Breaking the barriers for toxicogenomics in risk assessment

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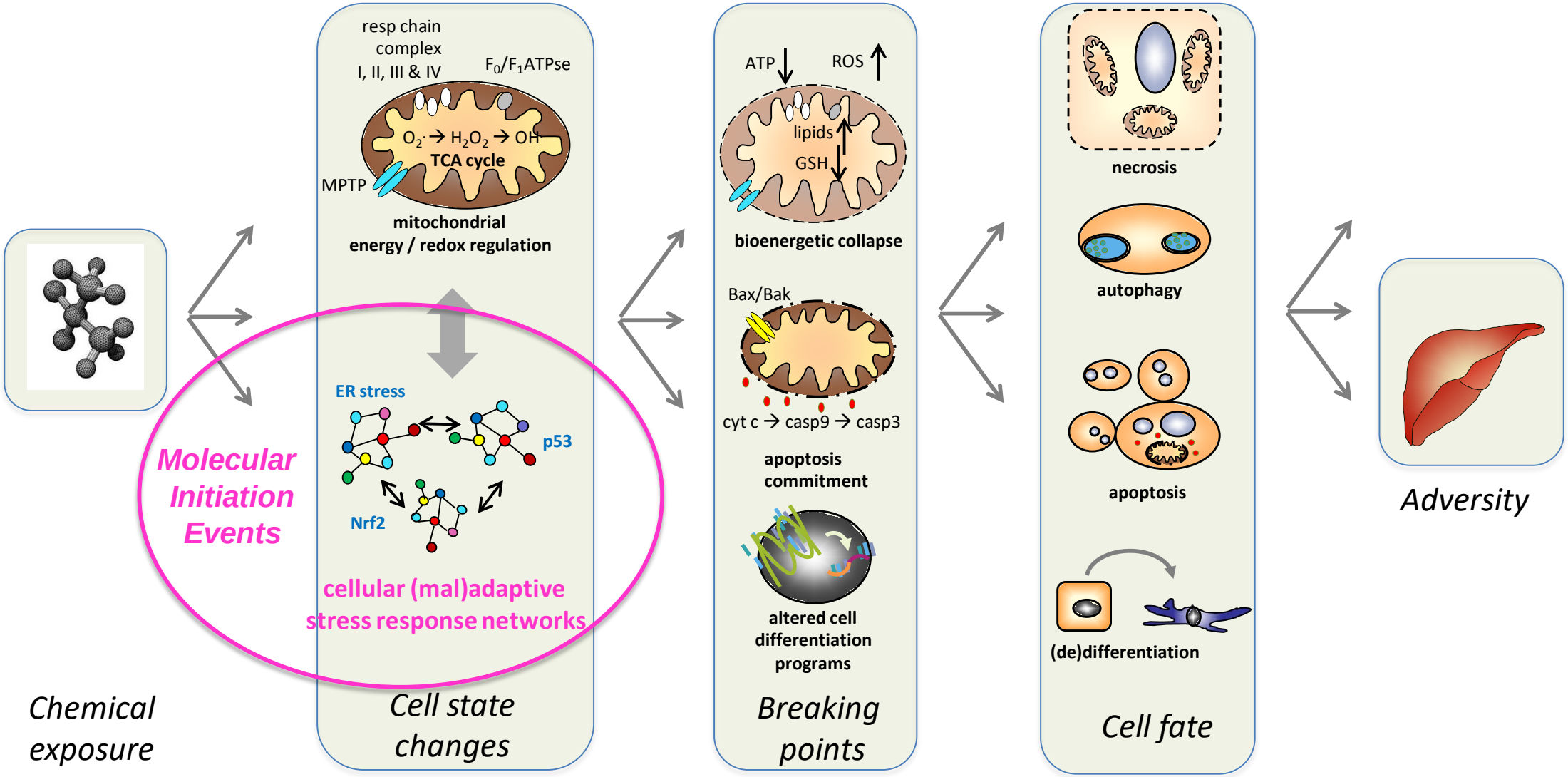
Universiteit Leiden
The Netherlands

LACDR
Leiden Academic Centre for Drug Research

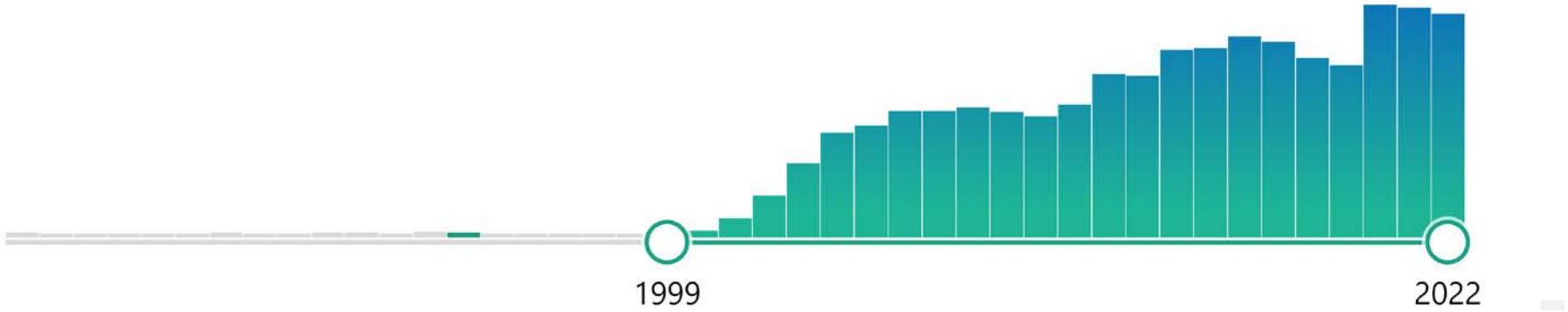
From chemical exposure to adversity: transcriptional changes representing KEs and driving Adverse Outcomes.



Wink et al. Chem Res Toxicol 2014

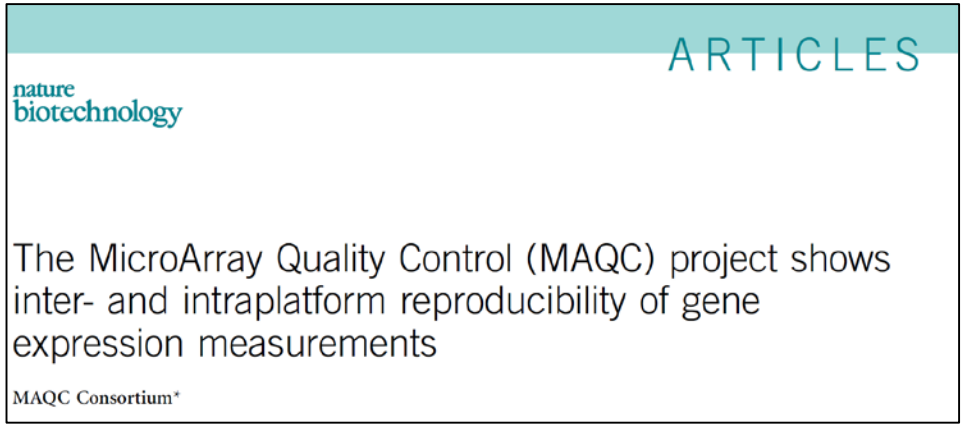
A brief historical perspective of toxicogenomics.

- ~3,000 publications mentioning toxicogenomics in PubMed
- 259 publications with toxicogenomics & risk assessment terms
- High expectations at early stage in pharma - voluntary inclusion of TXG data in FDA approvals
- Toxicogenomics so far largely for deriving mechanistic hypothesis for mode of action



Spotted arrays → Affymatrix arrays → RNAseq → targeted RNAseq

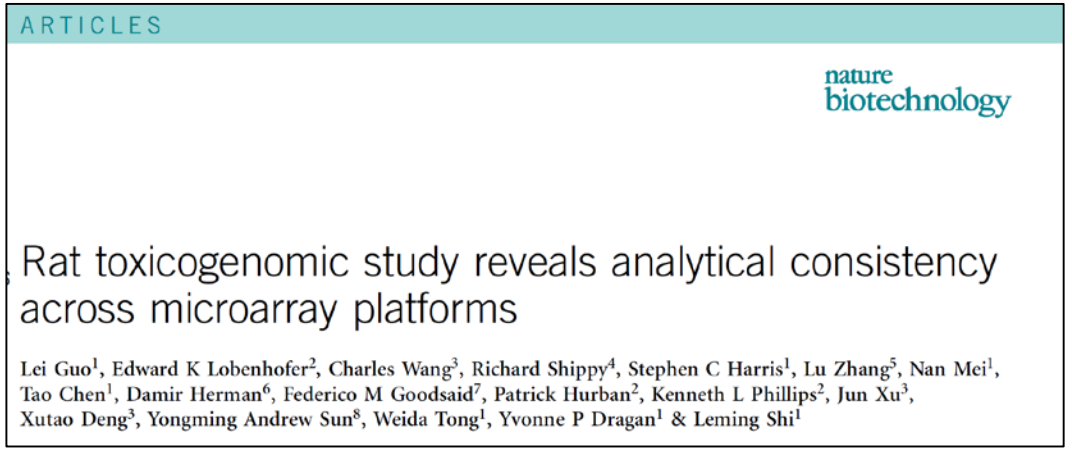
Systematic evaluation of transcriptomics technology: MAQC consortium.


ARTICLES

nature biotechnology

The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements

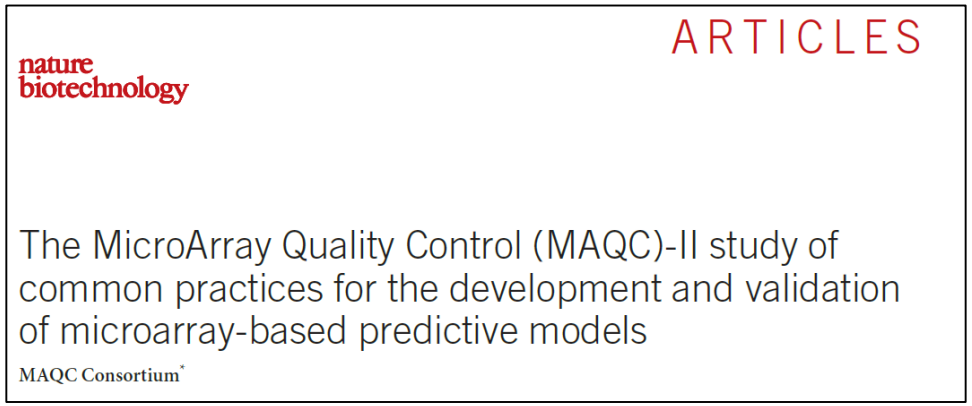
MAQC Consortium*


ARTICLES

nature biotechnology

Rat toxicogenomic study reveals analytical consistency across microarray platforms

