Emulating human organ interactions on a universal multi-organ-chip platform

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2010

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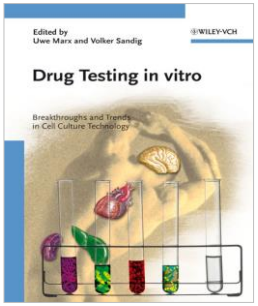
From a Vision Towards a Universal MPS-based Platform

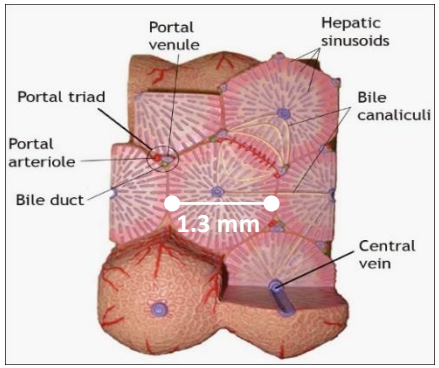
Vision 2007



Chapter 11
"How drug development of the 21st century could benefit from human micro-organoid in vitro technologies" © 2007
ISBN: 978-3-527-31488-1

- Organs are built up by multiple, identical, functionally self-reliant structural units
- Such micro-organoids are evolutionarily conserved and subject to genetically encoded self-assembly

1 million liver lobules per person



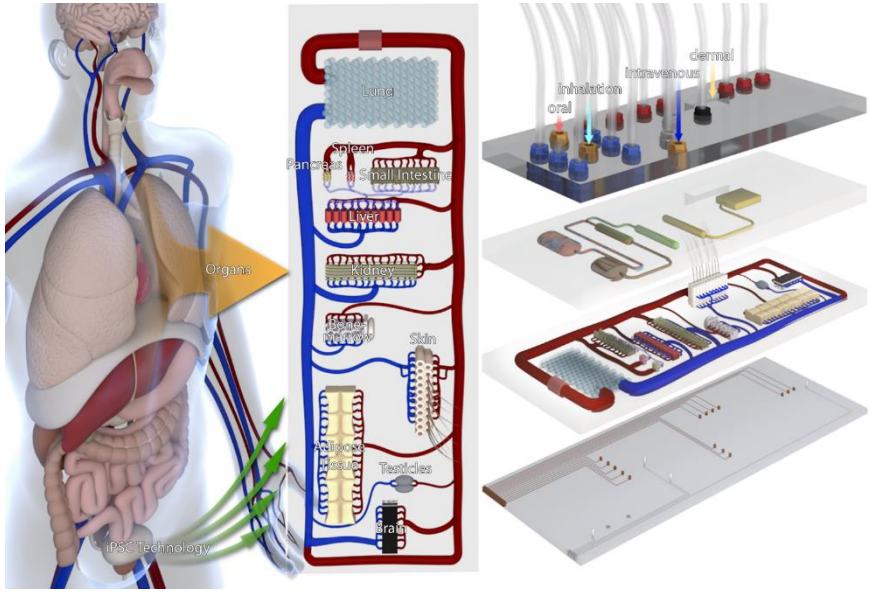
Marx et al., *ATLA* 2012

Marx et al. *ALTEX* 2020
Dehne & Marx, *Curr Opinion Tox* 2020

"Human"-on-a-chip

(Universal Physiological Template)

"Patient"-on-a-chip



Materne et al., *LabChip* 2013

https://www.youtube.com/watch?v=nkkBu8GrExk

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Components of the HUMIMIC® MPS Platform

Commercial Equip

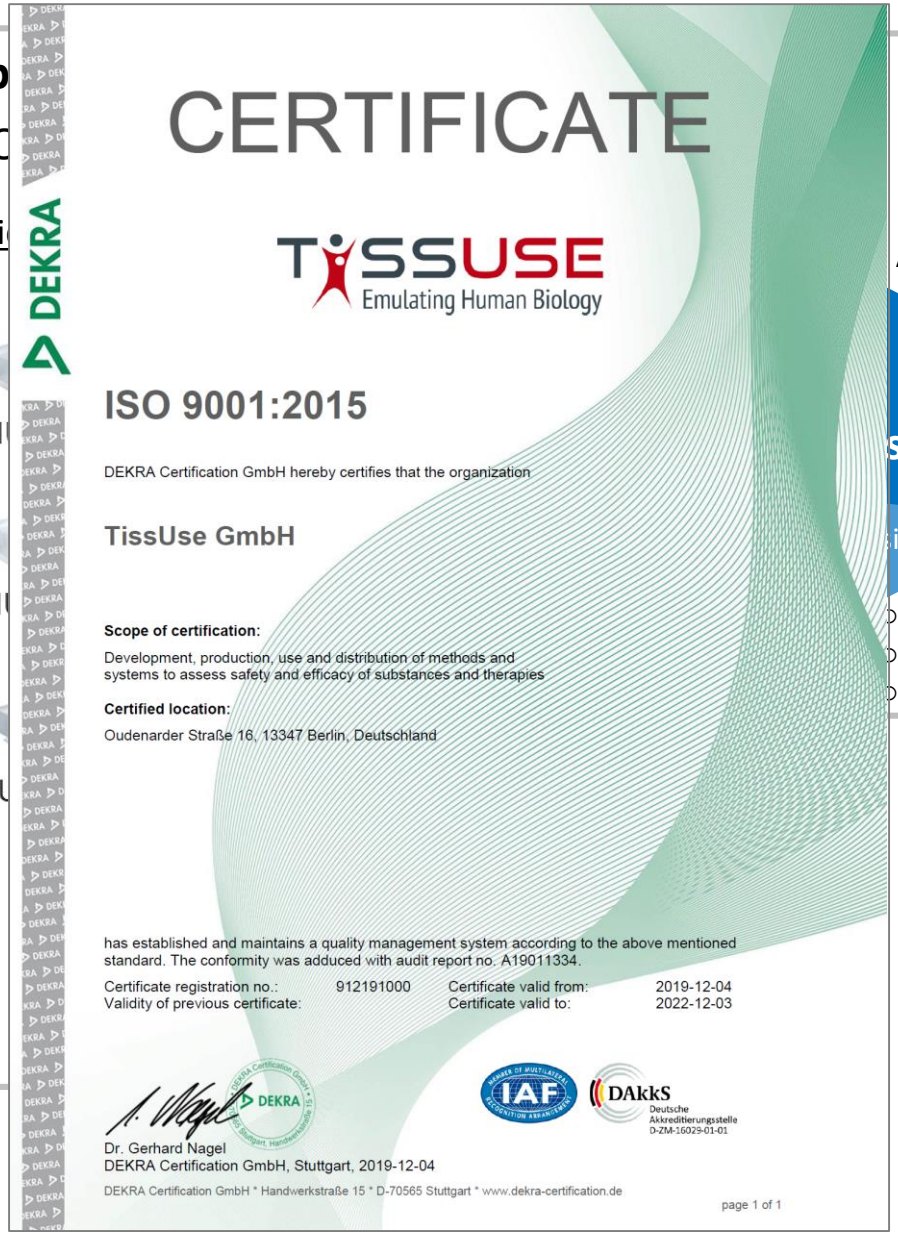
European IQ, OQ, PC

Rapi

HUMIMIC Starter
(4-8 circuits)

HUMIMIC AutoLab
(24-48 circuits)

HUMIMIC XX/XY



Qualified Assays (Context-of-Use)

GCCP standards

Administration, Exposure time, Dosing regimen, Controls

- internal portfolio decision making
- hazard identification, tier 3
- supportive data for IND/IMPD

Regulators

Regidstrations, Approvals

Regulatory-acceptable options to validate assays?
(Voluntary DDT validation pathway? OECD guideline validation? Others?)

