINS BLOOMBERG SCHOOL of PUBLIC HEALTH

Universität Konstanz

urope

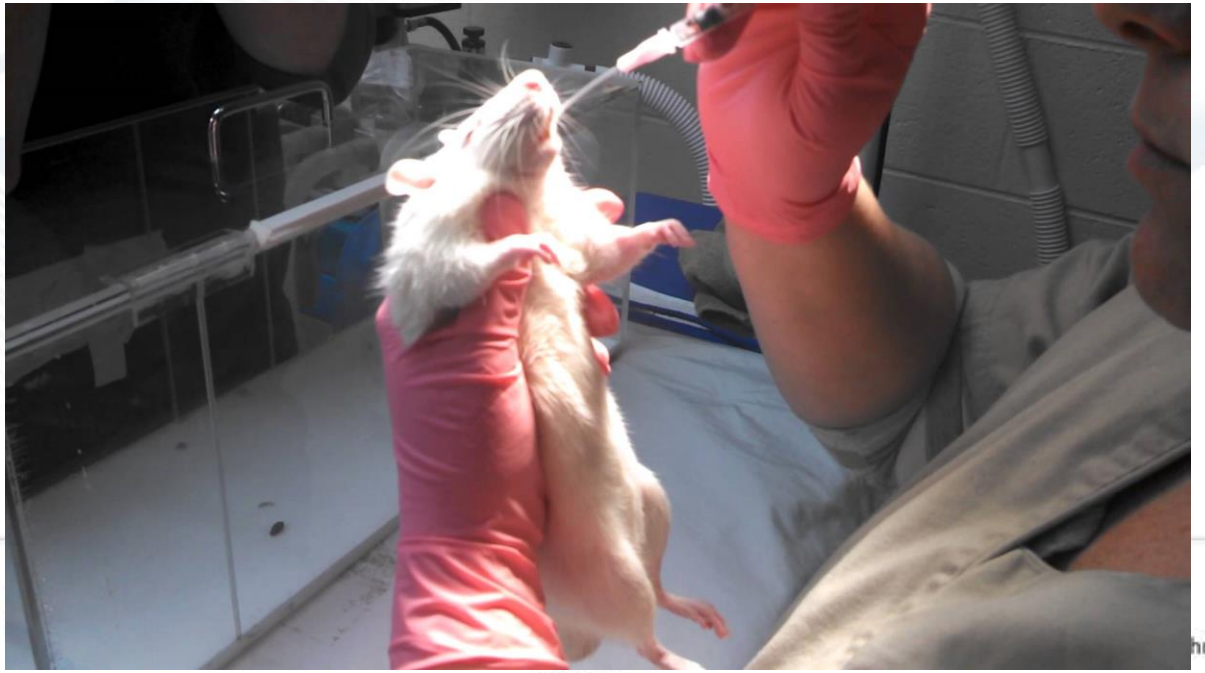
Regulation EC 1107/2009 on Plant Protection Products

Regulation EC 1223/2009 on cosmetics Products

Regulation EC 528/2012 on Biocidal Products

Toxicology: traditional in vivo approach

- Skin/Eye irritation
- Skin sensitisation
- Acute toxicity
- Repeated Dose Toxicity
- Reproductive Toxicity
- Genotoxicity
- Carcinogenicity



We are not 70kg rats !!!

Age 0 -100 years

Mostly 3 months, max 2 years

2-200 kg

20-500 g

Different ethnics, both gender

≠

Mostly twins, one gender

Diverse food, environment

Standardized chow and cage

Disease history, Comorbidities, Multiple treatments

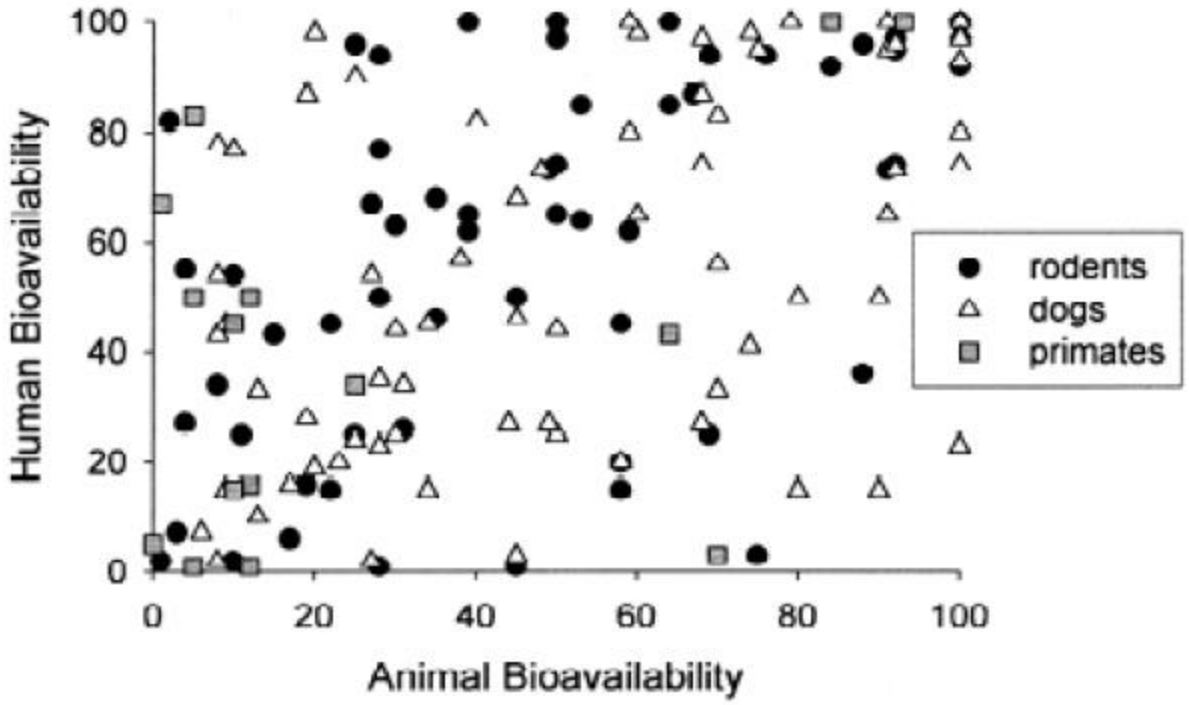
Healthy, Artificial diseases, Mono-treatments

RELIABILITY OF ANIMAL TESTS

GLP, standardized protocols, some validated, high doses, substance effects in healthy animals:

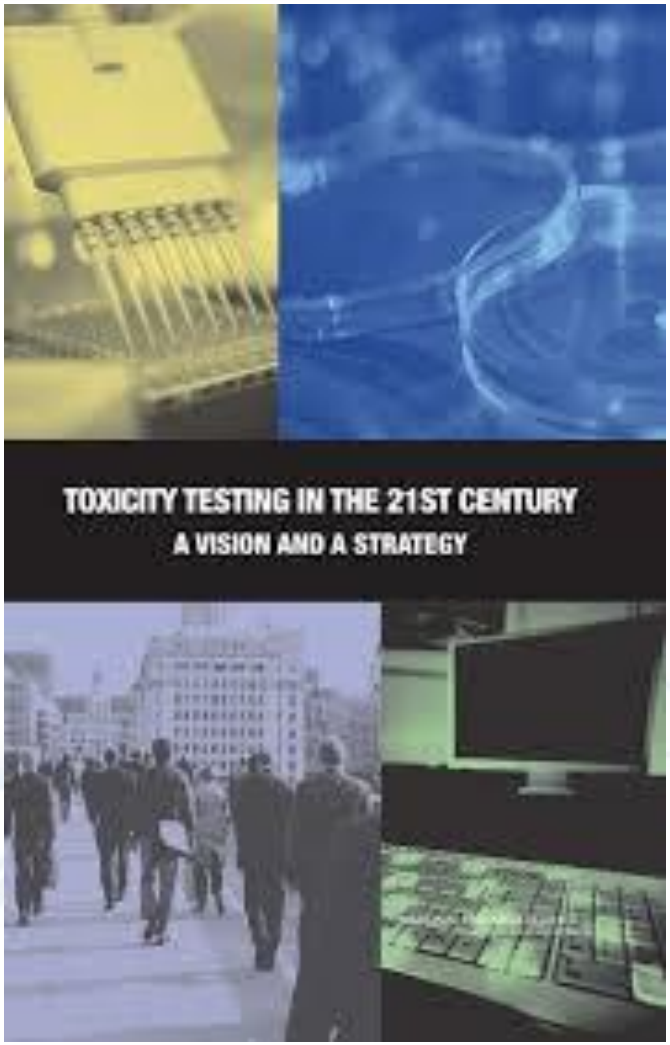
- **Cancer bioassay: 57% (repeat or mouse vs. rat)**
- **Reproductive Tox: 60% between species**
- **Uterotrophic assay: 26% contradictory**
- **Skin sensitization: 77% guinea pig vs. mouse**
- **Severs eye irritation: 70% reproducible**
- **Acute fish tox: up to 6 log orders different**
- **Chronic tox: no correlation between mouse and rats or genders**

How can we do quantitative risk assessment, if already oral bioavailability differs dramatically?



