The Final EUTOXRISK Symposium 7

Advanced test methods developed in the EU-ToxRisk project (WP8)

WP8 partners

Coordinated by: InSphero

4-organ ADME/Tox chip

Beren Attac
Uwe Marx

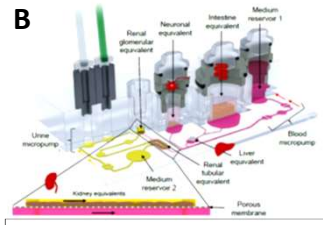
TISSUSE

4-organ chip (intestine, liver, neuronal, and kidney) build-up and blood flow simulation

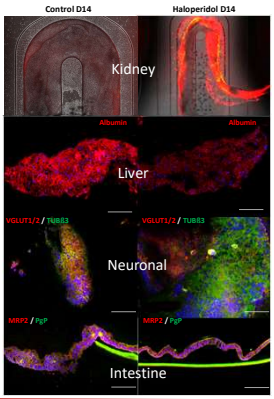
A

Organ	Human Data*		Chip Data	
	Blood flow in L/h (L/min)	Ratio of blood flow in %	Simulation Proportion in %	Theoretical volumetric flow in µl/min**
Portal system: Pancreas/Intestine	64.3 (1.07)	35.3	35	10.6
Liver	82.0 (1.37)	45.2	45	13.6
through portal vein	~64.3 (1.07)	35.3	35	10.6
trough hepatic artery	~17.7 (0.30)	9.9	10	3
Kidney	61.1 (1.02)	33.7	34	10.3
X	38.6 (0.64)	21.1	21	6.4
Total (arterial blood)	181.7 (3.03)	100	100	30.3

B

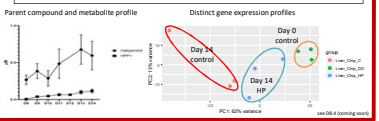


14 Days treatment with Haloperidol (3 µM)



PgP inhibition

no signs of morpholoical changes

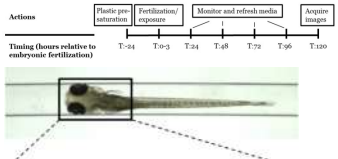


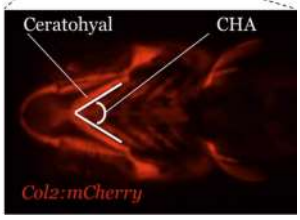
- Human ADME chip at 1:100.000 scale
- Accommodates gut, liver, neuro and kidney
- Metabolic competence to assess metabolite effects
- Barrier function (gut, kidney) stable over 14 days
- No signs of necrosis observed over 14-days culture
- Stable glucose, lactate and LDH values over 14d
- Increasing metabolite formation over time (HPP+)
- Only subtle effects observed upon HP-treatment

Reporter Zebra Fish

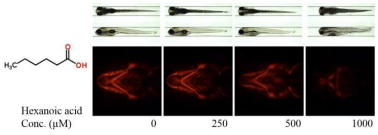
Bjorn Koch
Annemarie H. Meijer
Hermann Spaink

Universiteit Leiden

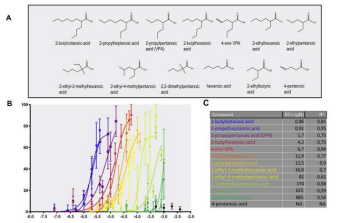




Col2:mCherry reporter visualizes ceratohyal angle during craniofacial development



- Developmental toxicity cannot be investigated in simple cell culture assays, as it involves concurrent cell migration and differentiation processes
- The v-shaped ceratohyal cartilage around day 5-post fertilization is highly dynamic and therefore represents a sensitive readout for DART
- CS-1 compounds (VPA and analogues) could be sorted for their relative potency of inflicting developmental toxicity



CS1 data, comparing effects of 13 compounds with chemical structures similar to valproic acid, and their relative potency in causing craniofacial developmental toxicity.

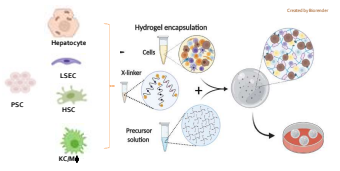
B.E.V. Koch et al. / Toxicology Letters 342 (2021) 20–25

Use of complex *in vitro* models for the study of complex drug-target and off-target interactions

Universiteit Leiden

Tissue-engineered iPSC-derived liver model

KU LEUVEN



PSC-derived hepatocytes and NPCs are further differentiated and maturated supported by engineered ECM scaffold and improved media.