Lei Guo[1], Edward K Lobenhofer[2], Charles Wang[3], Richard Shippy[4], Stephen C Harris[1], Lu Zhang[5], Nan Mei[1], Tao Chen[1], Damir Herman[6], Federico M Goodsaid[7], Patrick Hurban[2], Kenneth L Phillips[2], Jun Xu[3], Xutao Deng[3], Yongming Andrew Sun[8], Weida Tong[1], Yvonne P Dragan[1] & Leming Shi[1]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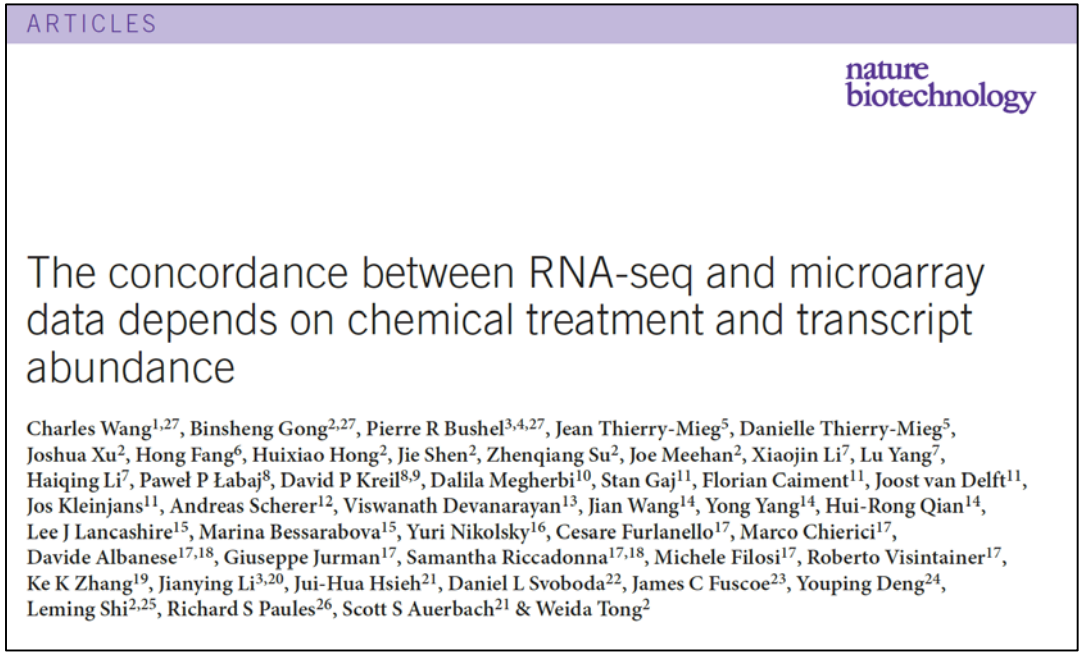
nature biotechnology

The MicroArray Quality Control (MAQC)-II study of common practices for the development and validation of microarray-based predictive models

MAQC Consortium*


ARTICLES

nature biotechnology

The concordance between RNA-seq and microarray data depends on chemical treatment and transcript abundance

Charles Wang[1,27], Binsheng Gong[2,27], Pierre R Bushel[3,4,27], Jean Thierry-Mieg[5], Danielle Thierry-Mieg[5], Joshua Xu[2], Hong Fang[6], Huixiao Hong[2], Jie Shen[2], Zhenqiang Su[2], Joe Meehan[2], Xiaojin Li[7], Lu Yang[7], Haiqing Li[7], Paweł P Łabaj[8], David P Kreil[8,9], Dalila Megherbi[10], Stan Gaj[11], Florian Caiment[11], Joost van Delft[11], Jos Kleinjans[11], Andreas Scherer[12], Viswanath Devanarayan[13], Jian Wang[14], Yong Yang[14], Hui-Rong Qian[14], Lee J Lancashire[15], Marina Bessarabova[15], Yuri Nikolsky[16], Cesare Furlanello[17], Marco Chierici[17], Davide Albanese[17,18], Giuseppe Jurman[17], Samantha Riccadonna[17,18], Michele Filosi[17], Roberto Visintainer[17], Ke K Zhang[19], Jianying Li[3,20], Jui-Hua Hsieh[21], Daniel L Svoboda[22], James C Fuscoe[23], Youping Deng[24], Leming Shi[2,25], Richard S Paules[26], Scott S Auerbach[21] & Weida Tong[2]

Systematic reporting of toxicogenomics data: OECD EAGMST omics reporting working group.

Regulatory Toxicology and Pharmacology 125 (2021) 105020

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Commentary

Progress towards an OECD reporting framework for transcriptomics and metabolomics in regulatory toxicology

Joshua A. Harrill[a,1,*], Mark R. Viant[b,c,**,1], Carole L. Yauk[d,1,***], Magdalini Sachana[e], Timothy W. Gant[f], Scott S. Auerbach[g], Richard D. Beger[h], Mounir Bouhifd[i], Jason O'Brien[j], Lyle Burgoon[k], Florian Caiment[l], Donatella Carpi[m], Tao Chen[h], Brian N. Chorley[a], John Colbourne[b,c], Raffaella Corvi[m], Laurent Debrauwer[n,o], Claire O'Donovan[p], Timothy M. D. Ebbels[q], Drew R. Ekman[r], Frank Faulhammer[s], Laura Gribaldo[m], Gina M. Hilton[t], Stephanie P. Jones[j], Aniko Kende[u], Thomas N. Lawson[c], Sofia B. Leite[m], Pim E.G. Leonards[v],

Regulatory Toxicology and Pharmacology 112 (2020) 104621

Contents lists available at ScienceDirect

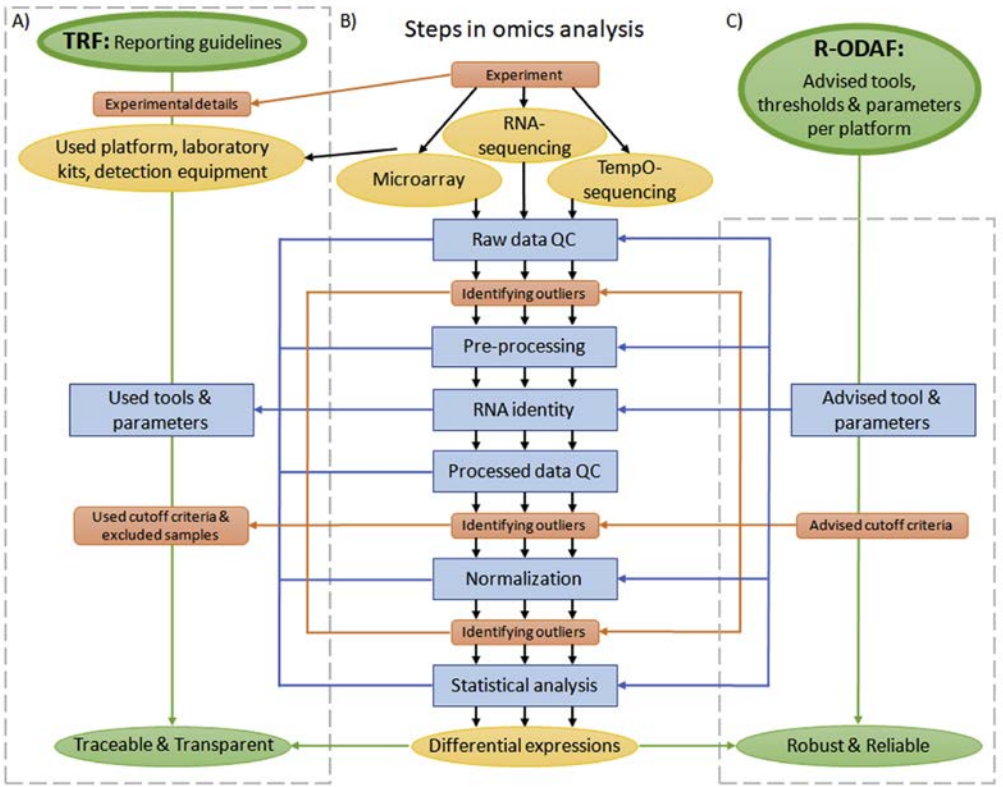
Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Towards the development of an omics data analysis framework

Marcha Verheijen[a], Weida Tong[b], Leming Shi[c], Timothy W. Gant[d], Bruce Seligman[e], Florian Caiment[a,*]

[a] Maastricht University, Maastricht, the Netherlands
[b] National Center for Toxicological Research, U.S. Food and Drug Administration (FDA), Jefferson, AR, USA
[c] Fudan University (FU), Yangpu District, Shanghai, China
[d] Public Health England (PHE), Harwell Science and Innovation Campus, Oxfordshire, UK
[e] Biospyder, Tucson, USA



Current gap: toxicogenomics interpretation framework for implementation in risk assessment.

Hurdles for qualitative and quantitative interpretation of toxicogenomics datasets

- Mechanistic interpretation based **only known biology:** an emphasis on cancer biology
- Commercial and public domain annotations (e.g. IPA and GSEA): gives **redundant information**.
- **Gene regulation and function is not identical** is diverse cells and tissues.
- Experimental variability and statistical thresholds may provide **different differential expressed gene sets**.

→ **Solution: test system and in vivo target organ co-regulated gene network analysis for quantitative toxicogenomics data interpretation.**

- Transcriptomics expensive **prohibiting concentration response** assessment for PoD

→ **Solution: high throughput transcriptomics using targeted RNAseq technology**

Gene co-expression network analysis

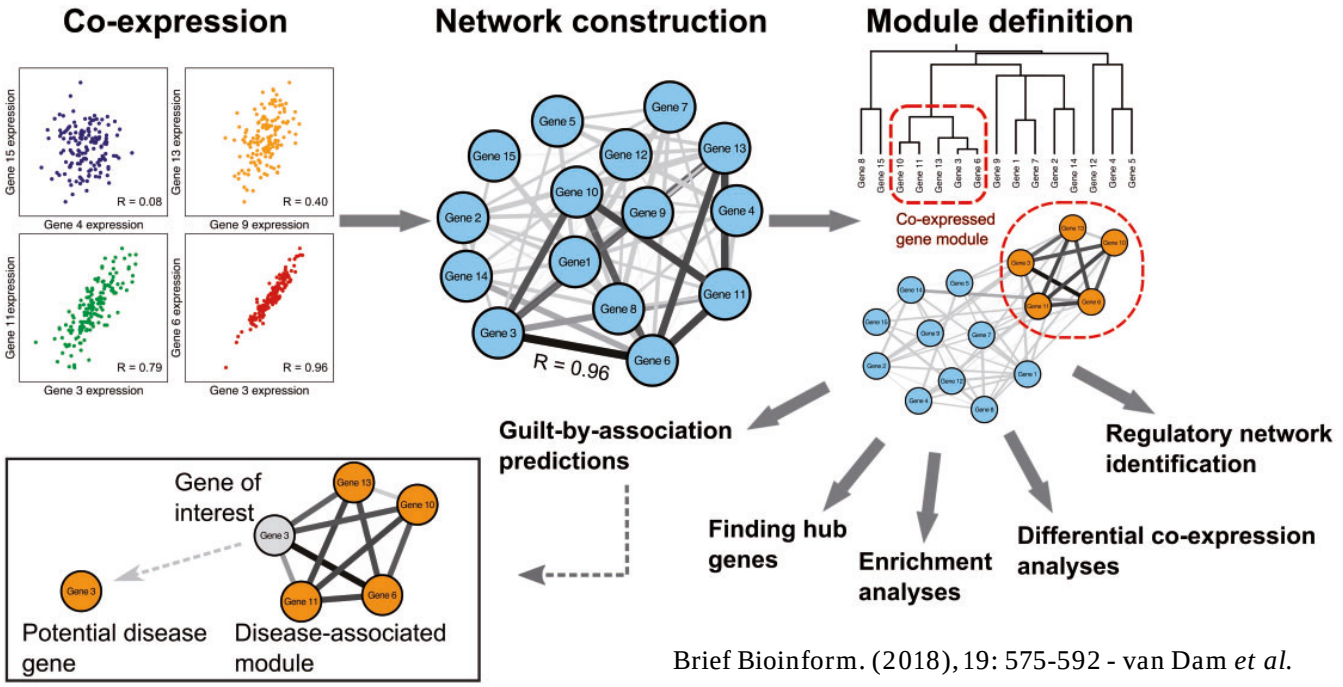
Weighted gene co-expression network analysis (WGCNA):