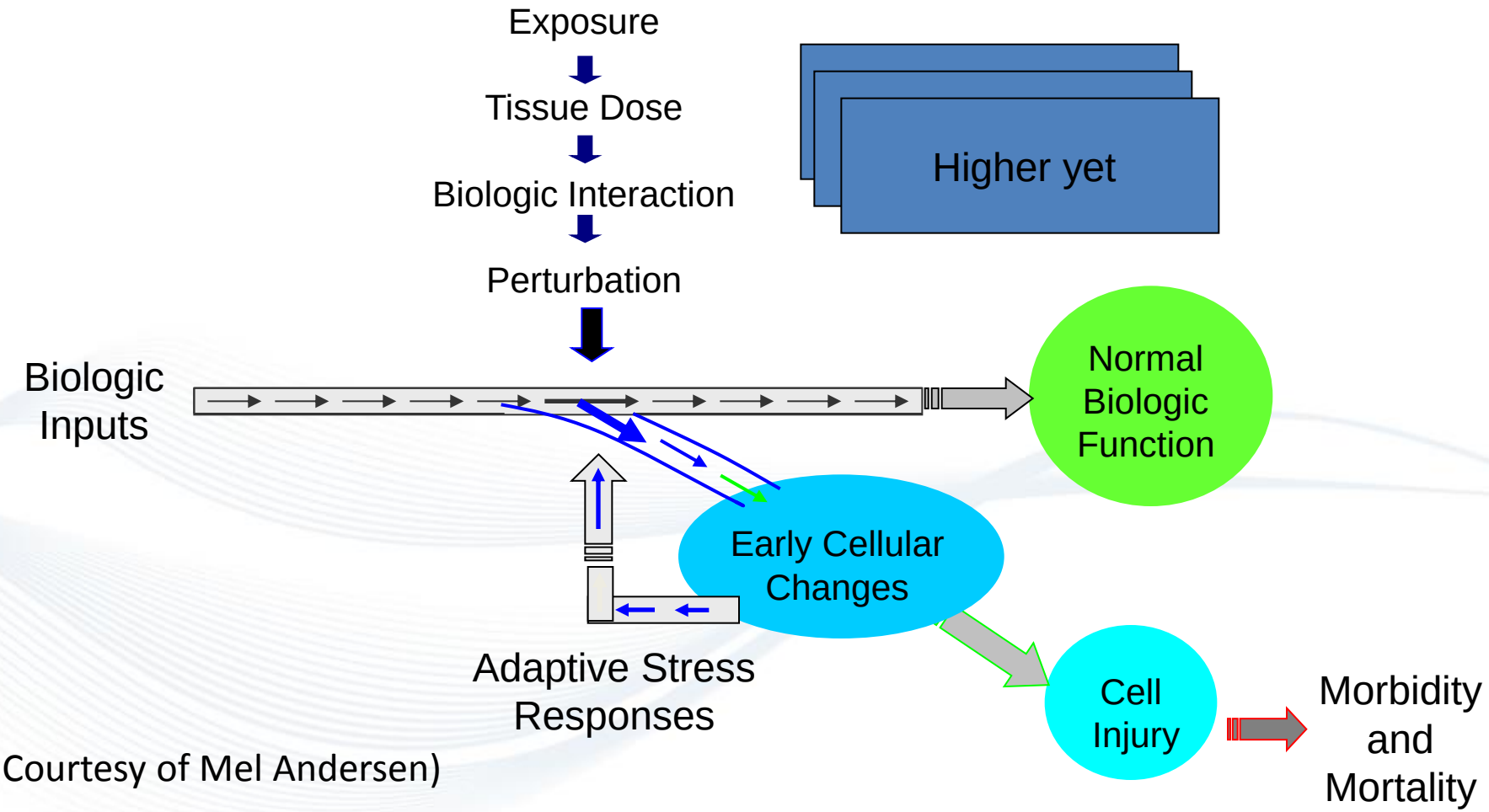
Grass GM and Sinko PJ. Adv Drug Delivery Rev 2002, 43:433-451

Report del National Research Council of the National Academies Published on 2007



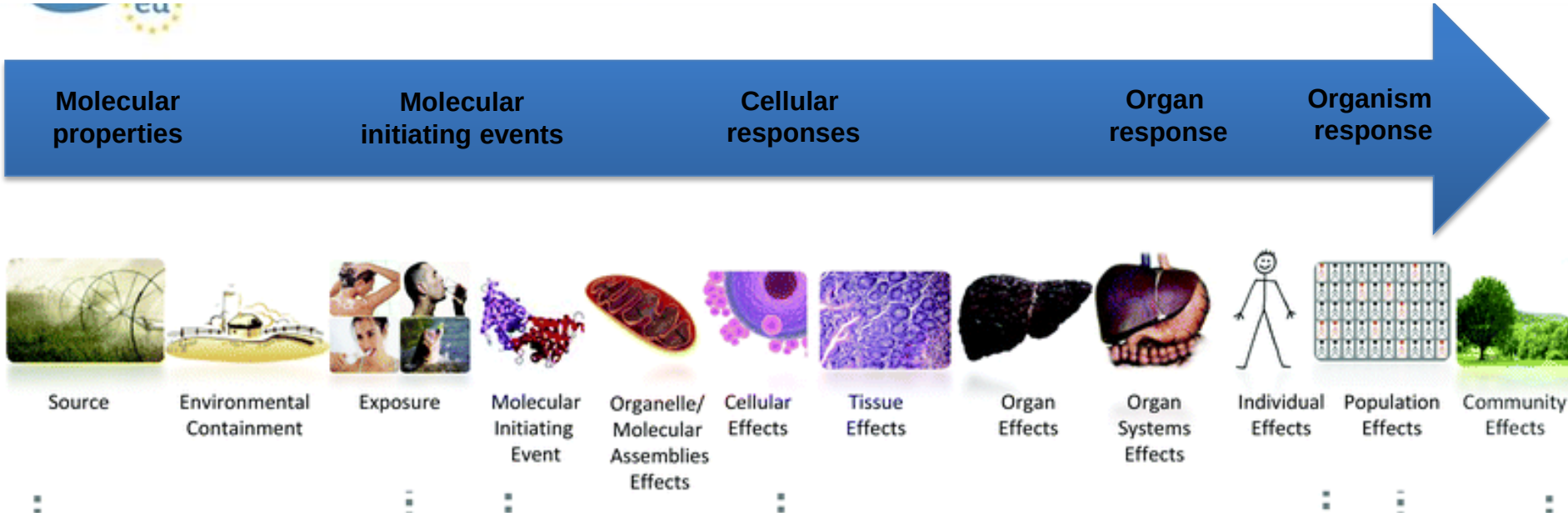
(Google: "Toxicity Testing in the 21st Century")

A New Paradigm: Activation of Toxicity Pathways



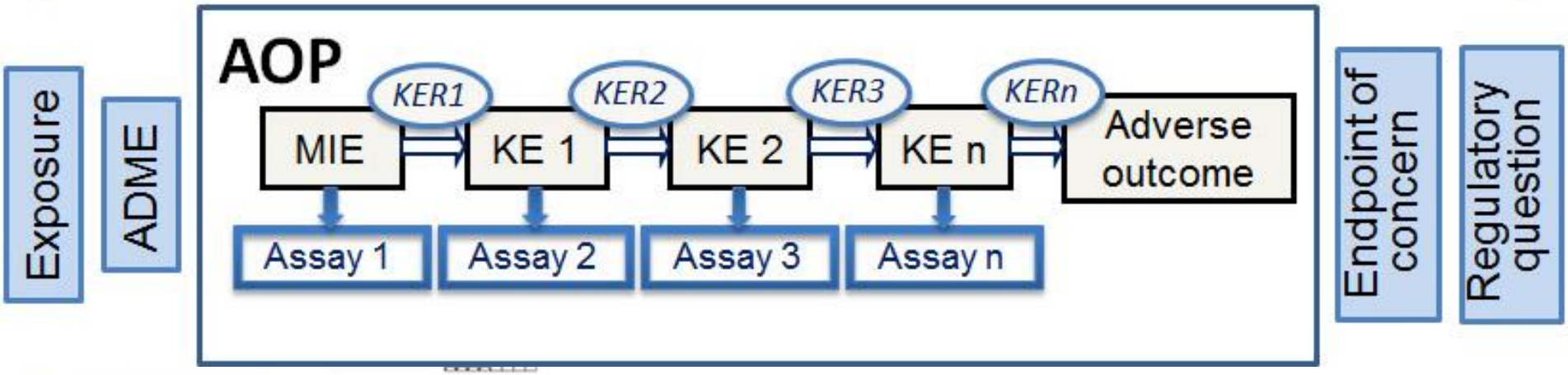
(Courtesy of Mel Andersen)

ADVERSE OUTCOME PATHWAY

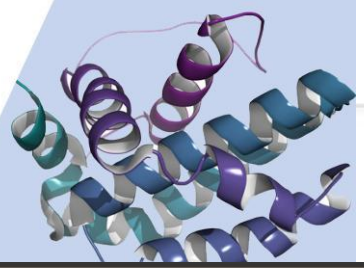


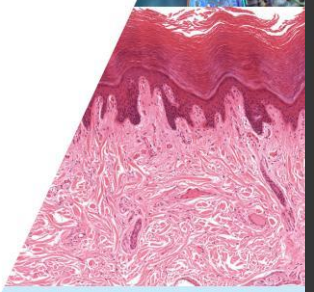
IATA

Decision Context



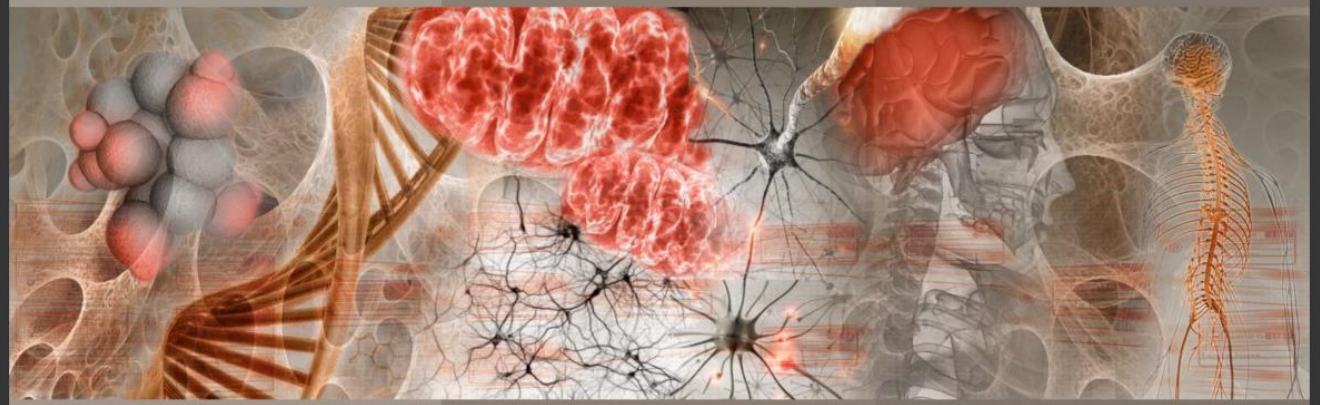
Guidance Document for the Use of Adverse Outcome Pathways in Developing Integrated Approaches to Testing and Assessment (IATA)





Series on Testing and Assessment
No. 260

Effectopedia is a collaborative research platform, facilitating knowledge exchange between **experimental biologists**, **modelers** and **chemical risk assessment communities**.