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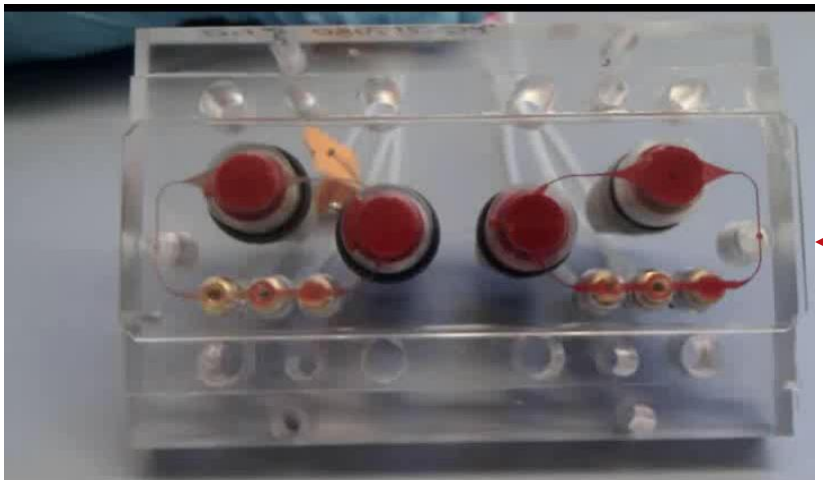
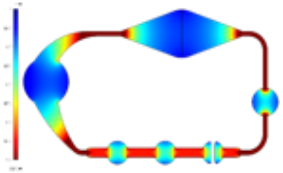
HUMIMIC Chip2 Features

- Size of a standard microscope slide
- On-chip micro-pump enabling pulsatile flow
- Suitable for iPSC-derived cells, primary cells, 3D tissues and cell lines
- Compatible with life tissue imaging
- Plug-in option for insert-based barrier models



Standard cell culture inserts *(96-/12-/24-well format)*

COMSOL Multiphysics® 5.2.



Selected Case Studies

Cosmetics Europe
the personal care association

BDF Beiersdorf

Nr.	Organ model	Schematic	Context of use	Level of readiness	Species
3	Skin - Liver		Hazard identification, Tier 3	III	
4	Intestine - Liver		Absorption, metabolism	III	
5	Lung - Liver		Hazard identification	III	
6	Liver - Pancreas		Diabetes drug substances	III	
7	Skin - Tumor		Anti-tumor antibodies	III	
8	Thyroid - Liver		Hazard identification, safety	II	vs
11	Skin - Leukocytes		Allograft rejection therapies	II	
12	Intestine - Muscle		Muscle growth agents	II	vs
13	vasc. Pancreas - Tumor		Anti-tumor therapy	II	
14	Bone		Nanoparticle toxicity	I	
15	Bone marrow		Erythropoiesis	I	
16	Skin - Hair follicles		Hair growth agents	I	
17	Liver - Cardio		Metabolite cardiotox	I	
18	Liver - Kidney		Kidney toxicity	I	
21	ADME-axis + 1		ADME-profile, PBPK, Tox	I	
22	Blood-Brain-Barrier		Permeability & Neurotoxicity	I	

BAYER

EUTOXRISK

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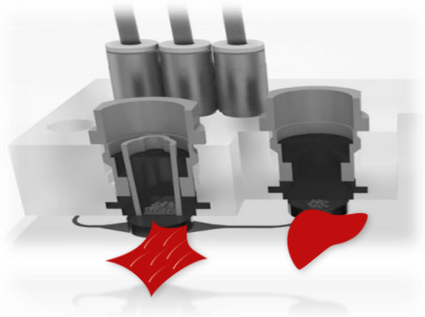
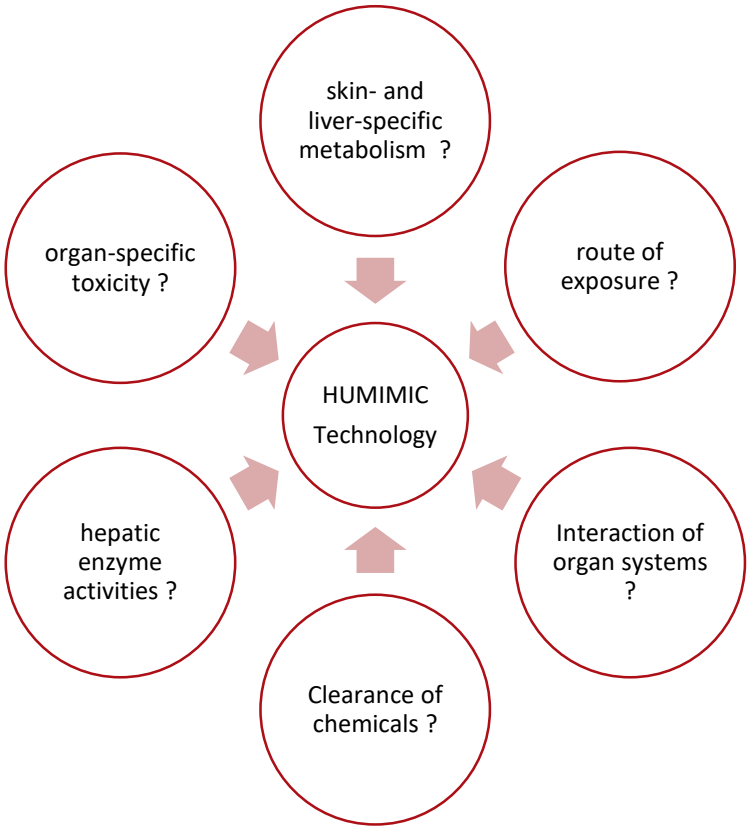
- DMPK / Safety/ Hazard identification
- Mode of Action /Efficacy

III - Assay established, available for testing/assay transfer
II - Model qualified, available for assay establishment
I - Proof of Concept, available for joint model & assay development

Aim of the Skin-Liver Model Project





- Evaluation of HUMIMIC Technology to contribute to safety assessment for subacute, repeated dose systemic toxicity
- Multi-Organ-Chip model to investigate the interaction of skin- and liver-specific metabolism of cosmetics chemicals after single and repeated dermal and systemic exposure

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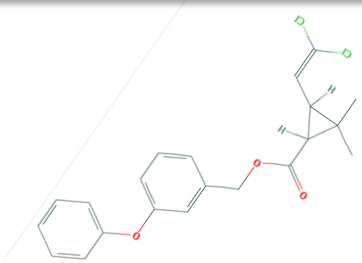
Kühnl et al., *Toxicology* 2021

Project Phase 1 – POC Chemical Selection

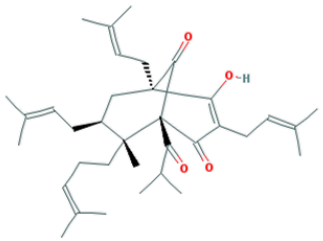
Permethrin



- Pesticide (occupational)
- Ointment against scabies

Focus: Metabolites

Hyperforin



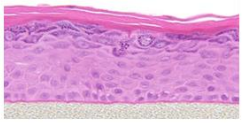
- Phytochemical – St. John's wort
- Activates CYP3A4 and CYP2C9 via PXR
- Cosmetics/Dermatics
- Antidepressants - NT reuptake inhibitor