- Group genes involved in the same pathway/process and regulated by similar transcription factors
- Not biased towards biological knowledge (unsupervised method)
- Functional enrichment provide insight into the underlying function/mechanism

Input for WGCNA → large and diverse transcriptomic datasets

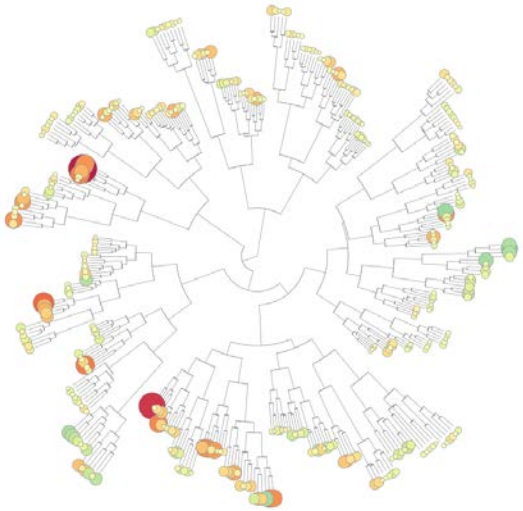
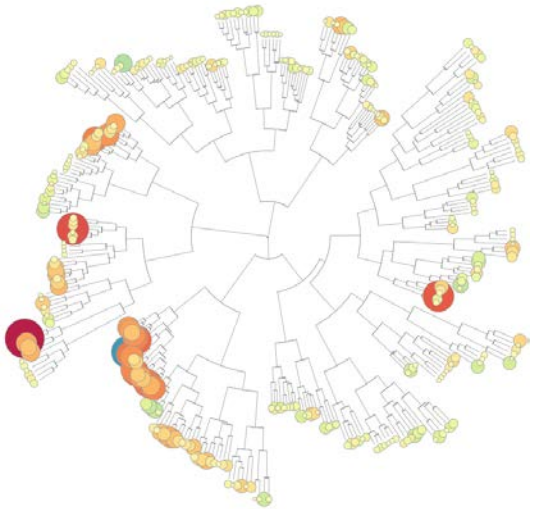
TG-GATEs

- **Primary human hepatocytes** **158 cmpds**
- **Rat liver (in vivo)** **160 cmpds**
- **Rat kidney (in vivo)** **41 cmpds**



Brief Bioinform. (2018), 19: 575-592 - van Dam *et al.*

TXG-MAPr: WGCNA on TG-GATEs datasets

Data	Primary human hepatocyte	Liver (in vivo)	Kidney (in vivo)
Conditions	941	3528	975
Compounds	158	160	41
Time-points	Single dose: 2, 8, 24 hr	Single dose: 3, 6, 9, 24 hr Repeat dose: 4, 8, 15, 29 d	Single dose: 3, 6, 9, 24 hr Repeat dose: 4, 8, 15, 29 d
Array	Affy Human U133 Plus 2.0	Affy Rat 230 2.0	Affy Rat 230 2.0
Genes in modules	10254	13095	11244
Modules	398	316	347
TXG-MAP **Shiny app:** **https://txg-mapr.eu/** **Docker version**			

(99+) Bob van de Water | LinkedI ✕ | The International Human Epigen ✕ | TXG-MAPr - WGCNA modules ba ✕ +

TXG-MAPr - WGCNA modules based on in vivo rat liver data (TG-GATEs)

LACDR - Leiden University - v 1.0

Welcome Bob

Log out

TXG Map | TXG Map enrichment | Compounds | Modules | Genes | TFs | Compound correlation | Module correlation | Pathology | Upload

Help

Visit the help section for more details

Compound:

ACETAMINOPHEN

Eigengene score:

-36 … 42

-36 -28 -20 -12 -4 4 12 20 28 36 42

Time-points:

0.125 day | 0.25 day | 0.375 day | 1 day | 4 day | 8 day | 15 day | 29 day | Other

Dose-level:

Low | Medium | High | Other

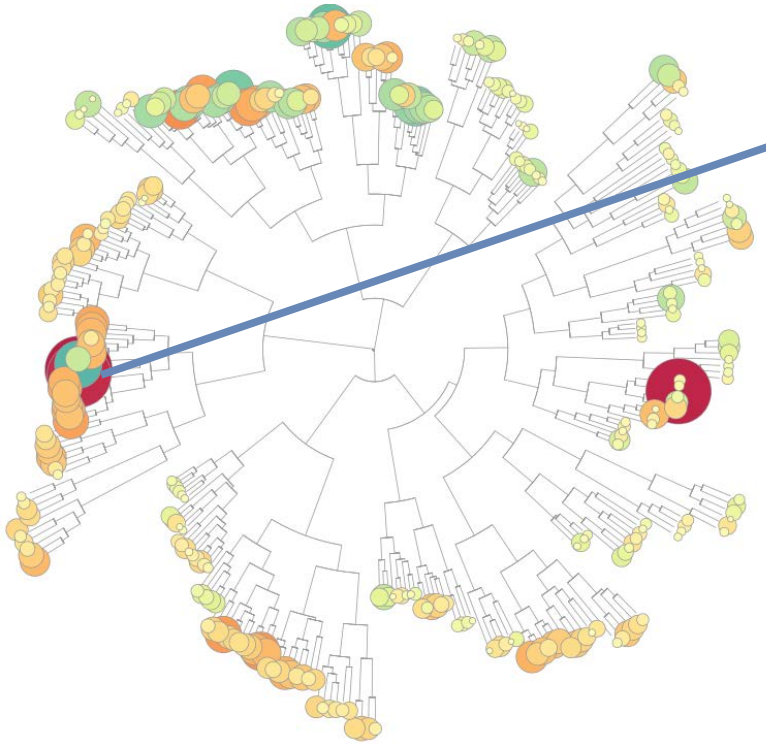
Single treatment

Callegaro et al. Arch Toxicol. 2021

Callegaro et al. Arch Toxicol. 2021

TXG-MAPr: WGCNA networks for kidney

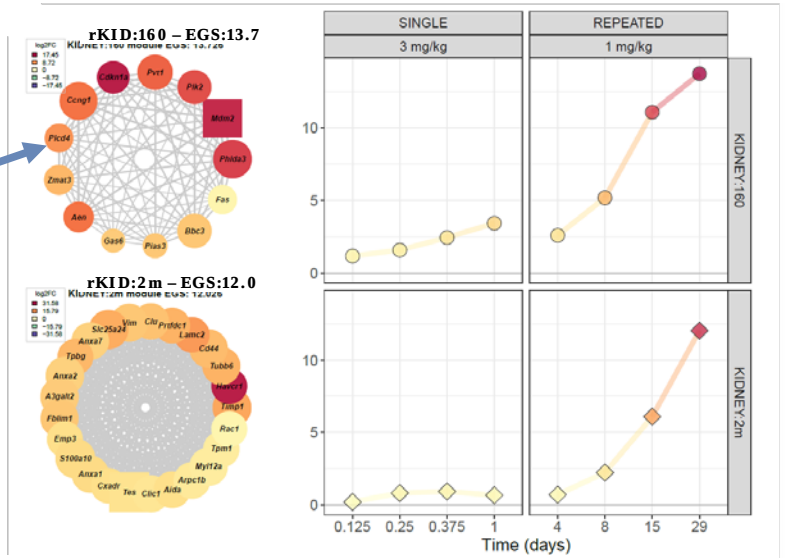
TXG-MAP: 15 days daily 1 mg/kg cisplatin



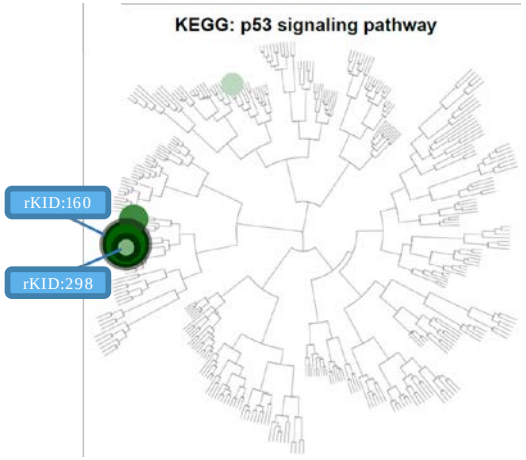
Gene co-expression (WGCNA) on TG-GATEs rat kidney data

Ward's hierarchical clustering

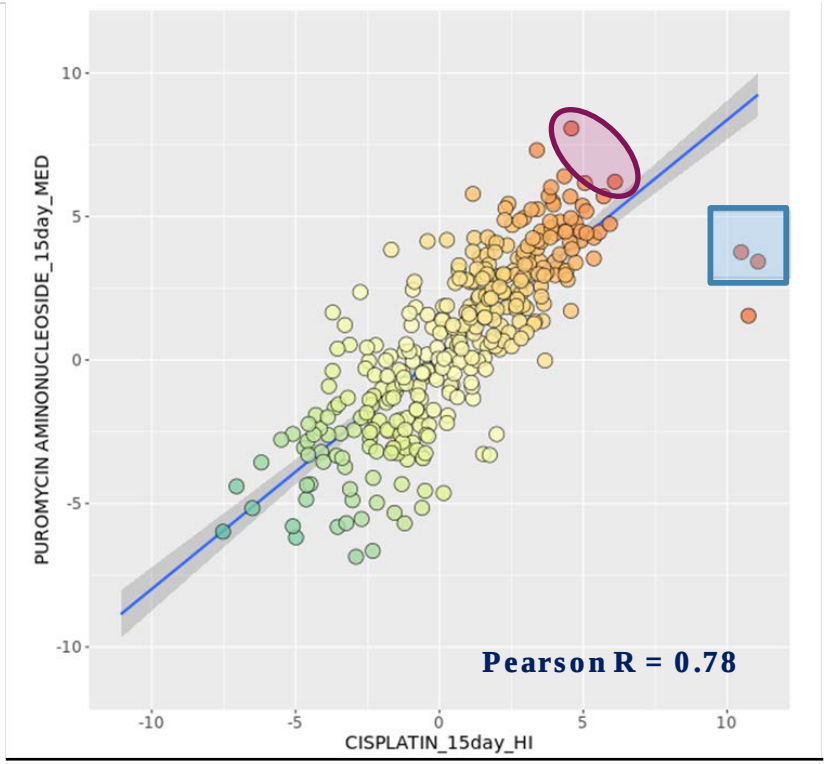
Module eigengene score (EGs) response



Module enrichment (GO, pathways, TFs)