An initial set of **training material** and videos are available on Effectopedia training website
http://training.effectopedia.org/

From the website you can **learn how to**:

- Create the visual diagram of an AOP
- Build Key Event and Key Event Relationship following the OECD Users' Handbook for AOP development.
- Save an AOP as a local file or publish it on the centralised Effectopedia database
- Build simple quantitative models using experimental data
- Design and implement interactive executable models using R or Matlab

Effectopedia is a free open source software distributed under GNU General Public License v3.0.
The most recent version is available for download from:

Effectopedia website
http://effectopedia.org

The source code is available on SourceForge :
https://sourceforge.net/projects/effectopedia/

Effectopedia is developed by:

OECD

with the financial support of

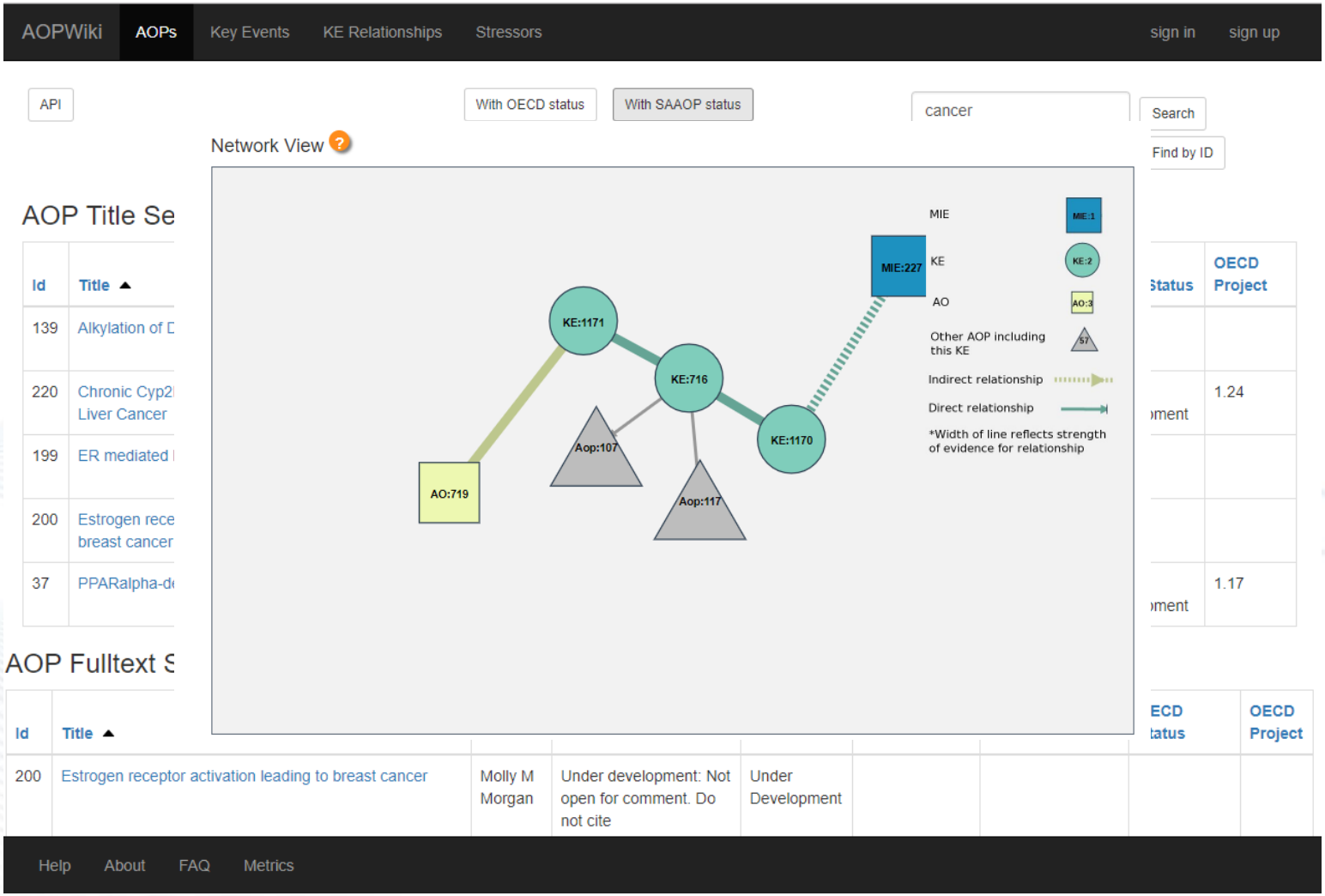
European Commission

Effectopedia
The Online Encyclopedia of Adverse Outcome Pathways

OECD
Jan, 2017

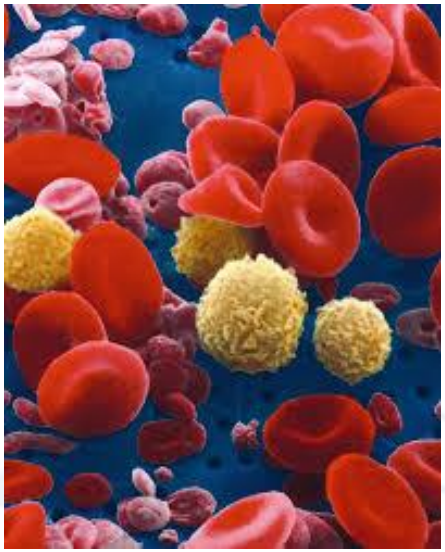
OECD
BETTER POLICIES FOR BETTER LIVES

AOP wiki – search for «cancer»



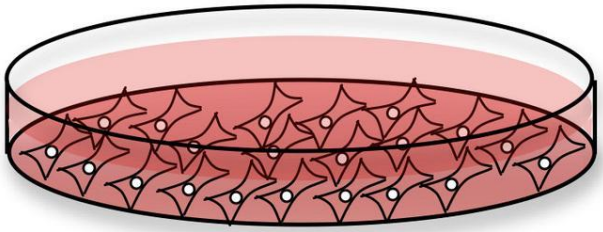
Type of Cells for Toxicity studies

- Cell Lines
  - Origine tumorale
- Primary cells
  - Biopsy
  - Volunteers (plasma, urine, etc.)
- Stem Cells
  - Adult Stem Cells
  - Embryo stem cells
  - Cord Blood Stem Cells
  - Embryos (animal origin)



Traditional culture: pan-fried eggs “sunny side up”

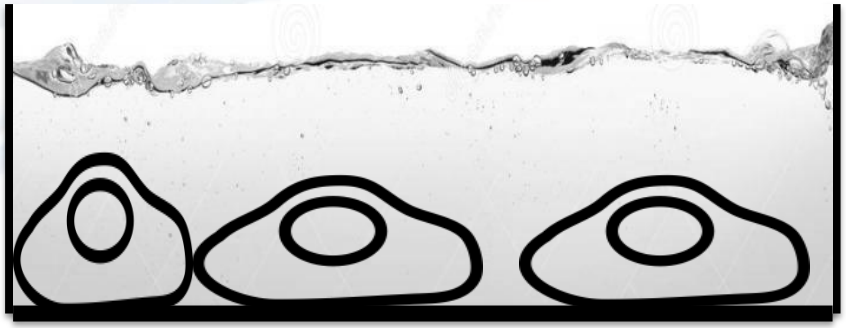
Cell density ca. 0.1% of tissue,
Dilution of all secreted factors

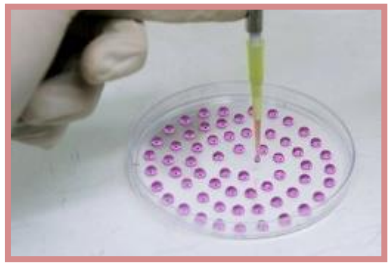


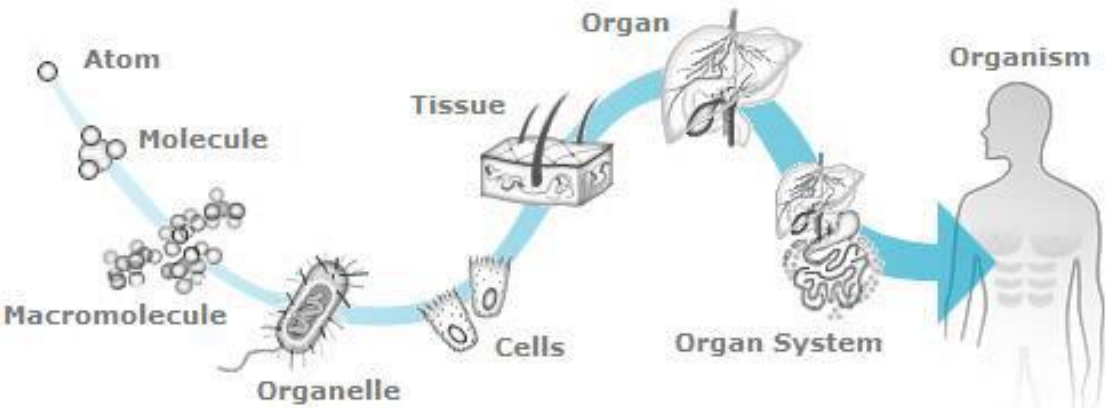
- **Ca. 25% of cell lines misidentified**
- **15-25% mycoplasma infected**
- **Genetic instability**
- **Culture artifacts**