Focus: XME induction

Selected Organ Models and Application Routes

EpiDerm Model

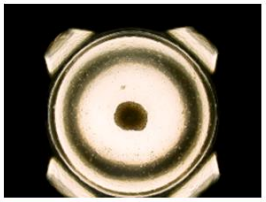
- epidermis model
- human epidermal keratinocytes
- exhibits human epidermal tissue structure and cellular morphology
- organized and proliferative basal cells, spinous and granular layers, and cornified epidermal layers

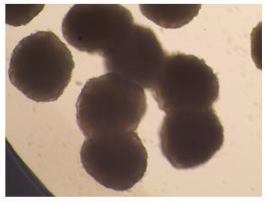


Liver spheroids

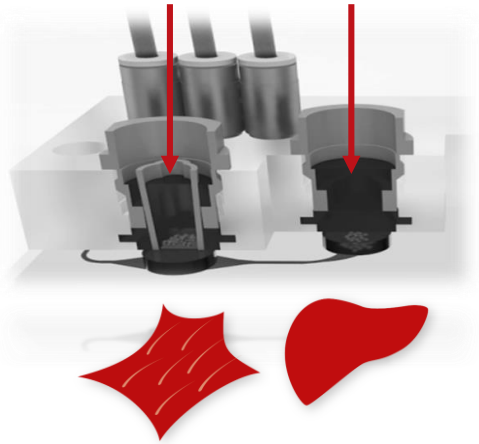
HepaRG + Stellate cells

- liver model
- Human hepatocytes (HepaRG cell line by Bioprodic) and human primary stellate cells
- → ratio 24:1
- 25,000 cells/spheroid

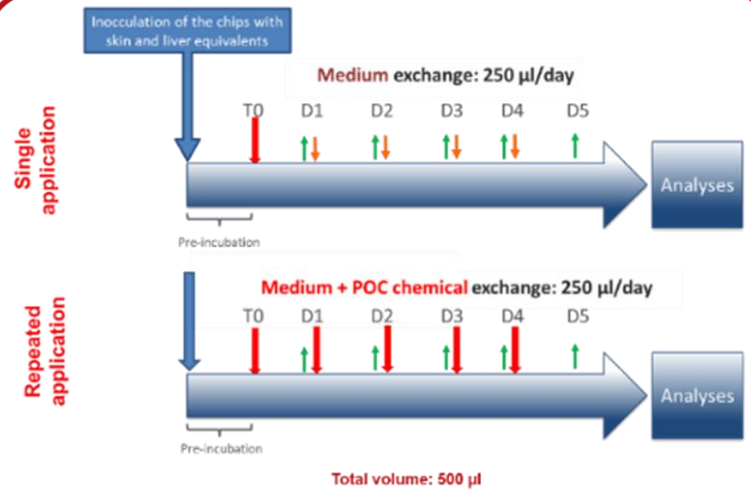




topical systemic



Experimental Design



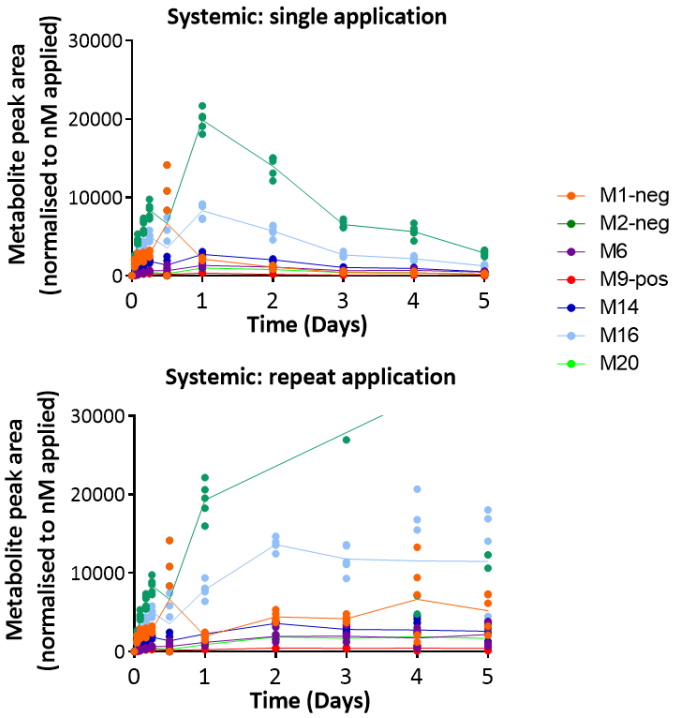
- 46 – 54 circuits per experiment, 3-5 circuits per condition
- Solvent control + negativ control

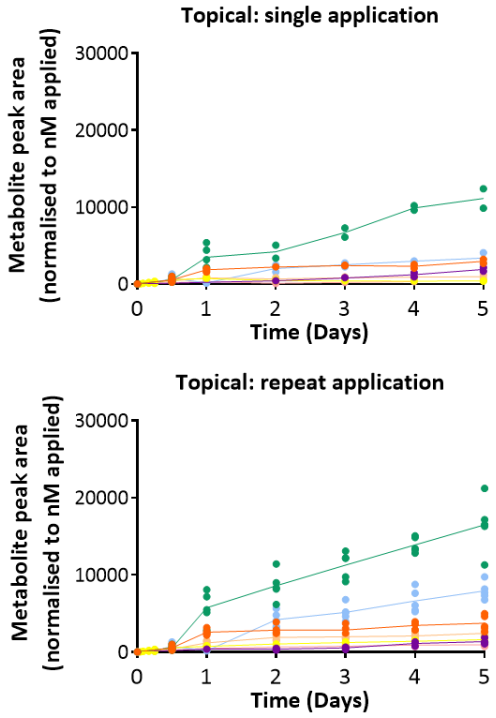
Different endpoints after 1st substance application: 24 hours, 48 hours, 5 days

Read-outs:

- metabolic analyses
- parent compound and metabolites quantification
- histology
- qPCR
- RNA sequencing

Systemic vs. Topical Application: Permethrin Metabolite Kinetics





- Metabolite kinetics of single topical application were different from a single systemic application
- Repeat topical application resulted in similar metabolic profile to repeated systemic application–only M2 and M16 were present at lower concentrations

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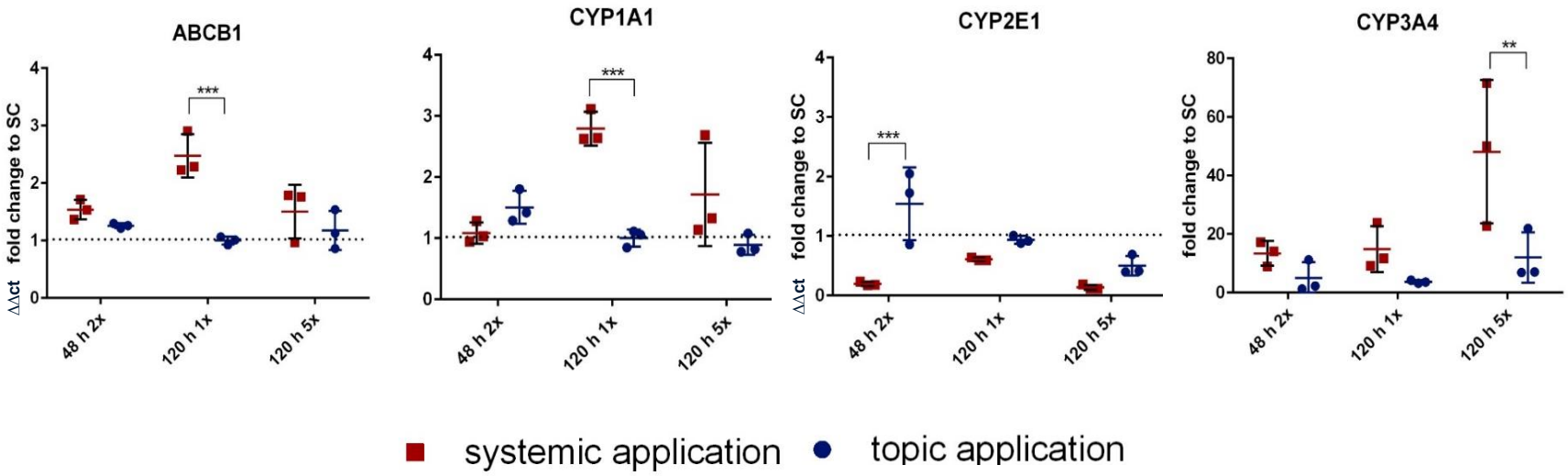
Hyperforin: XME Gene Modulation