Compound correlation



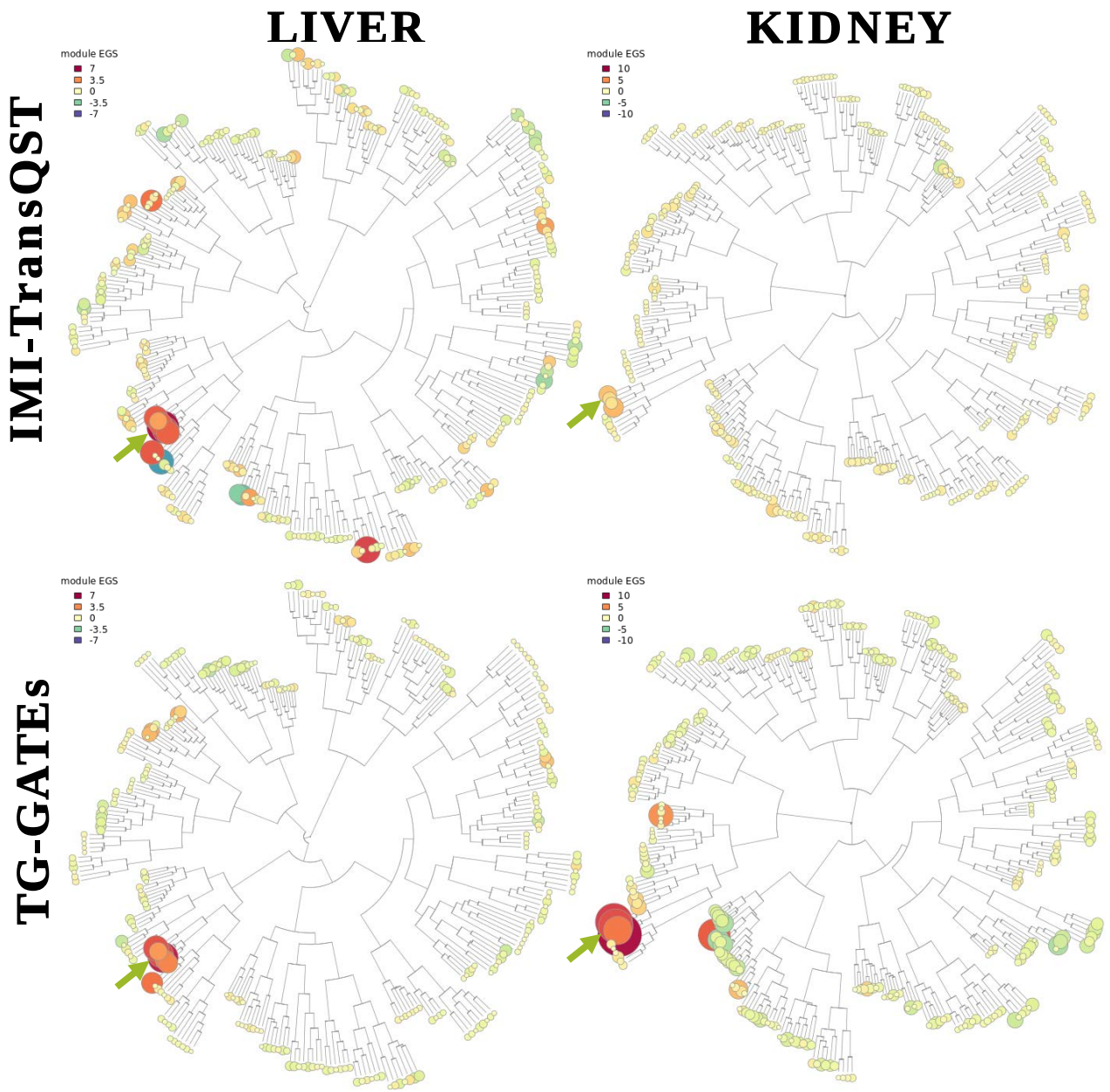
Module	Annotation	CSP_15day HI	PAN_15day MED
rKID:160	p53 signaling	11.07	3.43
rKID:298	p53 signaling	10.49	3.76
rKID:2m	Injury biomarkers	6.1	6.21
rKID:5m	Cell cycle	4.59	8.07

Kunnen et al. in preparation

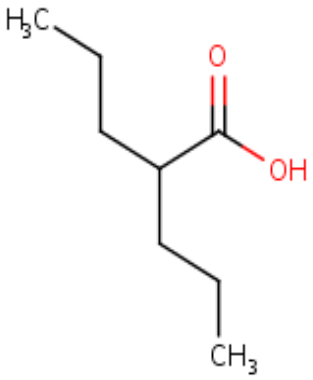
Cross transcriptomics platform compatibility and experimental robustness: cyclosporin A

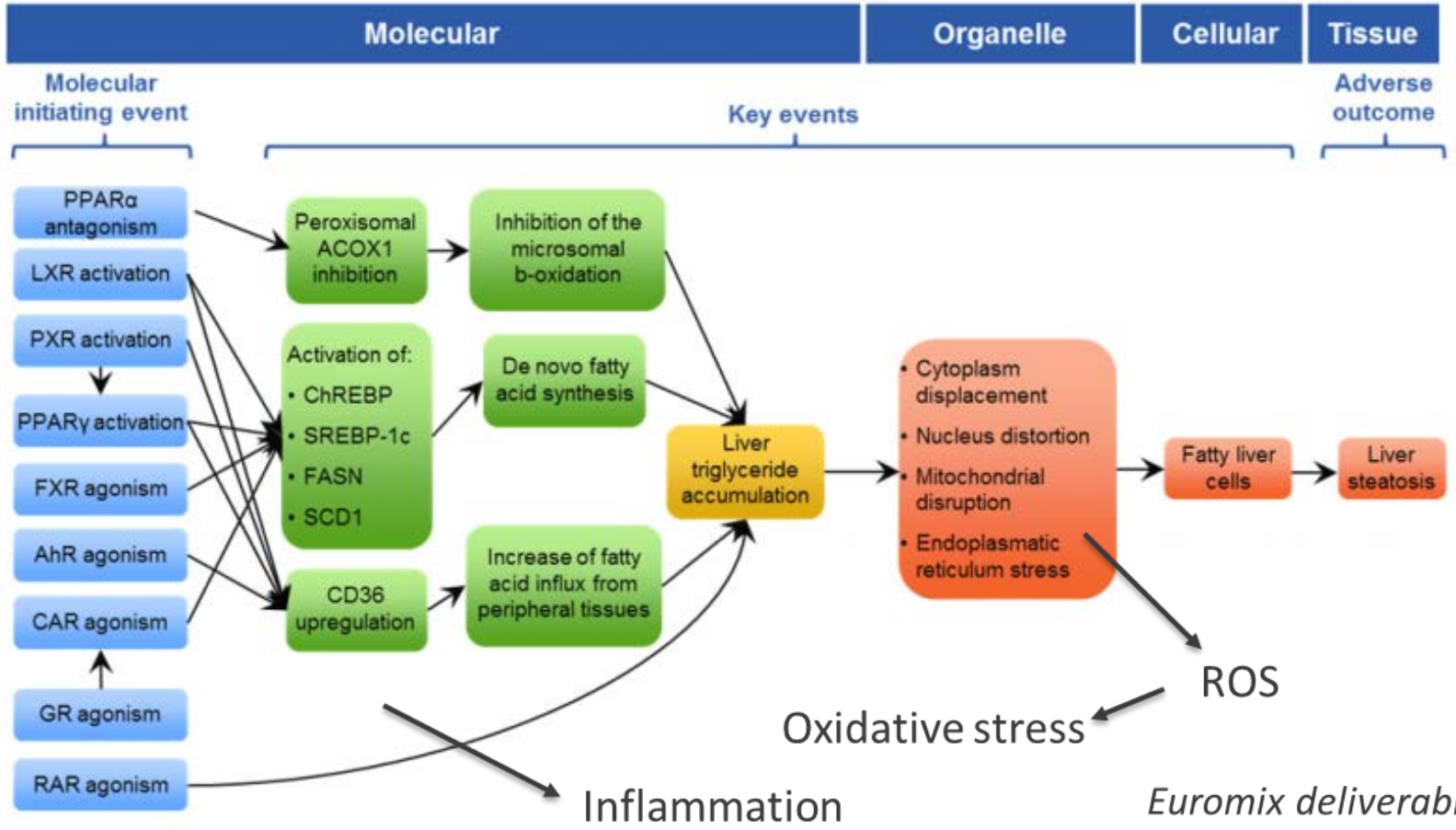
IMI-TransQST – 200 mg/kg CSA 10 hr
Whole transcriptome TempO-seq

TG-GATEs – 300 mg/kg CSA 9 hr
Affymetrix gene array

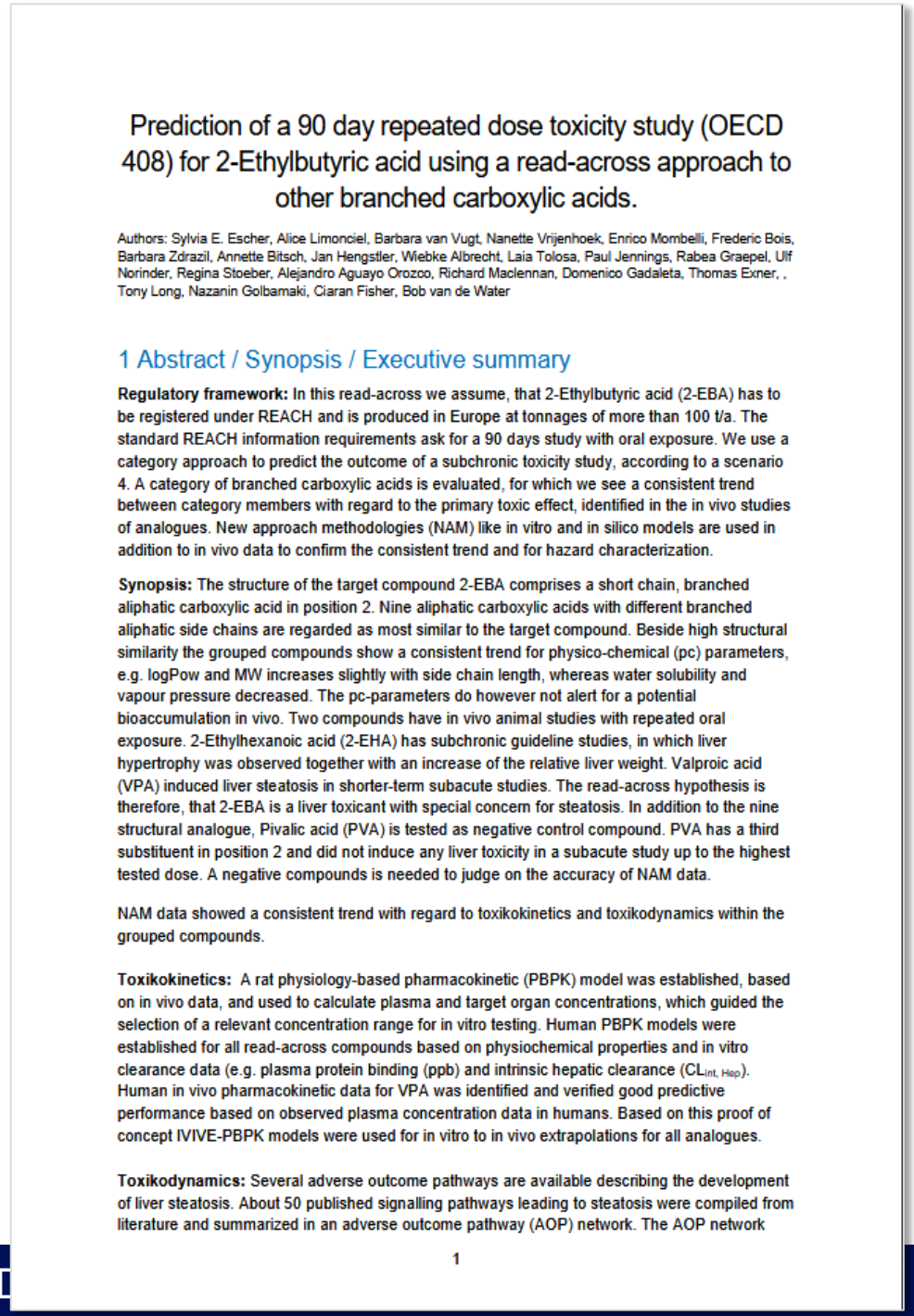


TXG-MAPr application for read across?
Valproic acid analogues and liver steatosis as a case study.