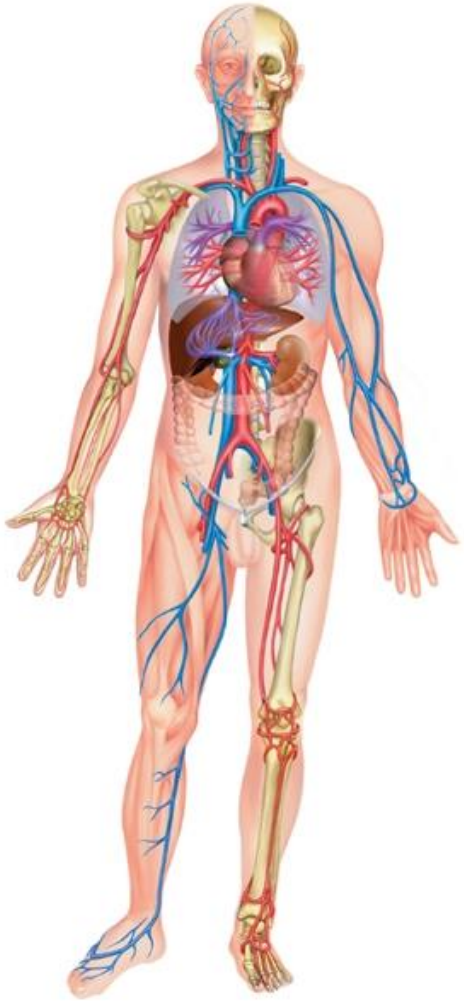
No flow
No steady ingredients,

Cell to cell contact about 2%,
49% plastic, 49% medium









validation

performance predictive

confidence

in vivo relevance

Application of the adverse outcome pathway concept

- Human-induced pluripotent stem cell technology
- Human organ-on-a chip
- Ex-vivo biopsy or post-mortem human tissue
- Bio-banks and advanced mathematical modelling
- Advanced clinical studies

Induced pluripotent stem cells (iPSC)

Embryotoxicity tests

Embryonic stem cells

DIFFERENTIATION

Induced pluripotent stem cells
(coming from somatic cells)

Reprogrammation

Somatic cells

test → Hepatotoxicity

Human hepatocytes

test → Cardiotoxicity

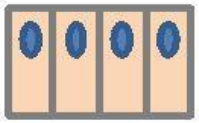
Human cardiomyocyte

test → Osteotoxicity

Human osteocytes

test → Neurotoxicity

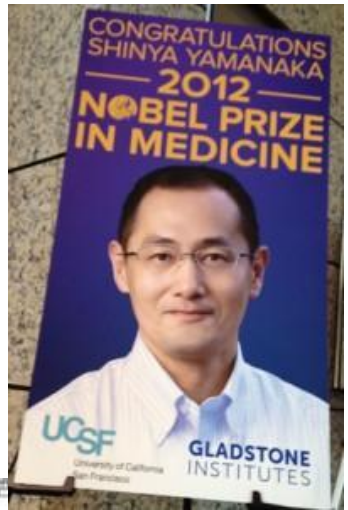
Human neurons



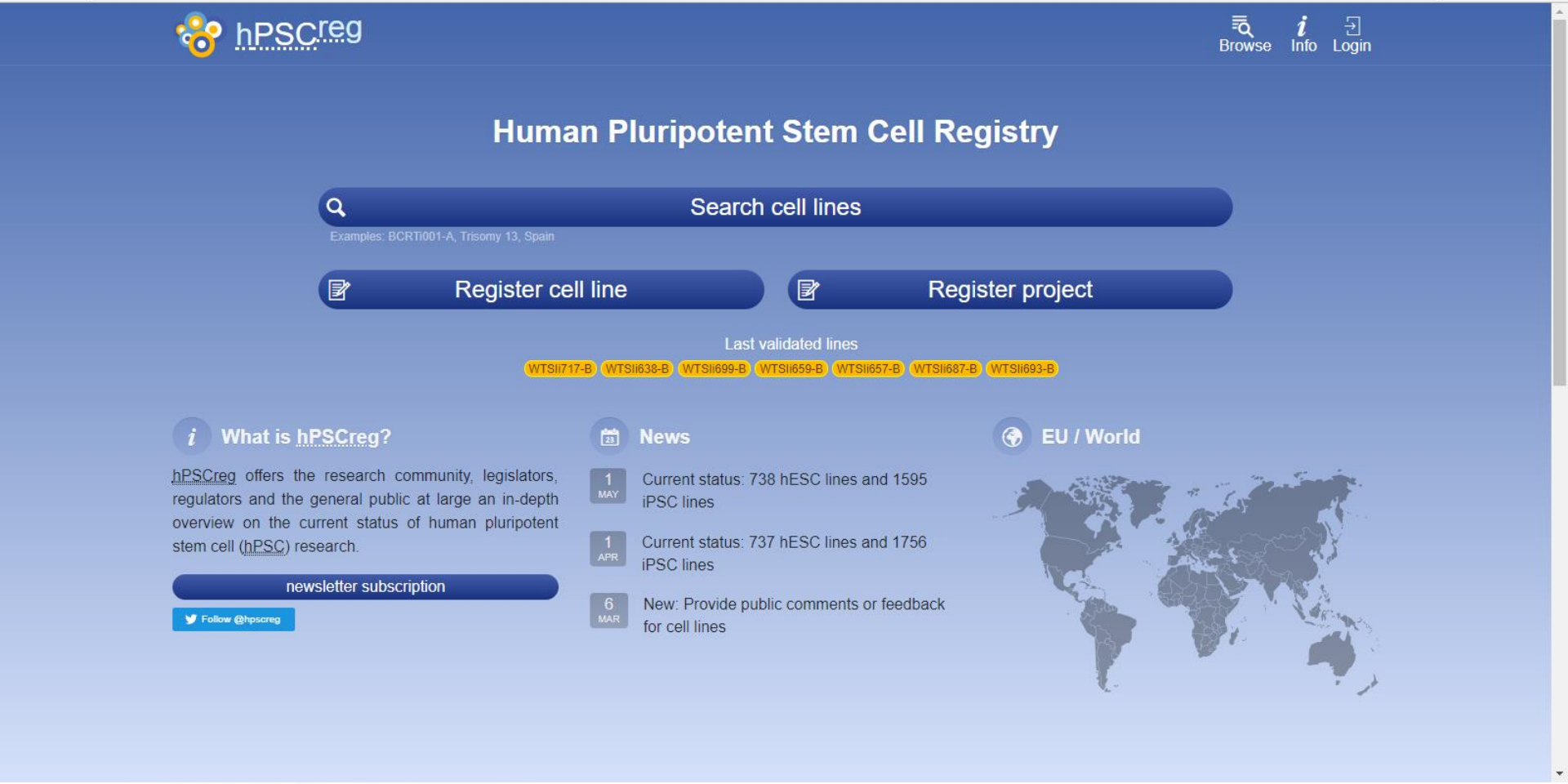
test → Dermatotoxicity

Human skin cells

Other human cells → test → Other organs target toxicity



iPSC Availability



https://hpscreg.eu/

Limitations and Challenges

2D *in vitro*

Lack organ function, structure and complexity

Cancer or immortalized cells

3D *in vitro*

Lower reproducibility

Endpoints generally need optimization from 2D

Challenge to study on a single cell level

Limited perfusion

Organ-on-chip

Costly and complex

Low availability

Demands engineering skills

Not suitable for high-throughput screening

Organ specific biomarkers

"All models are wrong, but some are useful"

George EP Box (2005): Statistics for Experimenters (2nd ed.) p 440

Your scientific question should determine which model to use

Why 3D cell cultures?


transatlantic think tank for toxicology

Alépée et al., ALTEX 2014, 31, 441-477.

t4 Workshop Report*

State-of-the-Art of 3D Cultures (Organs-on-a-Chip) in Safety Testing and Pathophysiology

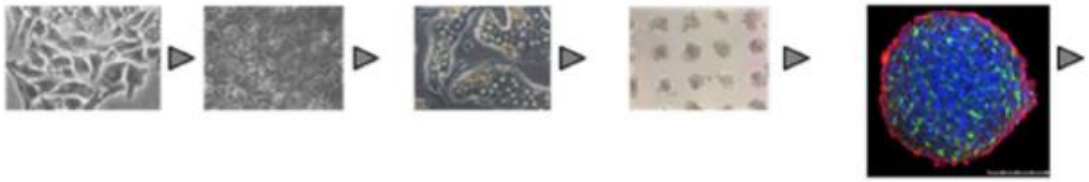
Natalie Alépée [1], Anthony Bahinski [2], Mardas Daneshian [3], Bart De Wever [4], Ellen Fritsche [5], Alan Goldberg [6], Jan Hansmann [7], Thomas Hartung [3,6], John Haycock [8], Helena T. Hogberg [6], Lisa Hoelting [9], Jens M. Kelm [10], Suzanne Kadereit [9], Emily McVey [11], Robert Landsiedel [12], Marcel Leist [3,9], Marc Lübberstedt [13], Fozia Noor [14], Christian Pellevoisin [1], Dirk Petersohn [15], Uwe Pfannenbecker [16], Kerstin Reisinger [15], Tzutzuy Ramirez [12], Barbara Rothen-Rutishauser [17], Monika Schäfer-Korting [18], Katrin Zeilinger [13] and Marie-Gabriele Zurich [19,20]