Liver organoids show XME gene modulation by hyperforin and respond differently to topical vs. systemic application at certain time points

Kühnl et al., ***Toxicology*** 2021
Characterization of application scenario-dependent pharmacokinetics and pharmacodynamic properties of permethrin and hyperforin in a dynamic skin and liver multi-organ-chip model

Predefined Success Criteria for Project Phase 1

Deliverable	
Maintenance of skin and liver organoid structure and functionality in MOC	✓
Transferability of MOC method to other labs	✓
High intra- and inter-laboratory reproducibility	✓
Demonstration of route effects on metabolism of POC chemicals	✓
Verification of application frequency effects on metabolism of POC chemicals	✓
Demonstrate that application route and frequency affects XME levels in liver organoids	✓

A second project phase has been successfully completed evaluating Genistein and 4-amino-2-hydroxytoluene (AHT). Data are processed.

Acknowledgements

Cosmetics Europe ADME Task Force

Cosmetics Europe
Nicky Hewitt
Martina Klaric (formerly)

Pierre Fabre Dermo-Cosmétique
Carine Jacques-Jamin
Camille Géniès
Hélène Duplan

L'ORÉAL Research & Innovation
Sébastien Grégoire

P&G
Corie Ellison
Cathy Lester

Beiersdorf
Andreas Schepky
Daniela Lange

Unilever
Ian Sorrell

BASF We create chemistry
Eric Fabian

kao
Yurika Fujita

Beiersdorf – TissUse Team

Katrin Brandmair


Thamée Rings
Silke Gerlach


Andreas Schepky


Nicky Hewitt



Thi Phuong Tao


Ilka Maschmeyer


Jochen Kühnl

Pharmacelsus CONTRACT RESEARCH ORGANISATION
for MS-analyses

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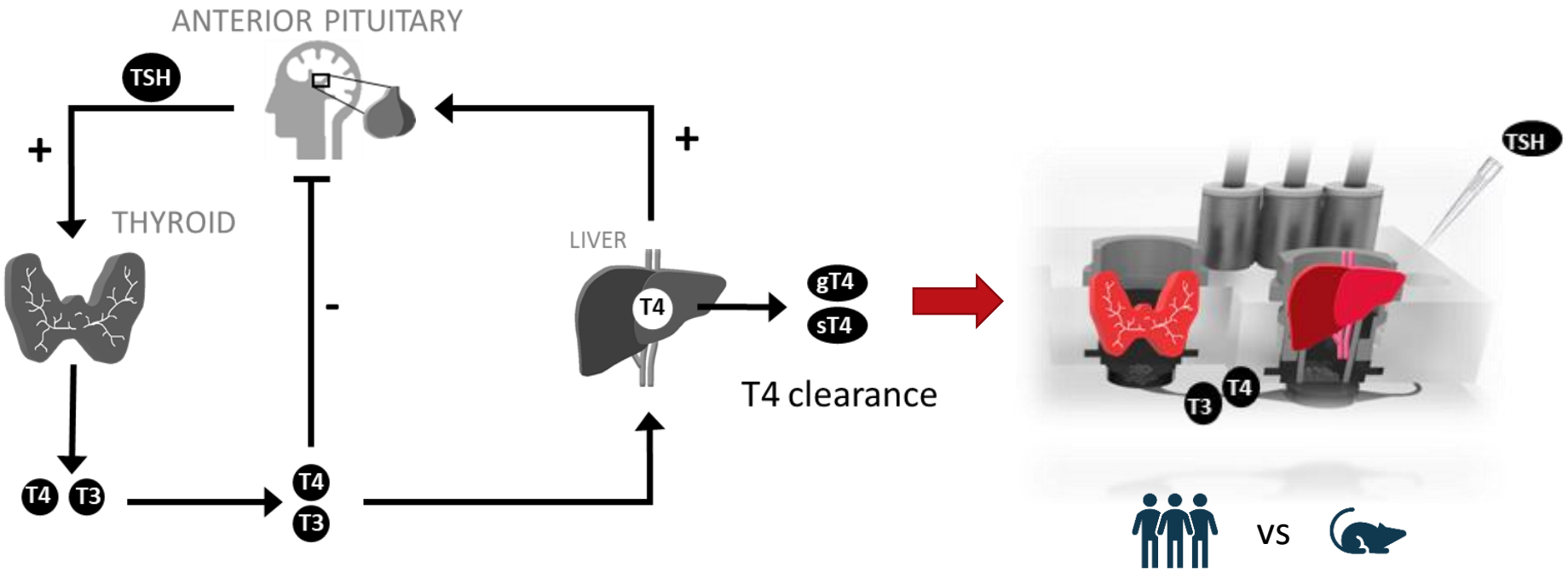
A HUMIMIC®-based Thyroid-Liver Model

Thomas Steger-Hartmann,
Marian Raschke
Remi Bars, Helen Tinwell
Diana Karwelat, Julia Kühnlenz

Hypo – and hyperthyroidism, Thyroid disruptors



TSH – thyroid-stimulating hormone, T4 – thyroxine, T3 – triiodothyronine
gT4 – glucuronidated thyroxine, sT4 – sulphated thyroxine

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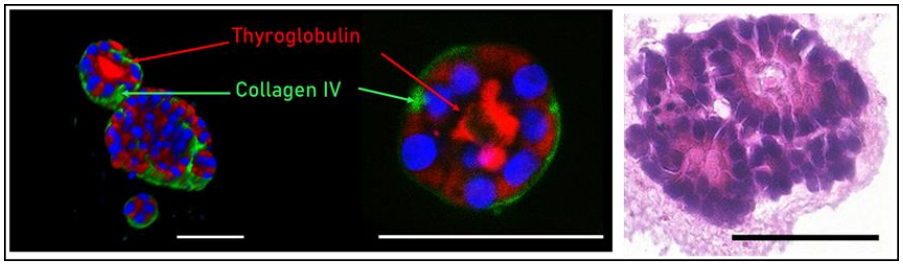
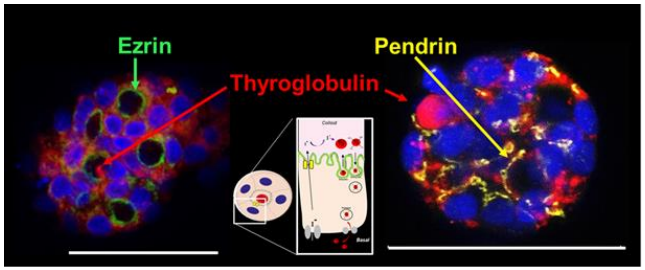
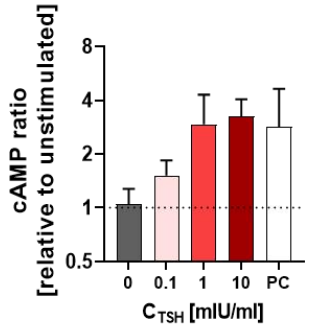
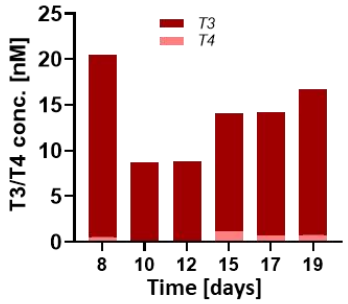
Establishing the Thyroid Models