Do carboxylic acid VPA analogues have similar mode of action?
Can HHTr TempOseq support biological RAx?



Prediction of a 90 day repeated dose toxicity study (OECD 408) for 2-Ethylbutyric acid using a read-across approach to other branched carboxylic acids.

Authors: Sylvia E. Escher, Alice Limonciel, Barbara van Vugt, Nanette Vrijenhoek, Enrico Mombelli, Frederic Bois, Barbara Zdrazil, Annette Bitsch, Jan Hengstler, Wiebke Albrecht, Laia Tolosa, Paul Jennings, Rabea Graepel, Ulf Norinder, Regina Stoeber, Alejandro Aguayo Orozco, Richard Maclennan, Domenico Gadaleta, Thomas Exner, , Tony Long, Nazanin Golbamaki, Ciaran Fisher, Bob van de Water

1 Abstract / Synopsis / Executive summary

Regulatory framework: In this read-across we assume, that 2-Ethylbutyric acid (2-EBA) has to be registered under REACH and is produced in Europe at tonnages of more than 100 t/a. The standard REACH information requirements ask for a 90 days study with oral exposure. We use a category approach to predict the outcome of a subchronic toxicity study, according to a scenario 4. A category of branched carboxylic acids is evaluated, for which we see a consistent trend between category members with regard to the primary toxic effect, identified in the in vivo studies of analogues. New approach methodologies (NAM) like in vitro and in silico models are used in addition to in vivo data to confirm the consistent trend and for hazard characterization.

Synopsis: The structure of the target compound 2-EBA comprises a short chain, branched aliphatic carboxylic acid in position 2. Nine aliphatic carboxylic acids with different branched aliphatic side chains are regarded as most similar to the target compound. Beside high structural similarity the grouped compounds show a consistent trend for physico-chemical (pc) parameters, e.g. logPow and MW increases slightly with side chain length, whereas water solubility and vapour pressure decreased. The pc-parameters do however not alert for a potential bioaccumulation in vivo. Two compounds have in vivo animal studies with repeated oral exposure. 2-Ethylhexanoic acid (2-EHA) has subchronic guideline studies, in which liver hypertrophy was observed together with an increase of the relative liver weight. Valproic acid (VPA) induced liver steatosis in shorter-term subacute studies. The read-across hypothesis is therefore, that 2-EBA is a liver toxicant with special concern for steatosis. In addition to the nine structural analogue, Pivalic acid (PVA) is tested as negative control compound. PVA has a third substituent in position 2 and did not induce any liver toxicity in a subacute study up to the highest tested dose. A negative compounds is needed to judge on the accuracy of NAM data.

NAM data showed a consistent trend with regard to toxikokinetics and toxikodynamics within the grouped compounds.

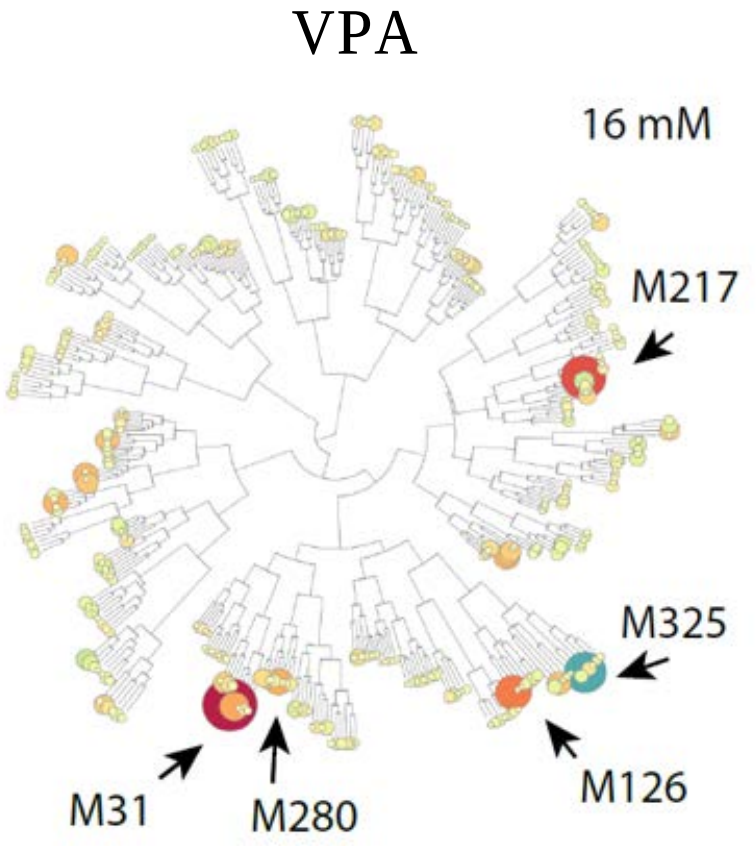
Toxikokinetics: A rat physiology-based pharmacokinetic (PBPK) model was established, based on in vivo data, and used to calculate plasma and target organ concentrations, which guided the selection of a relevant concentration range for in vitro testing. Human PBPK models were established for all read-across compounds based on physiochemical properties and in vitro clearance data (e.g. plasma protein binding (ppb) and intrinsic hepatic clearance ($CL_{int, Hep}$). Human in vivo pharmacokinetic data for VPA was identified and verified good predictive performance based on observed plasma concentration data in humans. Based on this proof of concept IVIVE-PBPK models were used for in vitro to in vivo extrapolations for all analogues.

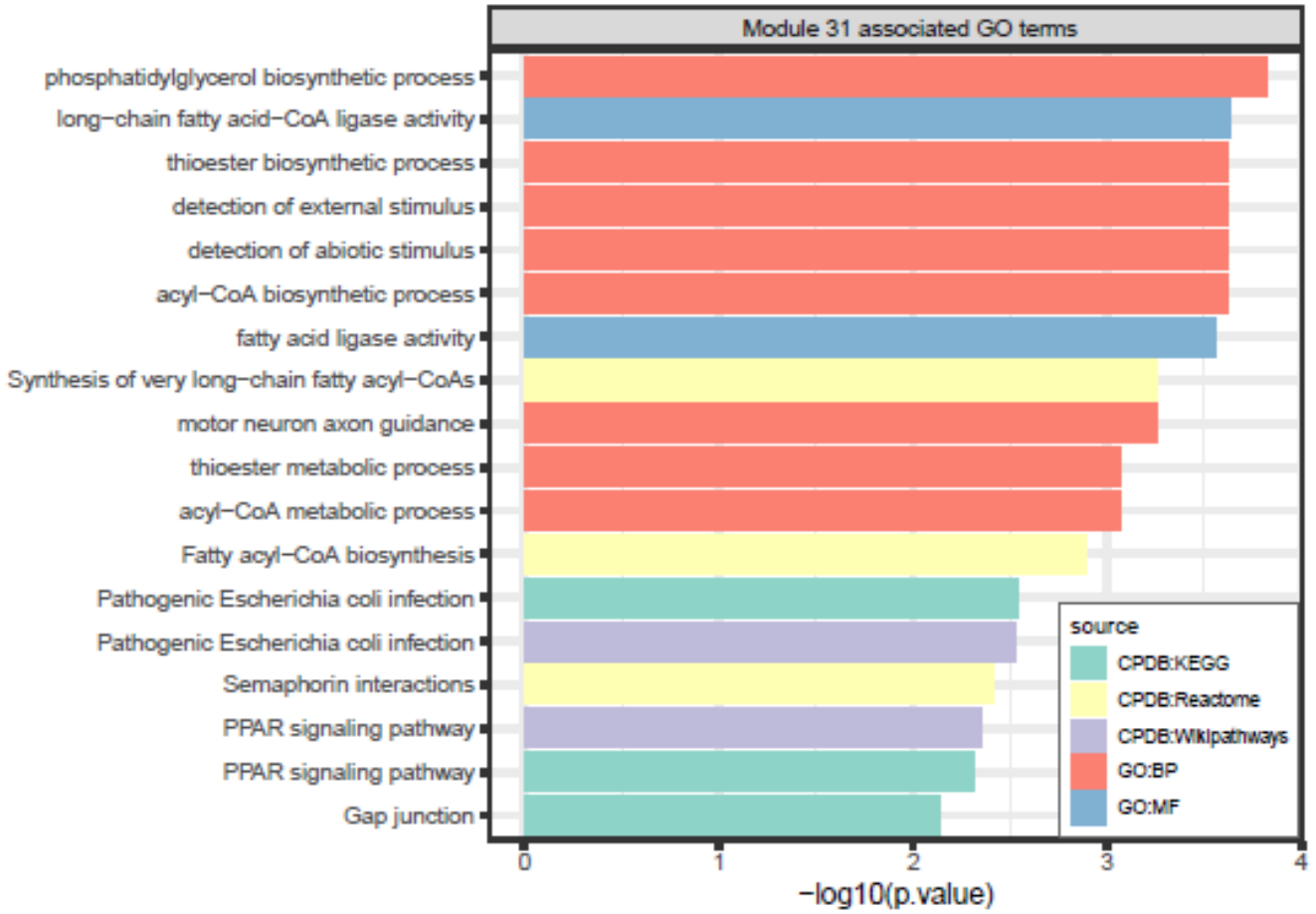
Toxikodynamics: Several adverse outcome pathways are available describing the development of liver steatosis. About 50 published signalling pathways leading to steatosis were compiled from literature and summarized in an adverse outcome pathway (AOP) network. The AOP network

- EU-ToxRisk RAx case study
- Liver steatosis AOP-based
- KE event analysis
- OECD IATA case study working group
- Official report published
- Gene expression profiling not involved

➢ **Can high throughput transcriptomics in combination with TXG-MAPr-based quantitative interpretation contribute to RAx of carboxylic acids**

VPA activates module 31: Representative of FFA acid oxidation.