- Increased cell survival
- Increased differentiation
- Increased cell – cell interaction
- Reproducing better the complexity of the organ
- Endpoints need optimization

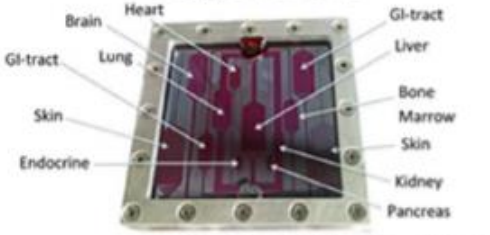
- More complex – lower reproducibility

Increasing complexity

Cell Lines → Primary Cells → Co-Cultures → Longterm 2D Cultures → Longterm 3D Co-Cultures



The Future:
Microphysiological Systems «Body-On-A-Chip»



from Cornell University and Hesperos, Inc

- ***Physiological Relevance***
- ***Interplay of Different Cell Types***
- ***Longterm Drug Exposure***

- ***Ease of Use: Throughput***
- ***Reproducibility***
- ***Genetic Manipulation***

Specific, established Mechanism can be assessed

Holistic Models for MoA Analysis & Hypothesis Generation

Funk & Roth. Arch Toxicol. 2017

ORGANOTYPIC CULTURES

Goal is to replicate human ORGAN-LEVEL functions *in vitro,*

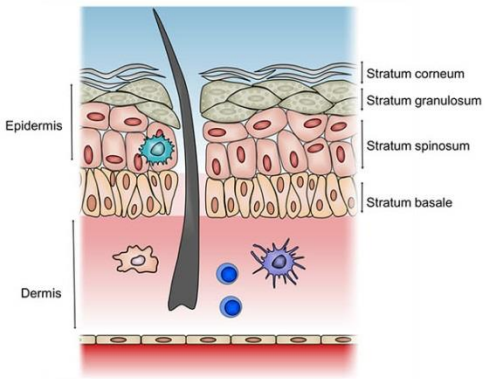
- **Composed of 2 or more tissues that exhibit unique functions when they are interfaced**
- **Perfused by blood flowing through endothelium-lined vessels**
- **Controlled by chemical and molecular factors produced by constituent cells or delivered through the vasculature**
- **Regulated by mechanical forces (e.g., due to motion, breathing, peristalsis) and blood flow**
- **Structured to secrete or transport factors in specific directions**
- **Infiltrated with immune cells during inflammatory responses**
- **Physiologically coupled to other organs via factors transmitted in blood flowing through linking vessels**