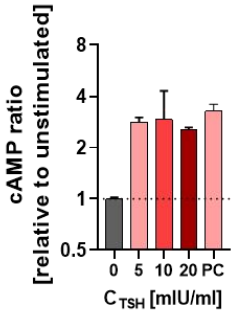
Thyroid

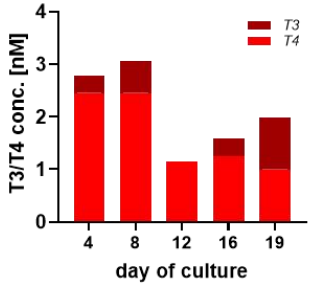












- ✓ Follicular architecture
- ✓ Correct cellular polarity
- ✓ **TSH-induced signal transduction and hormone secretion**

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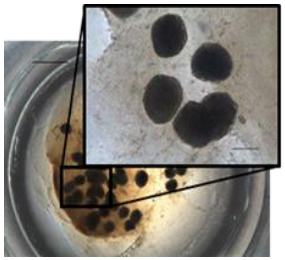
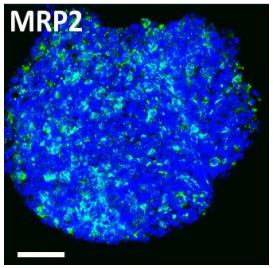
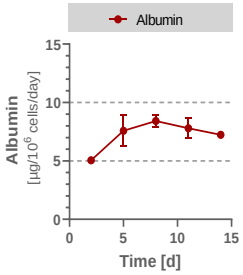
Establishing the Liver Models

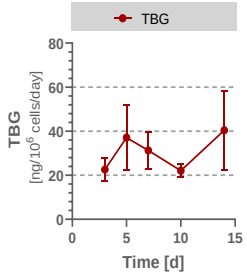
Liver

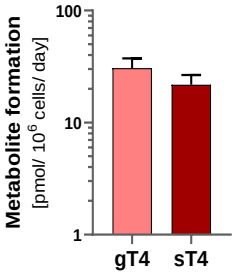
Spheroids composed of HepaRG & stellate cells





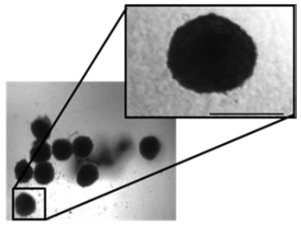


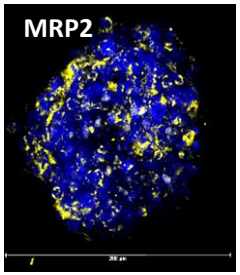


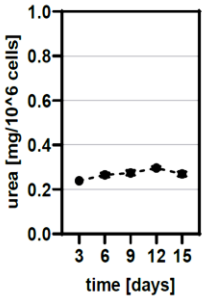


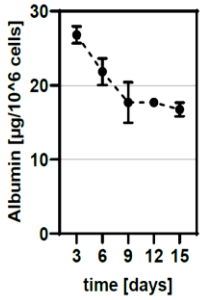
✓**Thyroid hormone glucuronidation and sulfation**

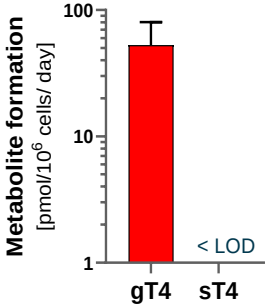
primary hepatocytes and non-parenchymal cells











✓**Thyroid hormone glucuronidation, no sulfation**

✓Basolateral and apical cell poles (bile canalicular network)

✓Stabile albumin and Thyroxin-binding globulin secretion

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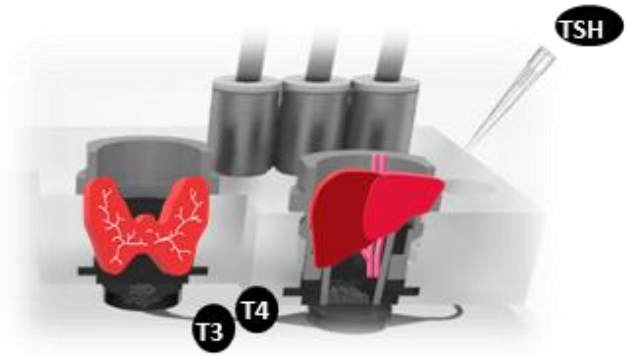
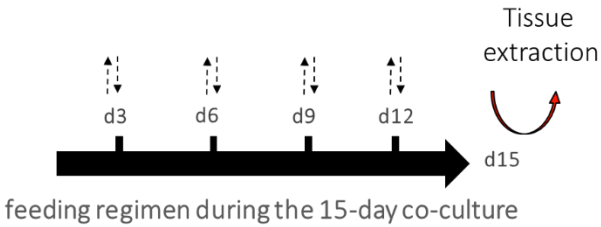
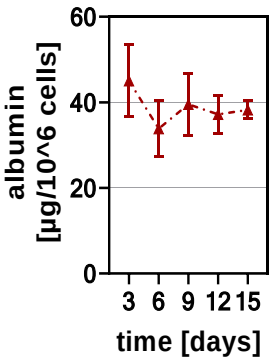
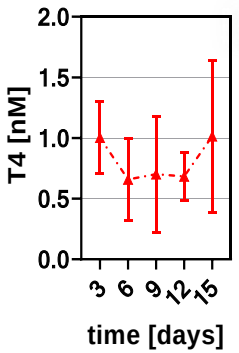
Thyroid-Liver Co-culture Data

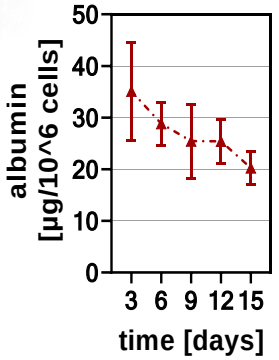
In vitro simulation of the hepatic-thyroid hormone axis with artificial administration of TSH (pitutary gland)

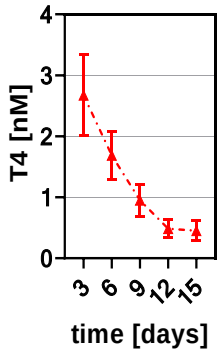












✓Thyroid hormone secretion
✓maintained albumin secretion

Successful co-culture for >14 days

Thyroid-Liver Model – Future Applications

Establishing a human assay to study the hepatic-thyroid axis in vitro

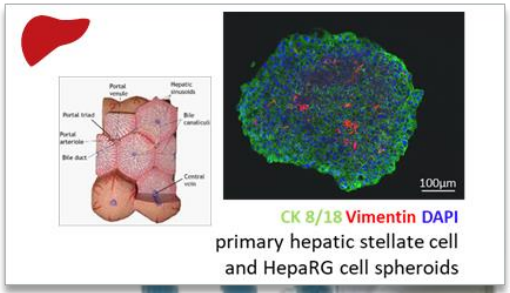
- **Direct perturbation** of the thyroid gland, e.g. by TPO inhibition
  - ► *reduced hormone synthesis*