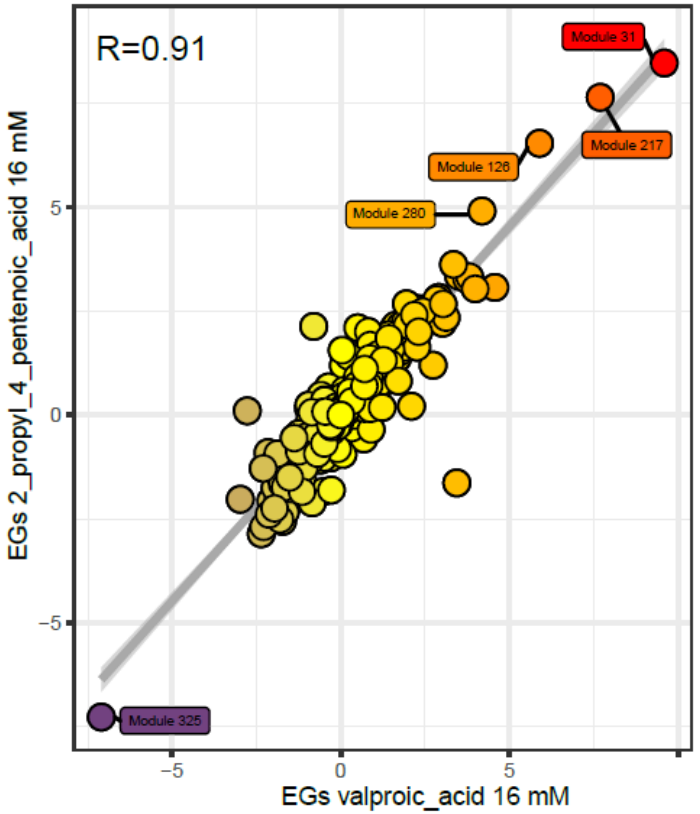


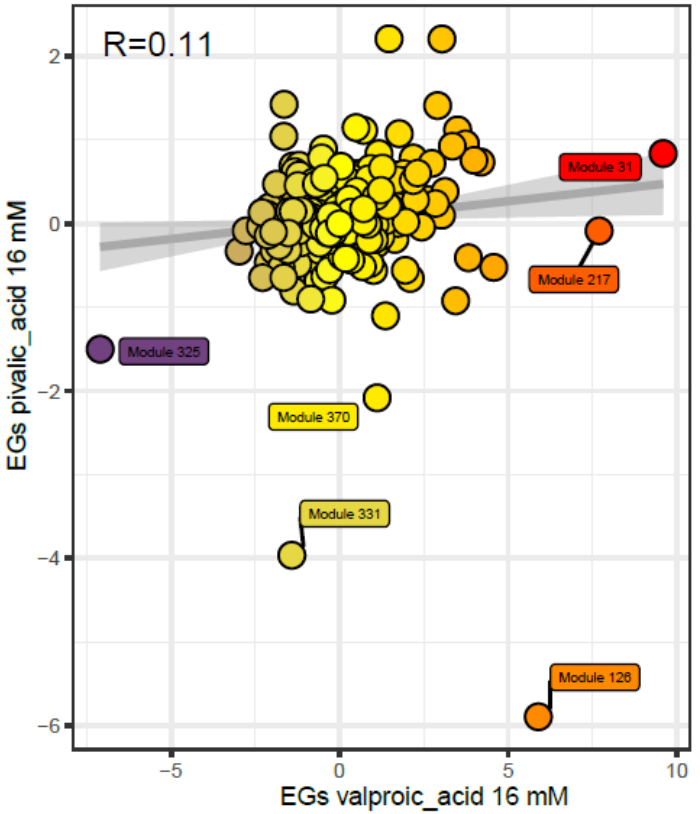


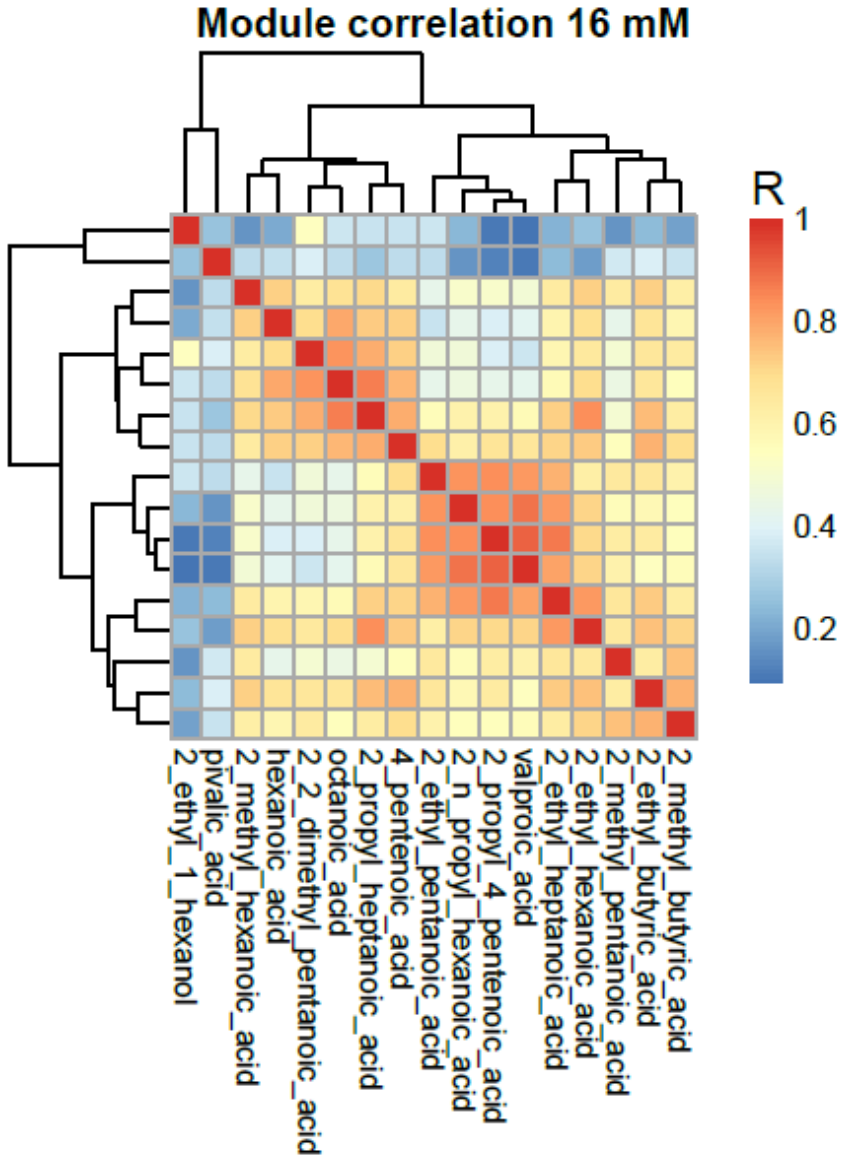
Vrijenhoek et al. ALTEX 2022

Vrijenhoek et al. in revision

PHH TXG-MAPr-based VPA analogue correlation for biological RAx



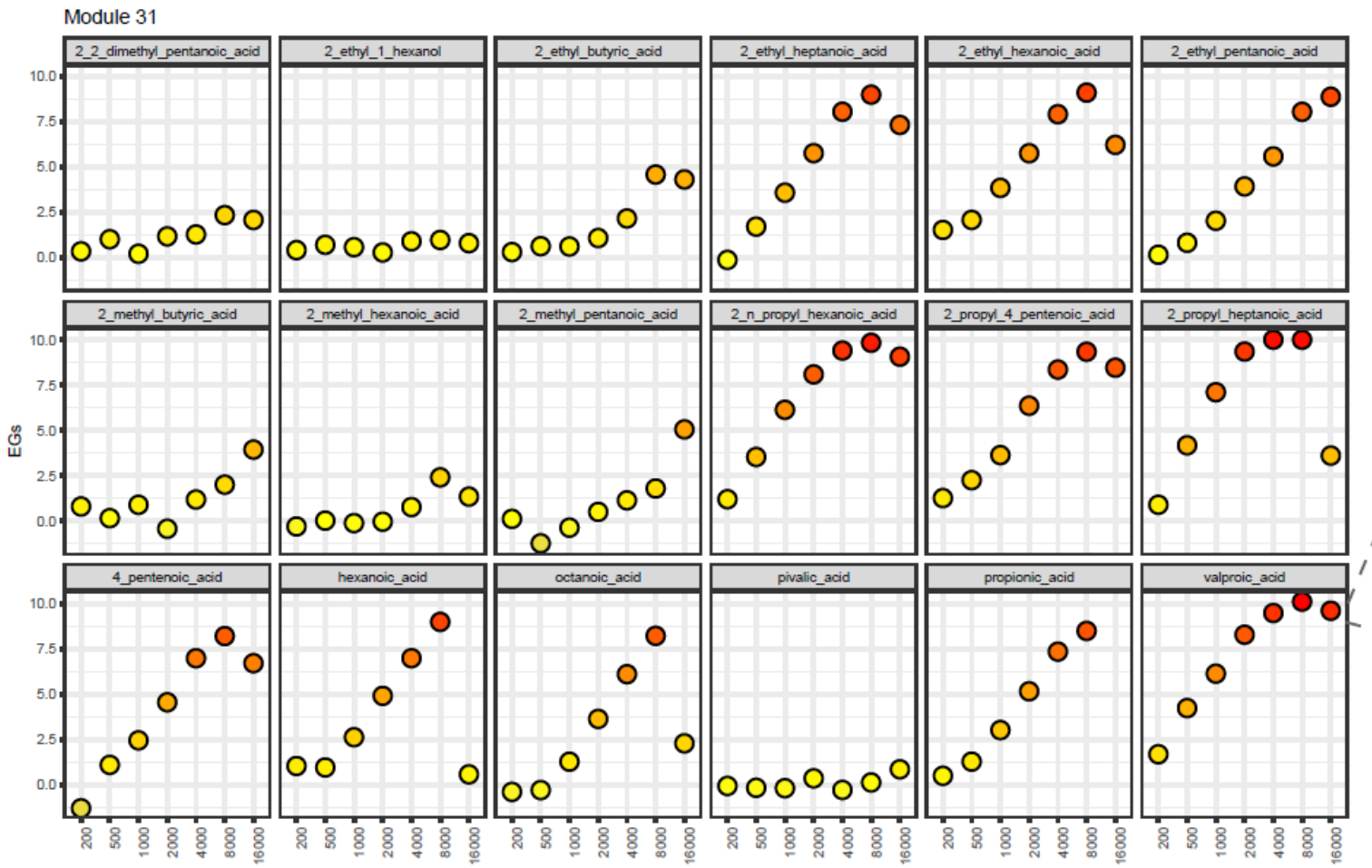


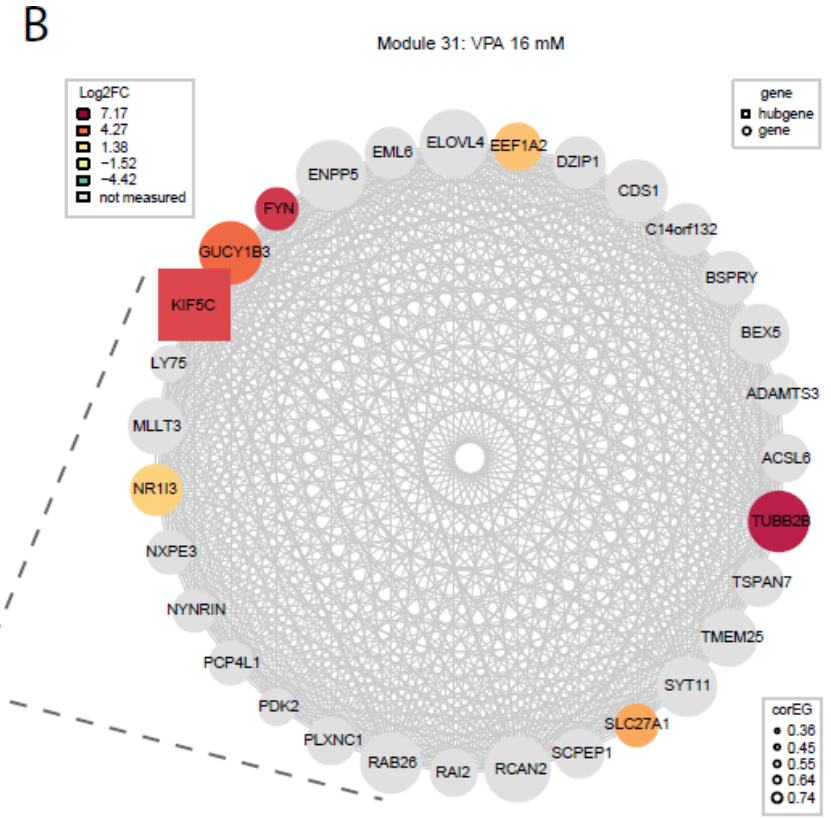


Vrijenhoek et al. ALTEX 2022

Potency evaluation of VPA analogues in PHH: TXG-MAPr Module 31 shows a dose response for VPA analogues