- A full-liver model was established by combining PSC-derived parenchymal (Heps) and non-parenchymal (endothelial, macrophages, and stellate) cells, cultured in bioengineered hydrogel promoting hepatocyte differentiation
- After a 40-day differentiation process, liver typical morphology and function could be observed
- The model is suitable to induce a NASH like phenotype and to test anti-NASH drugs

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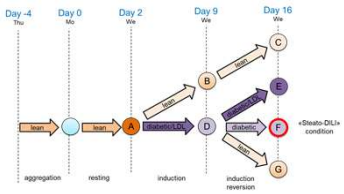
- Steatosis affects >25% of worldwide population, but is mostly ignored in pre-clin. and clinical safety
- 3D liver models can be induced for steatosis, which affects lipid and xenobiotic metabolism on gene expression level and enzymatic activity
- The models allow compound testing for 7 days
- Steatosis shows altered sensitivity to classical DILI compounds, which partially could be explained by changes in CYP activity

Steatotic liver induction scheme

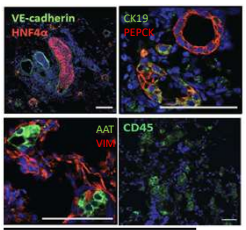


iPSC-derived liver model

Manoj Kumar
Cath. Verfaillie

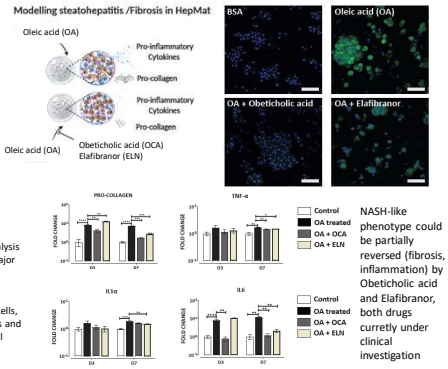
KU LEUVEN

Tissue-engineered iPSC-derived liver models to simulate NASH for drug candidate testing



Cell Type	Frequency	Percentage	Abs used
Cholangiocyte	913	4.68	KRT19
Endothelial	381	1.95	CD31
Macrophage	2455	12.57	CD68
Mesenchymal	2633	13.48	VIM
Hepatocyte Intermediate	895	4.58	HNF4a, PEPCK
Hepatocyte Mature	3051	15.62	CYP3A4, ALB
Hepatocyte Mesenchymal	1249	6.39	VIM, ALB
Progenitor	1265	6.48	AFP, KRT19
Blank/Other	6692	34.26	No Signal present

Cell-type analysis reveals all major liver cell constituents, hepatocytes, endothelial cells, macrophages and mesenchymal cells



NASH-like phenotype could be partially reversed (fibrosis, inflammation) by Obeticholic acid and Elafibranor, both drugs currently under clinical investigation

Kumar et al; j.biomaterials.2021.121006

Diseased liver model

Monika Kijanska
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Wolfgang Moritz

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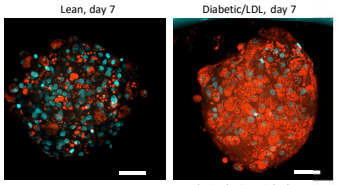
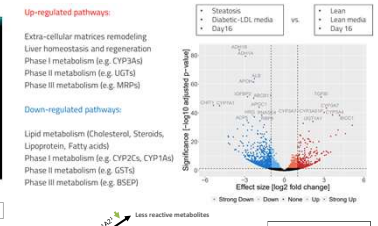
Induction of steatotic phenotype upon lipid loading

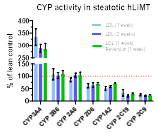
Nile Red O staining, CQ1 confocal imaging, MIP (lipids: red; nuclei: blue)



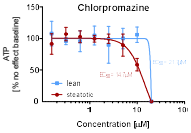
Altered gene expression profile relevant to drug metabolism

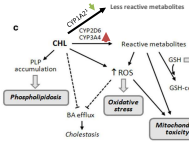


Steatosis affects CYP activities



Altered sensitivity to DILI cmpd





Potential mechanism of observed hypersensitivity towards Chlorpromazine in steatotic liver microtissues

modified from: Archives of Toxicology (2019) 93:519–532 doi.org/10.1016/j.bcp.2010.06.045

Contributing partners

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Several advanced *in vitro* models were established at current TRL 4-8. They all represent heterotypic multicellular systems, providing metabolic and immune-competence in health and disease for better hazard identification and risk stratification.

The EU-ToxRisk project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 681002.

www.eu-toxrisk.eu/