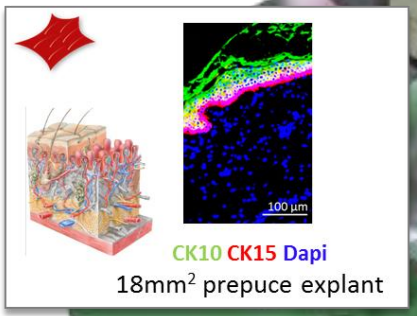
- **Indirect perturbation**, i.e. induced hepatic hormone elimination
  - ► *accelerated hormone turnover (gT4 & sT4)*

…. *Interpolation of thyroid toxicity findings from rodents to humans remains challenging.*

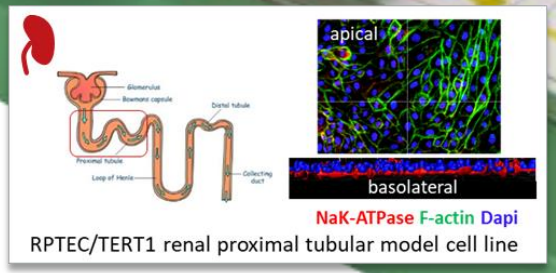
Aiming for a MPS-based Intestine-Liver-Kidney Axis

intestinal lumen: 250µl
surrogate blood circuit: 830µl
excretory circuit: 600µl





2 g/l Gluc
liver
skin
2.5 g/l Gluc
intestine
kidney
1 g/l Gluc



Maschmeyer et al.
Lab on Chip 2015

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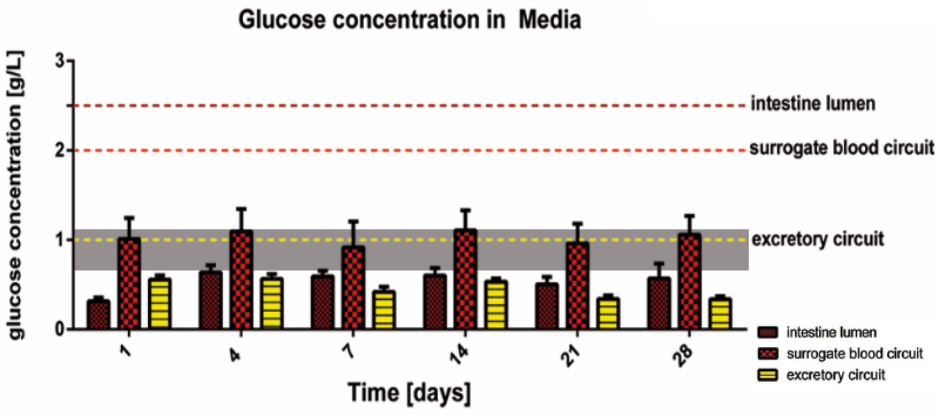
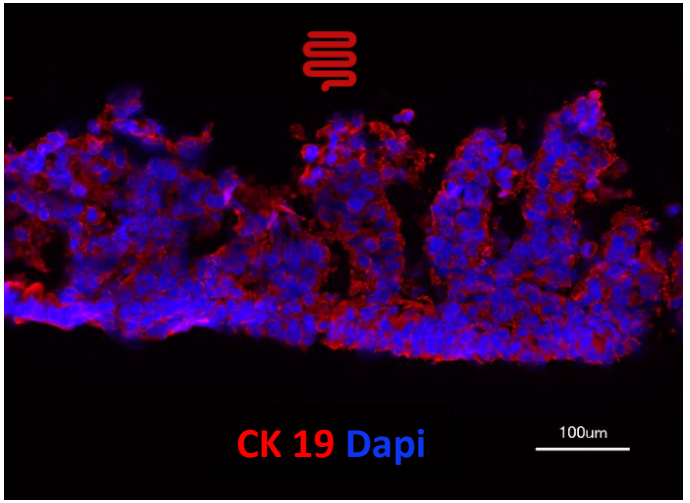
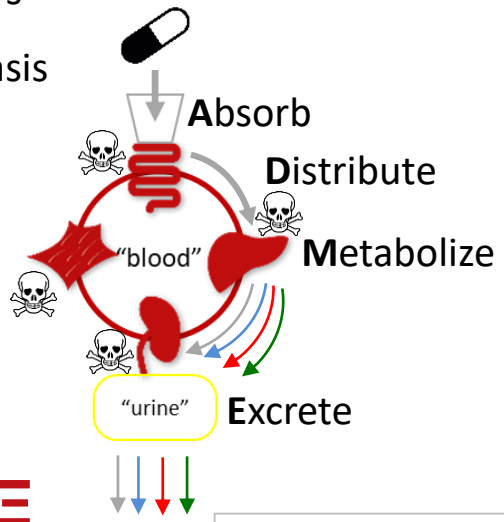
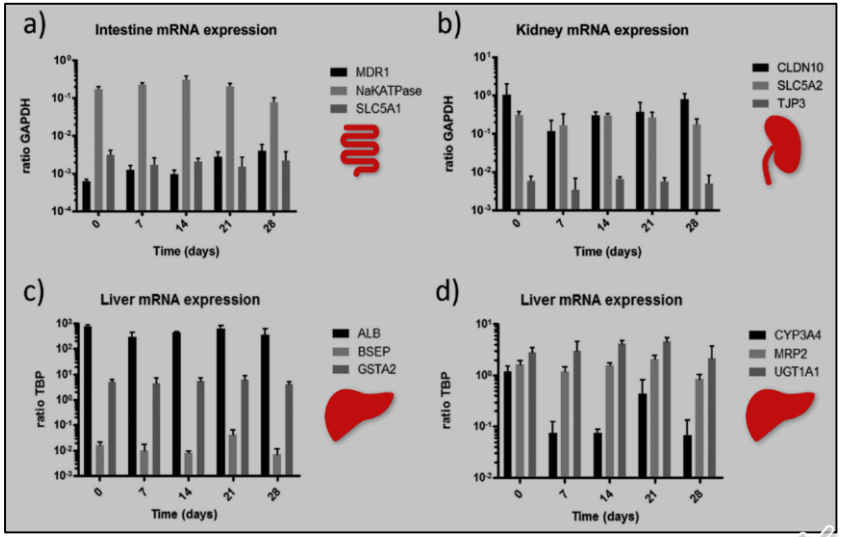
28-day Maintenance of Homeostatic Conditions





- normoglycemic blood glucose
- functional barriers
- 28-day homeostasis





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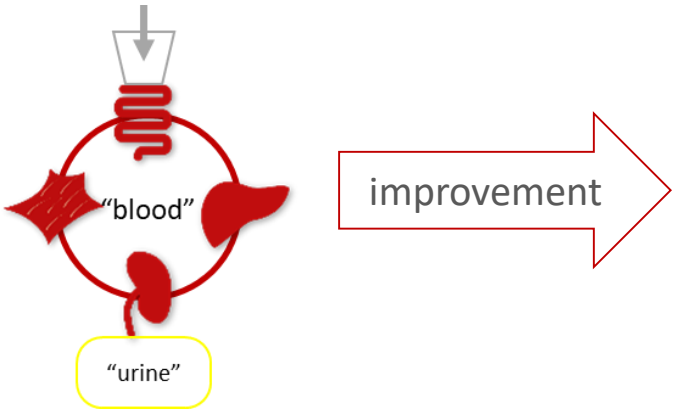
Maschmeyer et al. *Lab on Chip 2015*