~400 samples EU-ToxRisk targeted TempO-seq gene panel



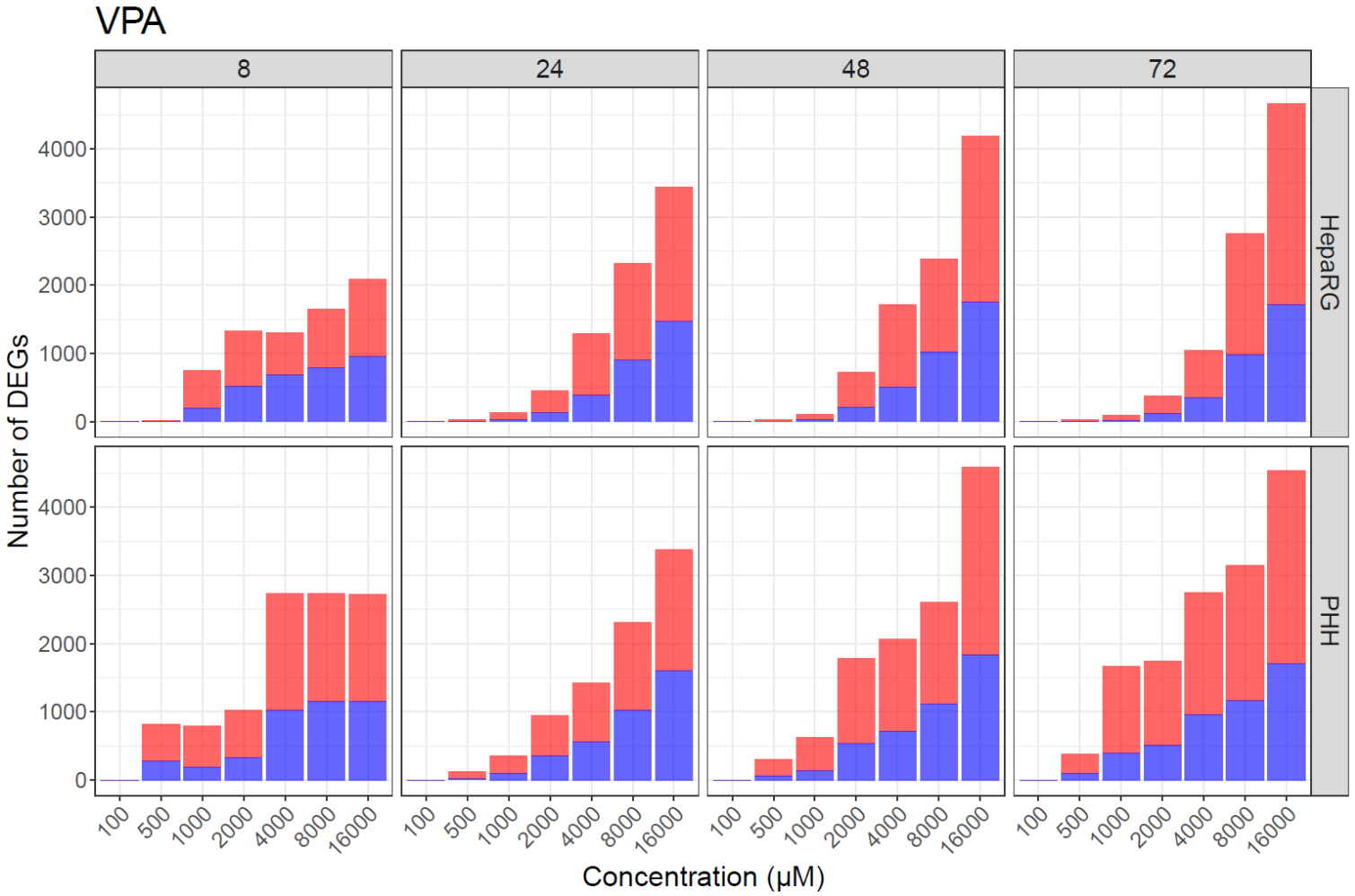


Vrijenhoek et al. ALTEX 2022

Vrijenhoek et al. in revision

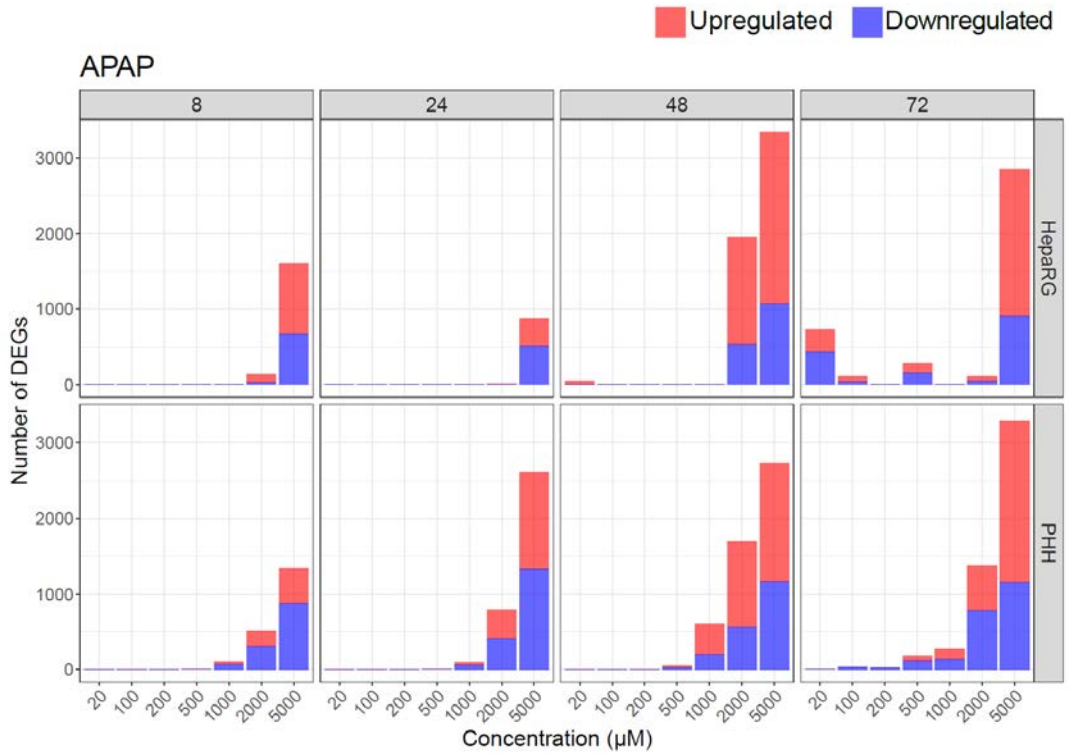
What are the differences between test systems?
Temperal VPA concentration response PHH versus HepRG

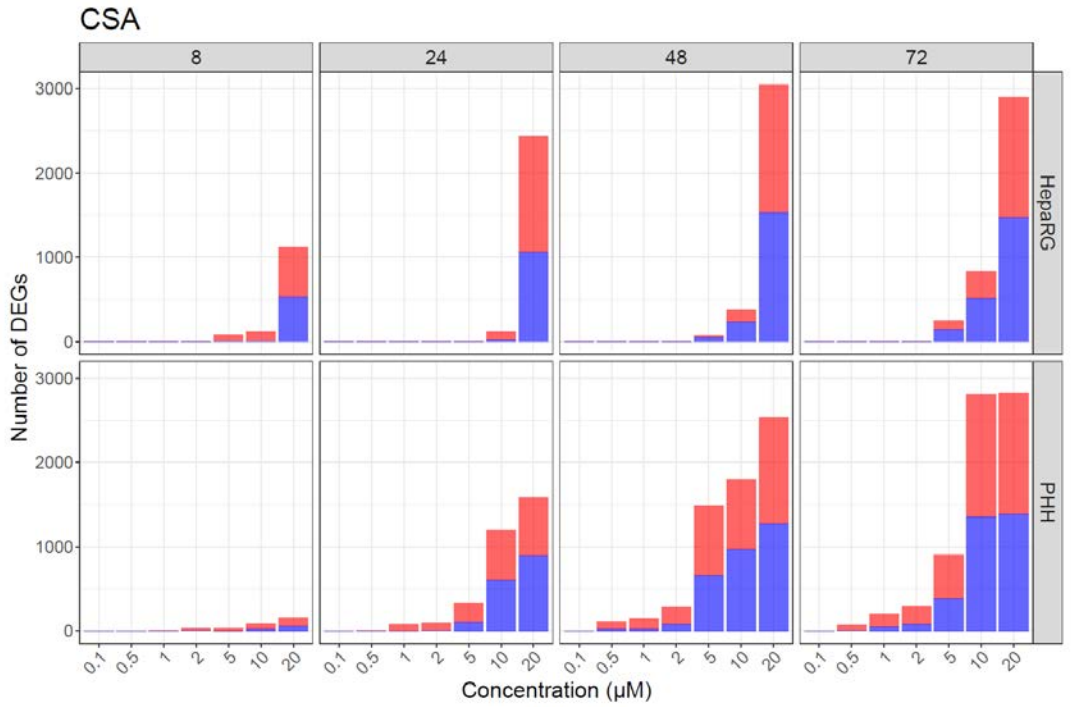
- PHH and HepaRG
- 8, 24, 48 and 72 hr
- 7 concentrations
- WT TempO-seq



How about other reference liver toxicants: APAP and cyclosporin A?