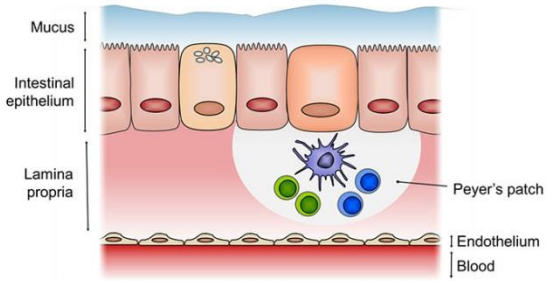
Courtesy of Wyss Institute

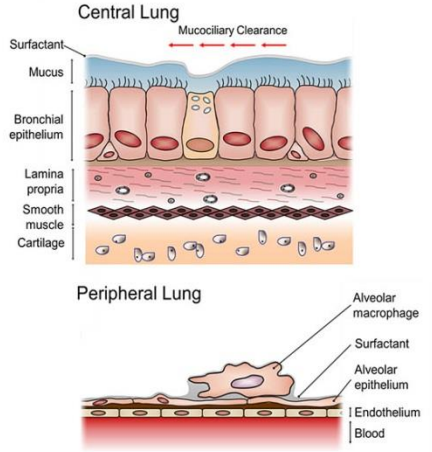
ROUTES OF EXPOSURE

Skin | **Intestine** | **Lung**

LOCAL EXPOSURE



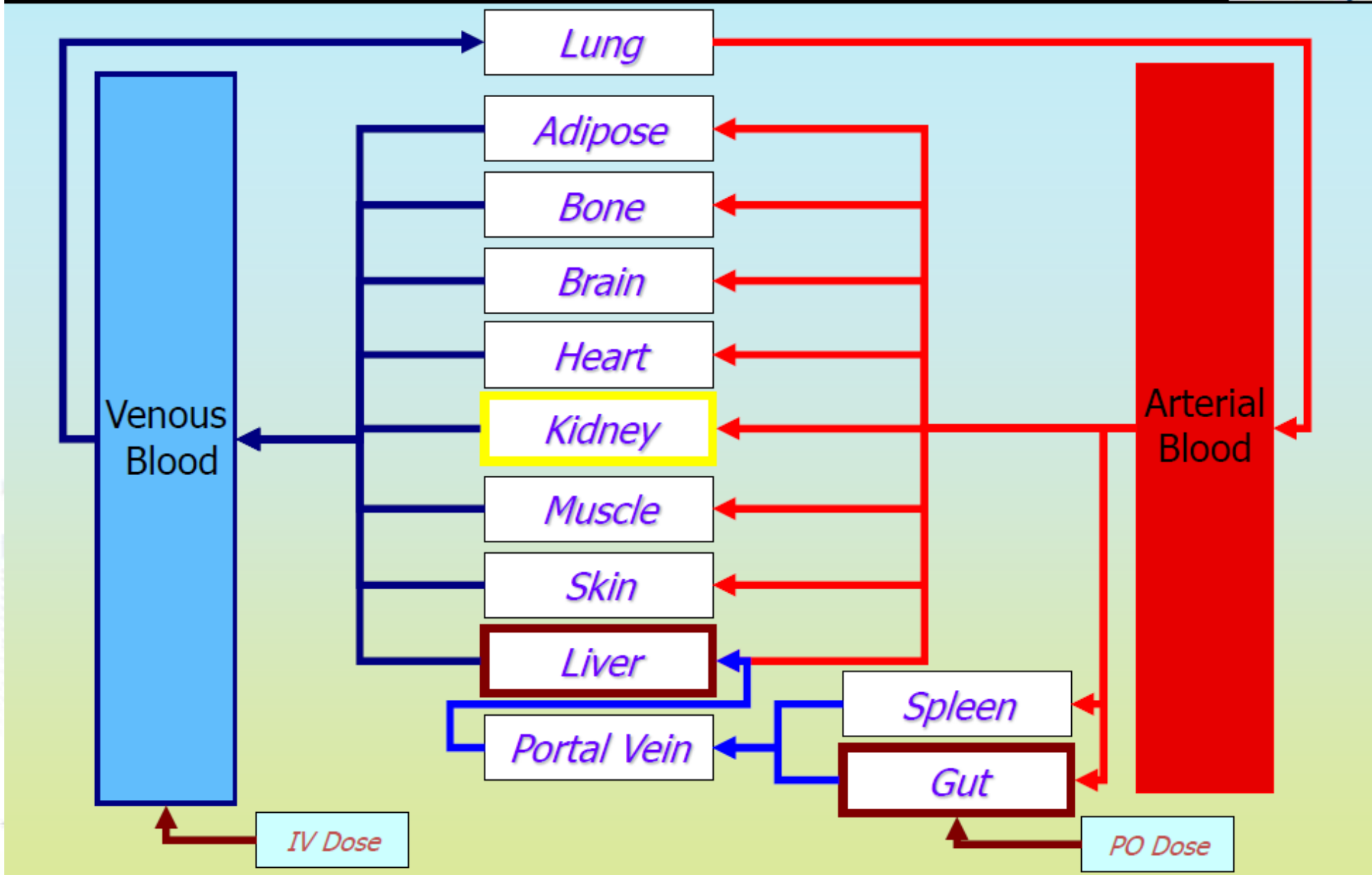




SYSTEMIC EXPOSURE

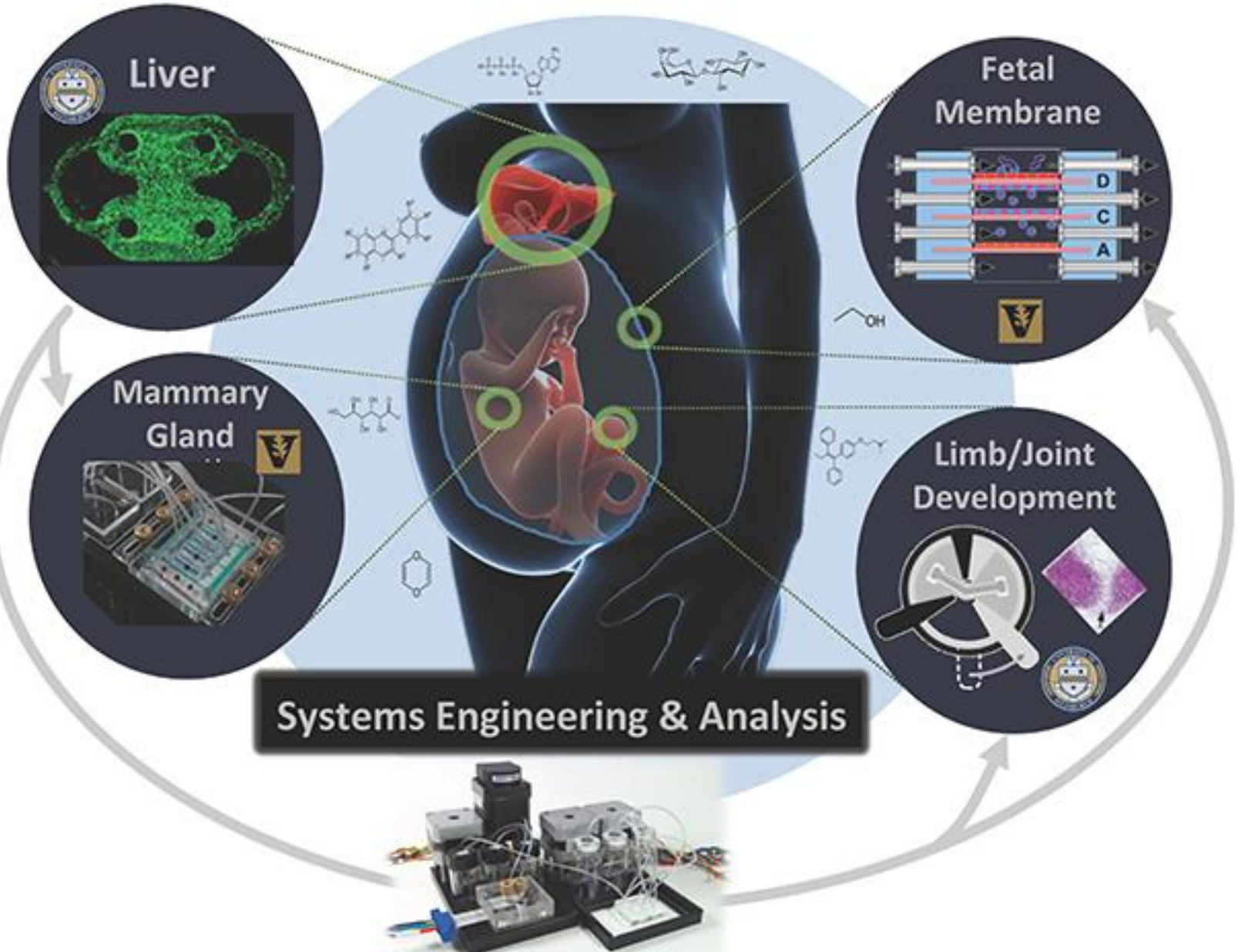
Ruge et al., 2013 de Souza Carvalho et al., 2014

Full PBPK Model with Time-Dependent Volume

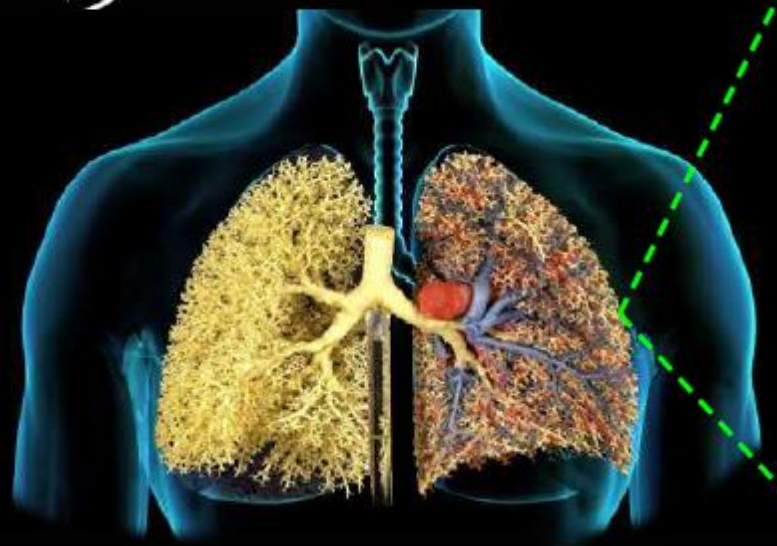


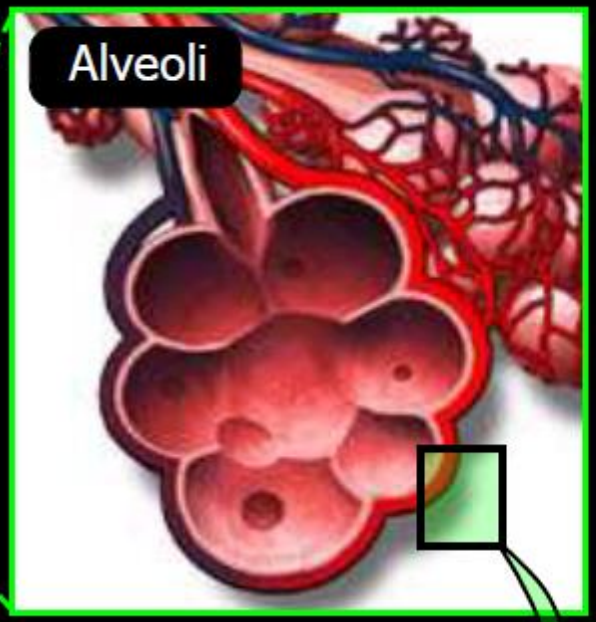
VPROMPT

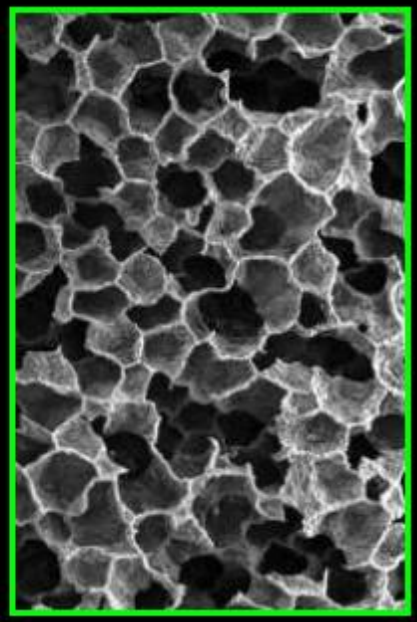
Vanderbilt-Pittsburgh Resource for Organotypic Models for Predictive Toxicology

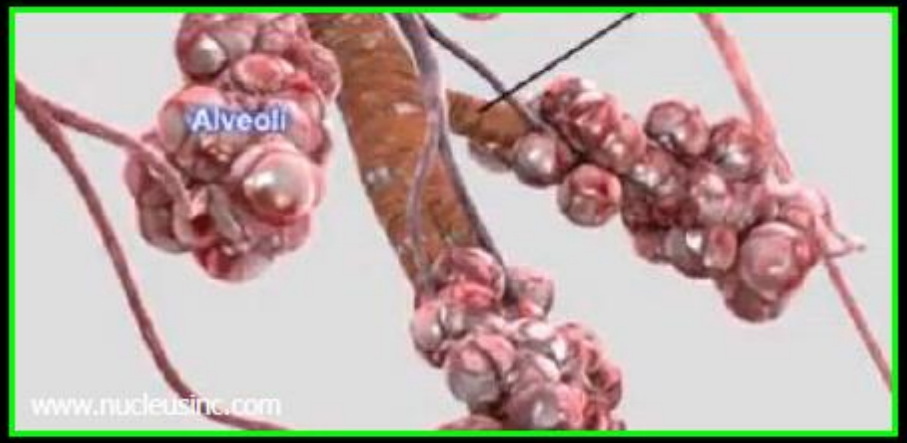


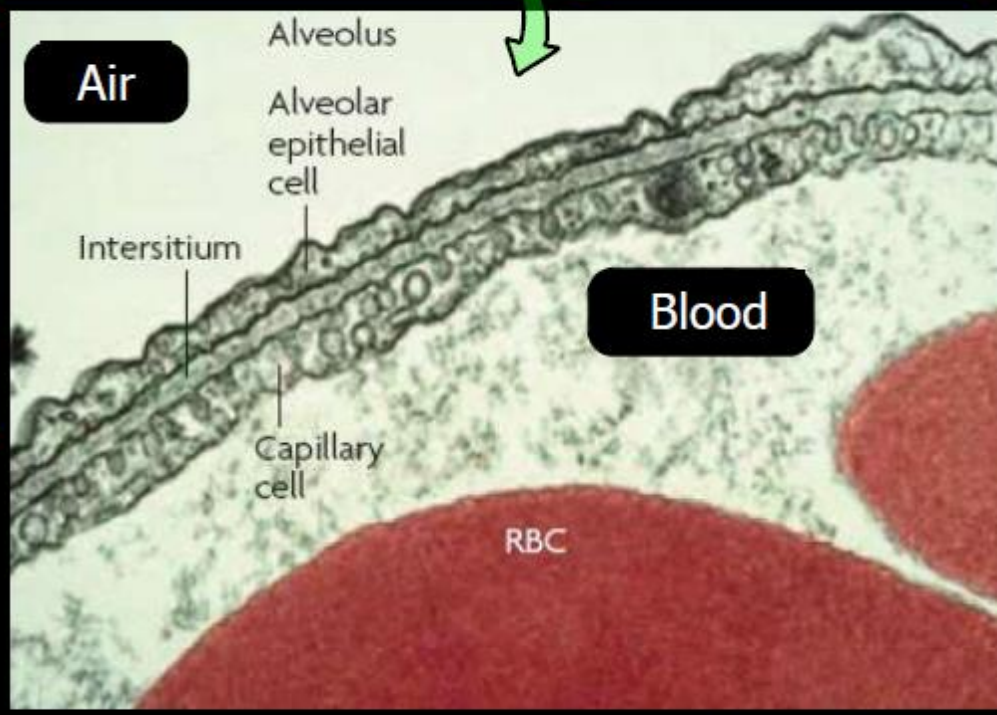
WYSS INSTITUTE











BIODESIGN PRINCIPLES:

- **Tissue-Tissue Interface**
- **Dynamic Flow**
- **Cyclic Breathing Movements**