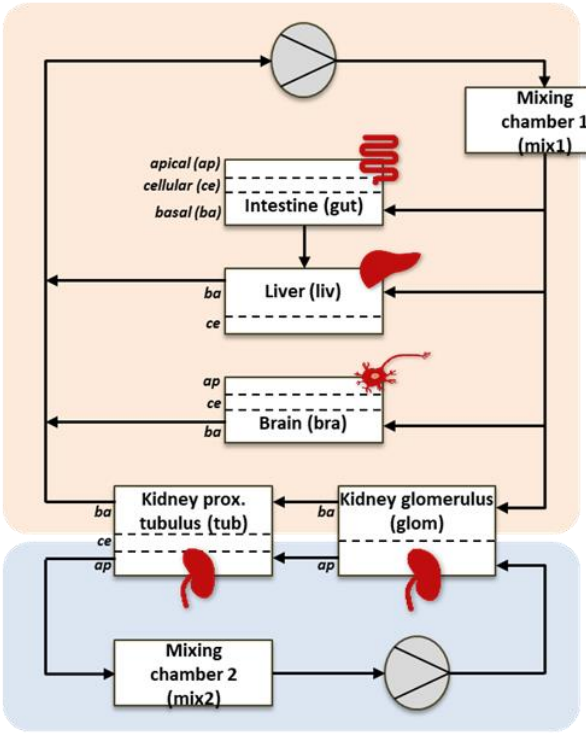
Industry-inspired Redesign

1. physiological organ arrangement
2. physiological “blood” flow rates
3. brain instead of skin
4. add glomerulus
5. autologous organ equivalents



EUTOXRISK

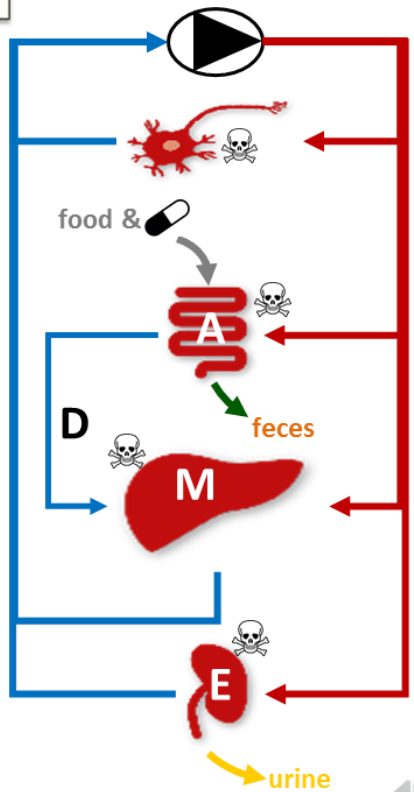
EU flagship on systemic toxicity testing



Frederik Bois, INERIS, France

Hamon et al.
Toxicology in vitro 2015

Physiology-based Four-Organ-Chip supporting QIVIVE (PBPK-compliant)



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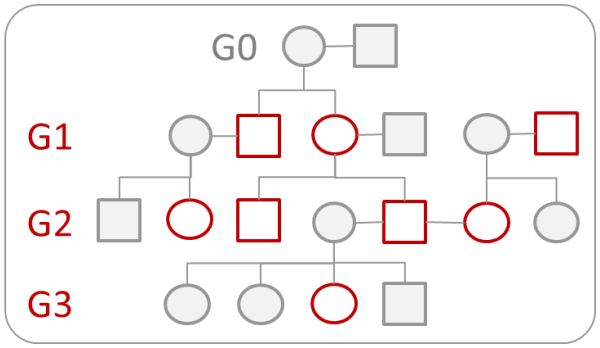
An Autologous Four-Organ-Model Co-culture

hPSCreg

Ramme et al.,
Stem Cell Research 2019

8 donors



Ethikvotum
Eth 25/16
EU compliant legal background for commercial use

induced pluripotency

TISSUi001-A
Commercial Biobank

7 MCBs

QC release

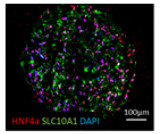
Q2 Q3 Q4 Q1 Q2
2016 **2017**

weeks -6 -5 -4 -3 -2 -1

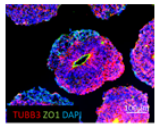
intestinal organoids
(Kaufmann et al. 2015)

connective tissue
(Zhou et al. 2013)

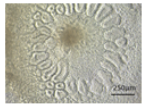
hepatocytes
(Szkolnika et al. 2014)

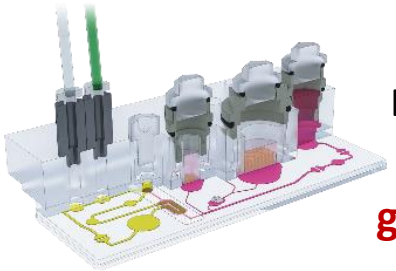


neurospheres
(in-house protocol)



renal vesicles
(Morinzane et al. 2017)





Day -6 0 14-day co-culture

growth factor-free medium with 5% human serum

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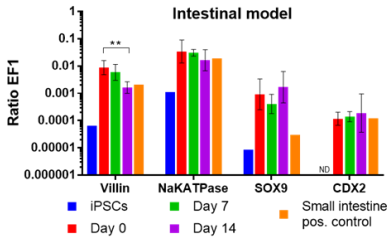
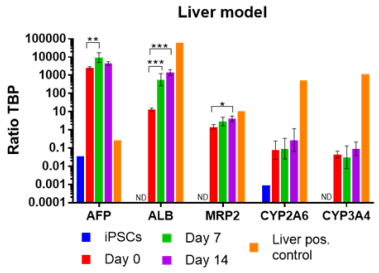
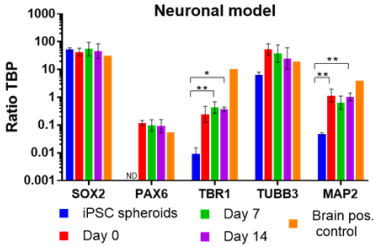
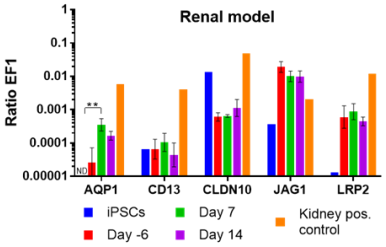
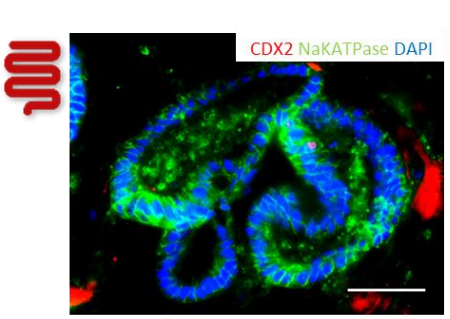
Gene Expression Analysis and Immunohistology