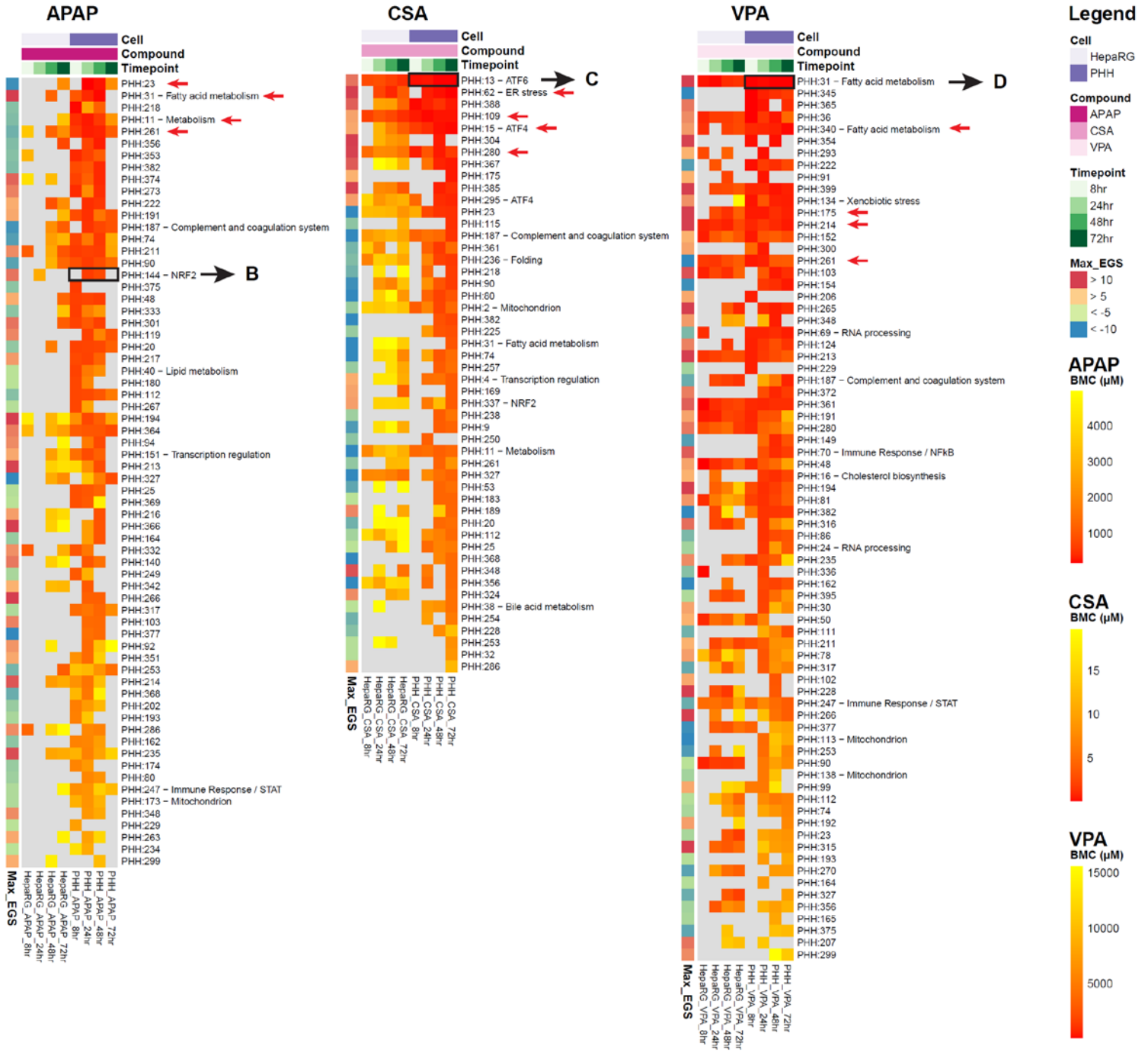
- PHH and HepaRG
- 8, 24, 48 and 72 hr
- 7 concentrations
- WT TempO-seq



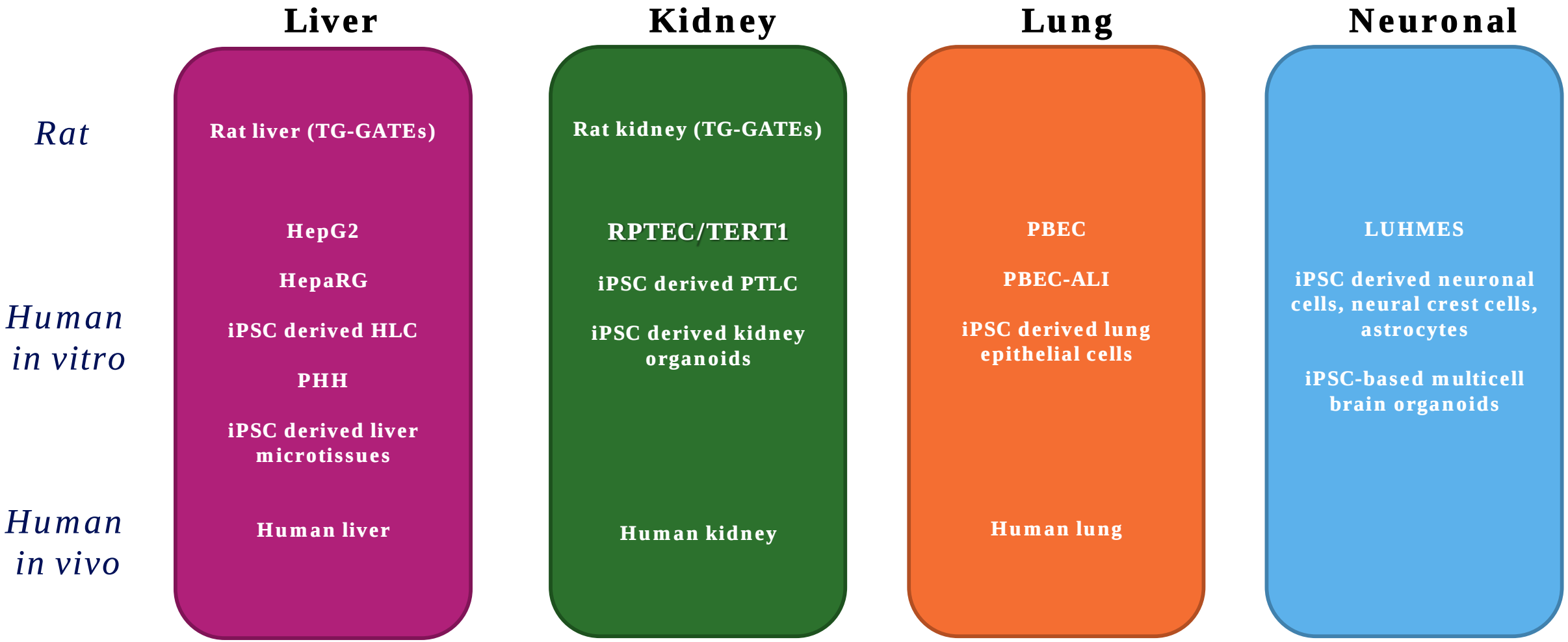


BMC modelling of gene network activity based on PHH TXG-MAPr

- BMC value is pathway dependent
- VPA activates module 31 FFA metabolism also in HepaRG
- CSA activates ER stress in both HepaRG and PHH
- PHH and HepaRG activate similar biology
- PHH more sensitive than HepaRG

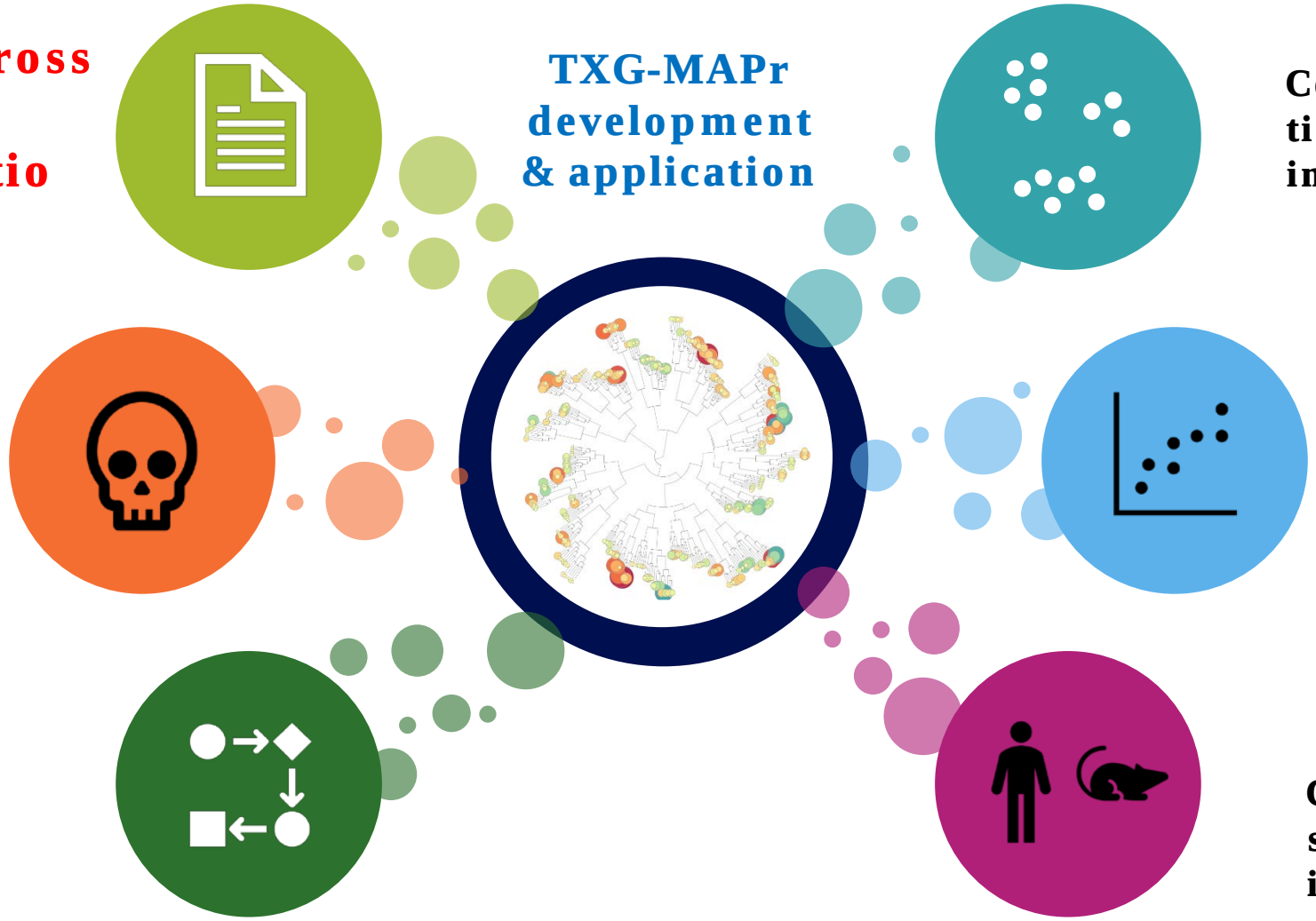


Our longterm objectives for TXG-MAPr development



EFSA TD-TRAQ project: interindividual variability in toxicodynamics → TXG-MAPr for PBMCs

Our vision: gene co-expression analysis for quantitative systems toxicology-based risk assessment



Thank you!

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