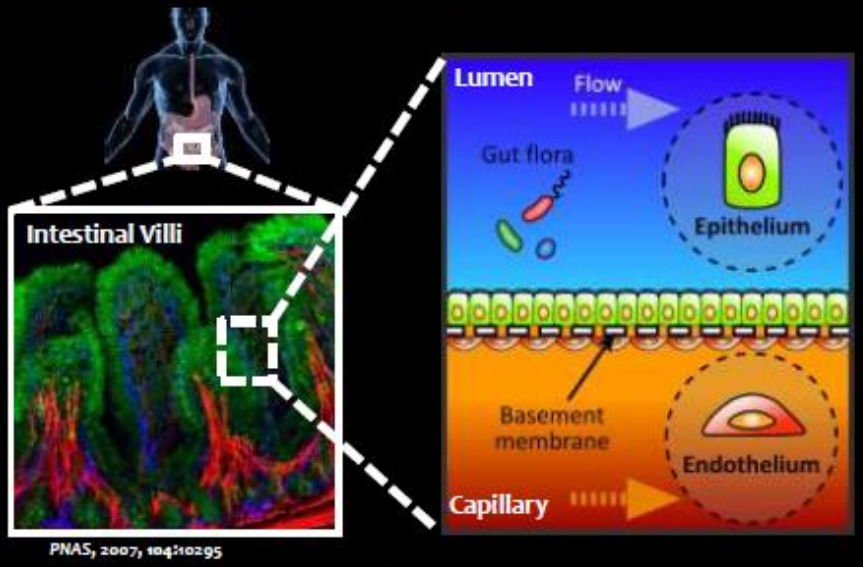
Paton & Byron, *Nat. Rev. Drug Discov.* 2007

WYSS INSTITUTE

Peristaltic Human Gut-on-a-Chip

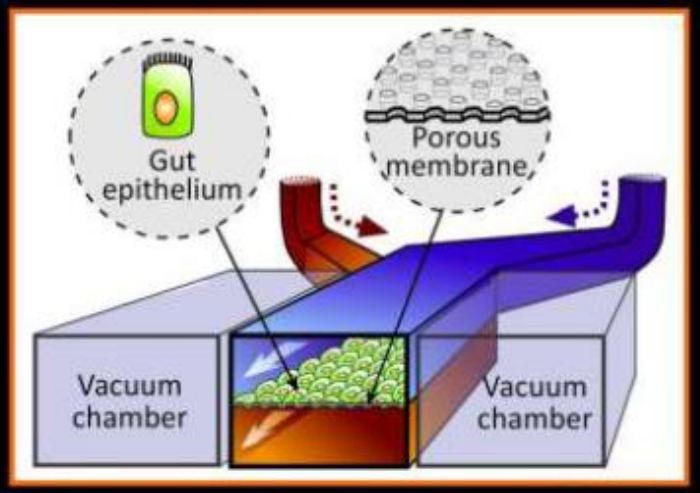
(Kim et al., *Lab on a Chip* 2012 & *Integrative Biology* 2013)

Human Intestine

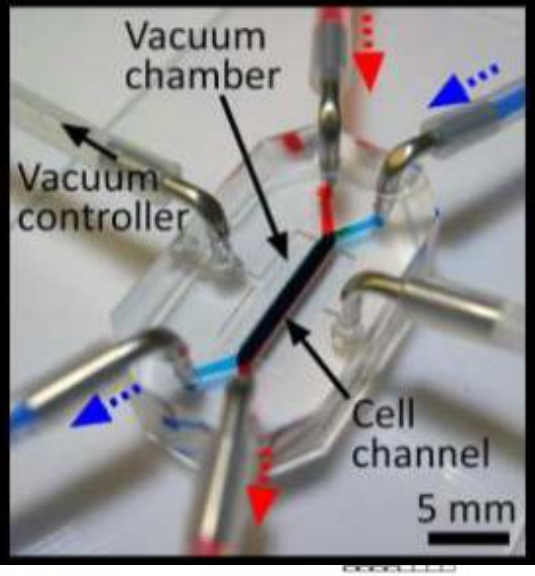


PNAS, 2007, 104:10295

Microfluidic Platform

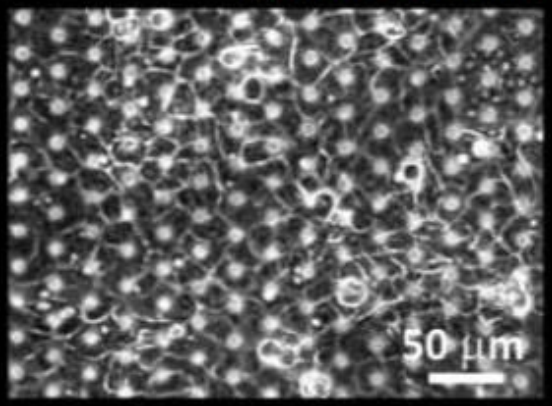


Gut Chip

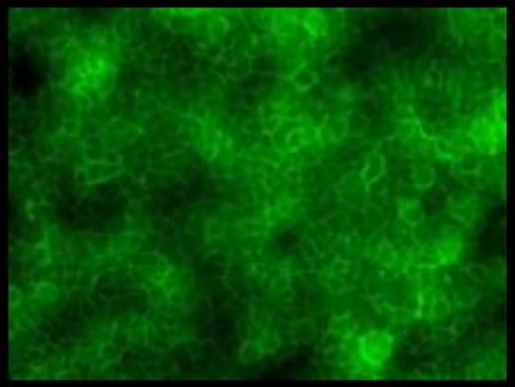


Human Gut Epithelium

(Caco-2 cell monolayer in Microfluidic)