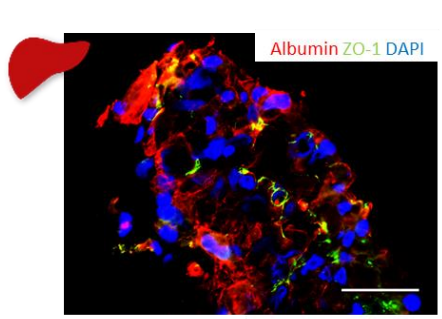


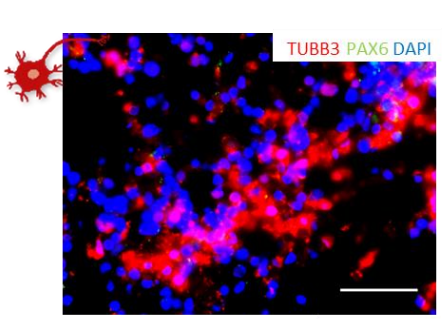


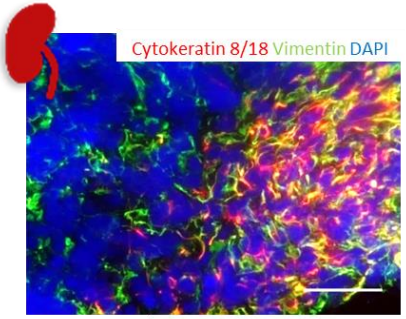


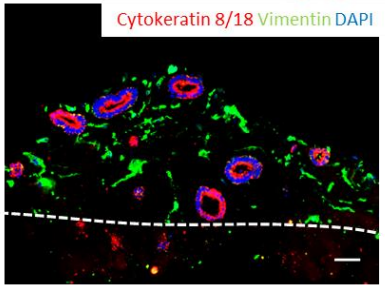


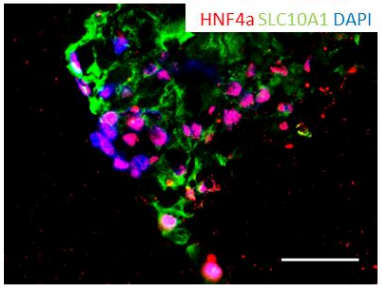


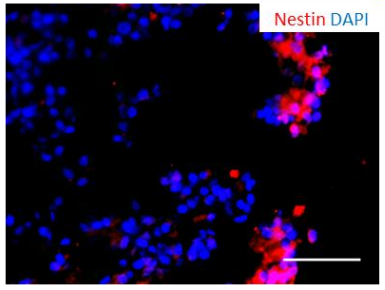


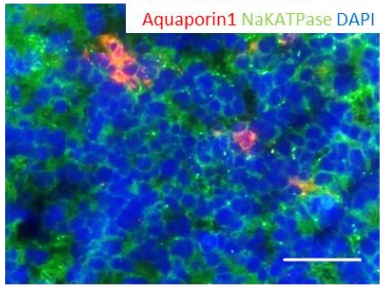












Scale = 50 µm

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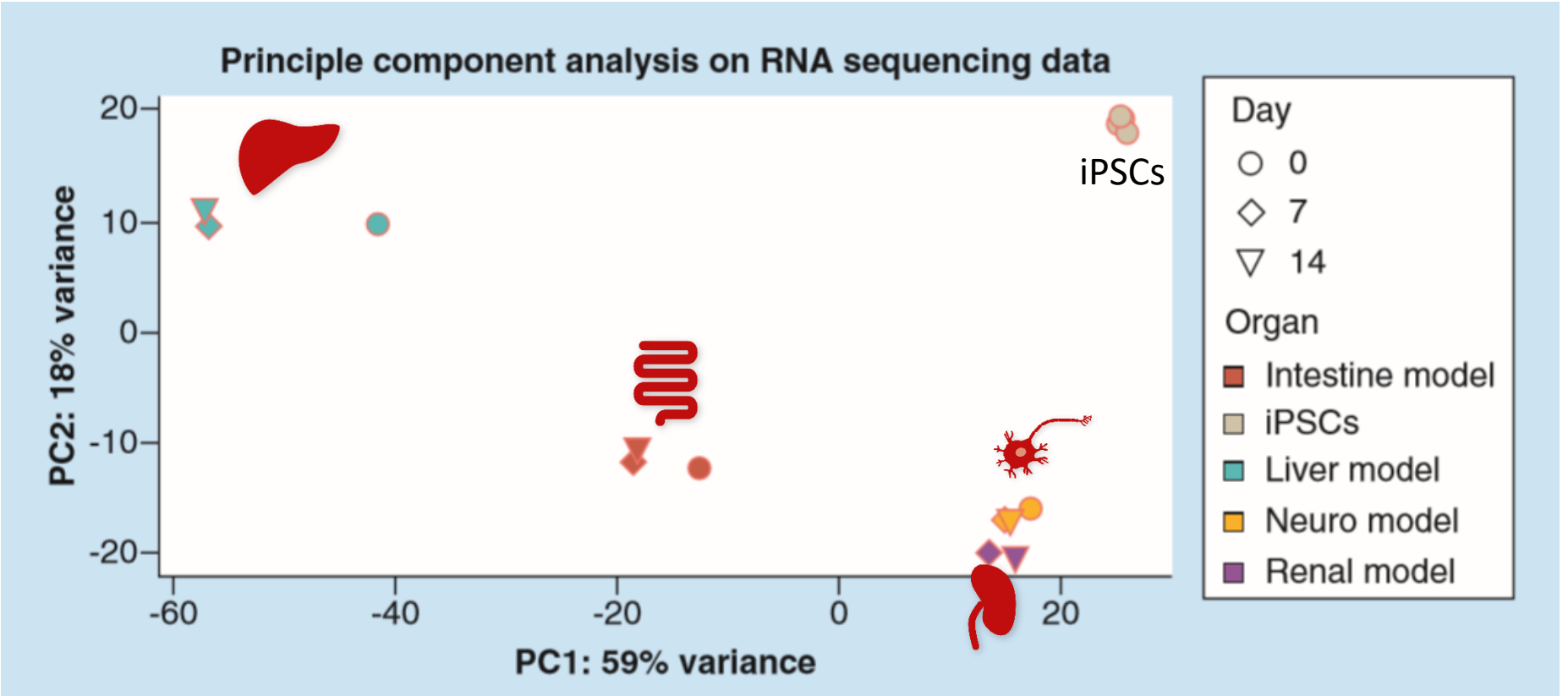
Ramme et al., *OA reviews* 2019

MPS Workshop, Jan 19-20, 2021

PC Analyses Revealed Distinct Clustering



A POC – study is ongoing to demonstrate usability of such a ADME axis model on the basis of the physiology based 4 organ chip

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Ramme et al., *OA reviews* 2019

Conclusion

Qualified MPS- based single- and multi-organ-models can provide qualified context of use assays for hazard identification, safety and efficacy tests

At academic level they support new discoveries and add to the investigation of mode of action of substances and therapies

The establishment of MPS-based animal models is possible but requires the same effort as the human models do.

Nr.	Organ model	Schematic	Context of use	Level of readiness	Species
1	Bone marrow		Bone marrow toxicity	III	
2	Hair follicle		Hair growth agents	III	
3	Skin - Liver		Hazard identification, Tier 3	III	
4	Intestine - Liver		Absorption, metabolism	III	
5	Lung - Liver		Hazard identification	III	
6	Liver - Pancreas		Diabetes drug substances	III	
7	Skin - Tumor		Anti-tumor antibodies	III	
8	Thyroid - Liver		Hazard identification, safety	II	
9	Testis - Liver		Testicular toxicity	II	
10	Liver - Neuro		Metabolite neurotoxicity	II	
11	Skin - Leukocytes		Allograft rejection therapies	II	
12	Intestine - Muscle		Muscle growth agents	II	
13	vasc. Pancreas - Tumor		Anti-tumor therapy	II	
14	Bone		Nanoparticle toxicity	I	
15	Bone marrow		Erythropoiesis	I	
16	Skin - Hair follicles		Hair growth agents	I	
17	Liver - Cardio		Metabolite cardiotox	I	
18	Liver - Kidney		Kidney toxicity	I	
19	Skin - Lymph node		Hazard identification, Tier 3	I	
20	vasc. Intestine – Lymph node - Tumor		Immuno-Oncology	I	
21	ADME-axis + 1		ADME-profile, PBPK, Tox	I	
22	Blood-Brain-Barrier		Permeability & Neurotoxicity	I	

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The joint Team is the Key for our Success

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THANK YOU!

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MPS Workshop, Jan 19-20, 2021

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Medizinische Biotechnologie