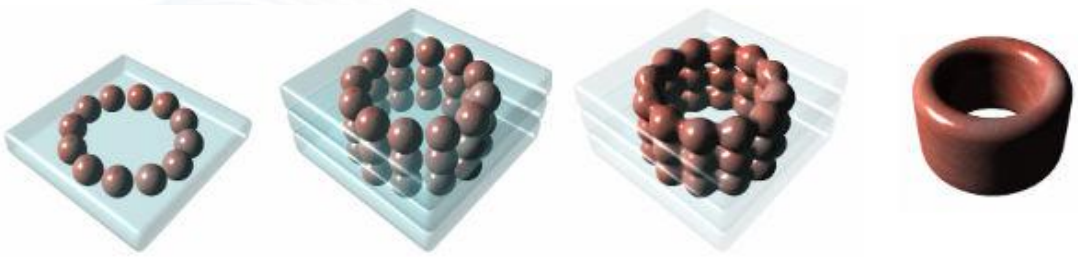
24 hr after seeding



+ Peristaltic-like motions

Bioprinter

- Living cells + Hydrogel
- Matrix for the scaffols
- Multilayers

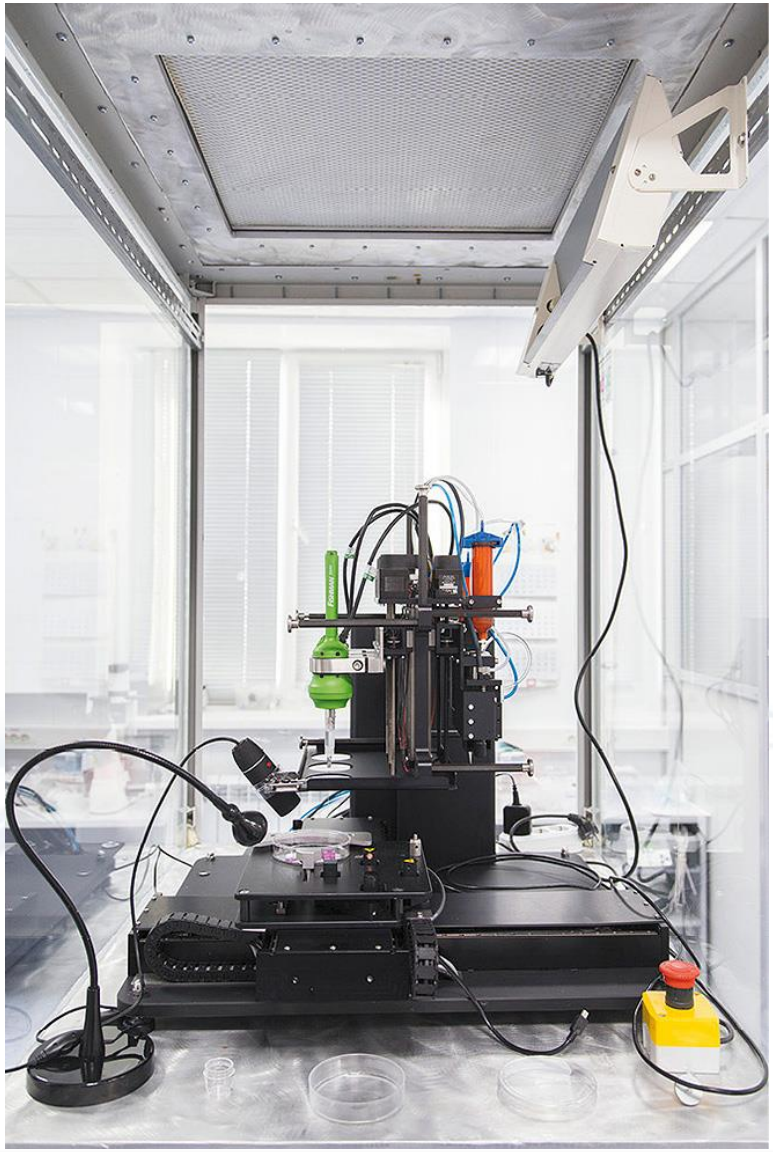


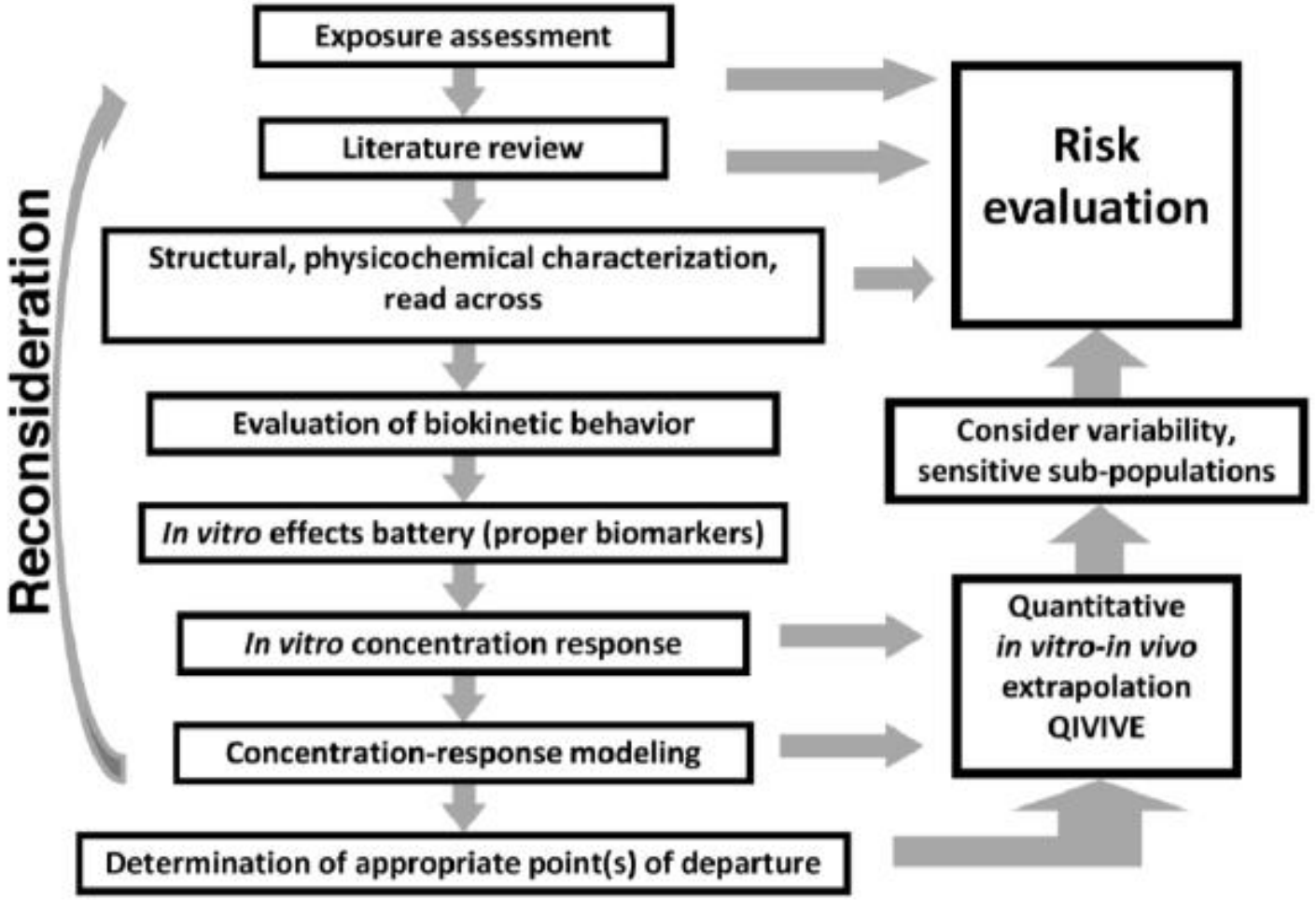
[A] Bioink spheroids printed into layer of biopaper gel

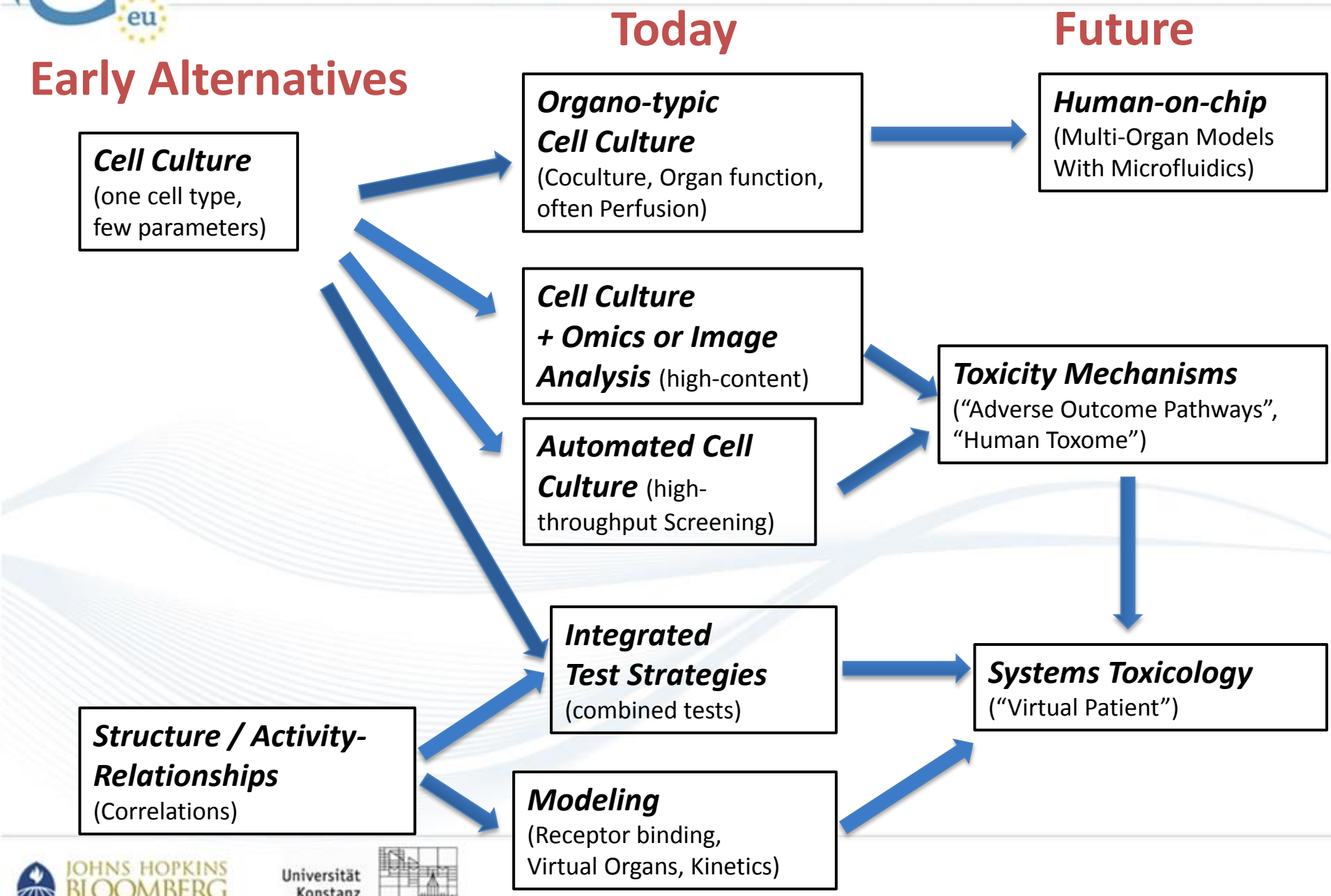
[B] Additional layers printed to build object

[C] Bioink spheroids fuse together and biopaper dissolves

[D] Final living tissue

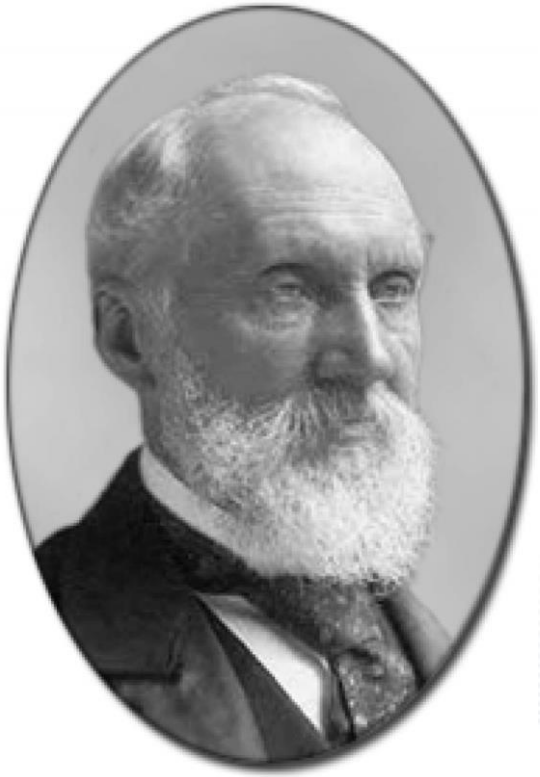



Exposure assessment
Literature review
Structural, physicochemical characterization, read across
Evaluation of biokinetic behavior
In vitro effects battery (proper biomarkers)
In vitro concentration response
Concentration-response modeling
Determination of appropriate point(s) of departure
Reconsideration
Risk evaluation
Consider variability, sensitive sub-populations
Quantitative in vitro-in vivo extrapolation QIVIVE



In case you want to know more

- **www.altex.ch**
- **http://altweb.jhsph.edu**
- **http://academy.altertox.be/**
- **www.estiv.org**



Heavier-than-air flying machines are impossible (1895)

No balloon and no aeroplane will ever be practically successful (1902)

William Thomson, known as Lord Kelvin (1824-1907),
President of the Royal Society and inventor of the absolute temperature scale

Thank you for the attention!!!