SEURAT-1 Achievements: Technologies, Integration, Realisation

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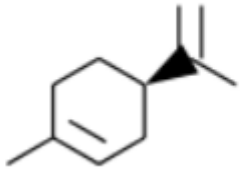
Coordination Action COACH: Grant Agreement N° : 26 7044

Assessment Without Animals: Utilising Novel SEURAT-1 Technologies

- The SEURAT-1 projects have developed a number of transferable technologies:
  - From computational approaches, cell based assays to simulations of organ function
  - Suitable to provide toxicological information for new and existing chemicals
  - Will inform risk assessment
  - Complementary to other global projects

COSMOS Database: Historical *In Vivo* Data

Structure #1

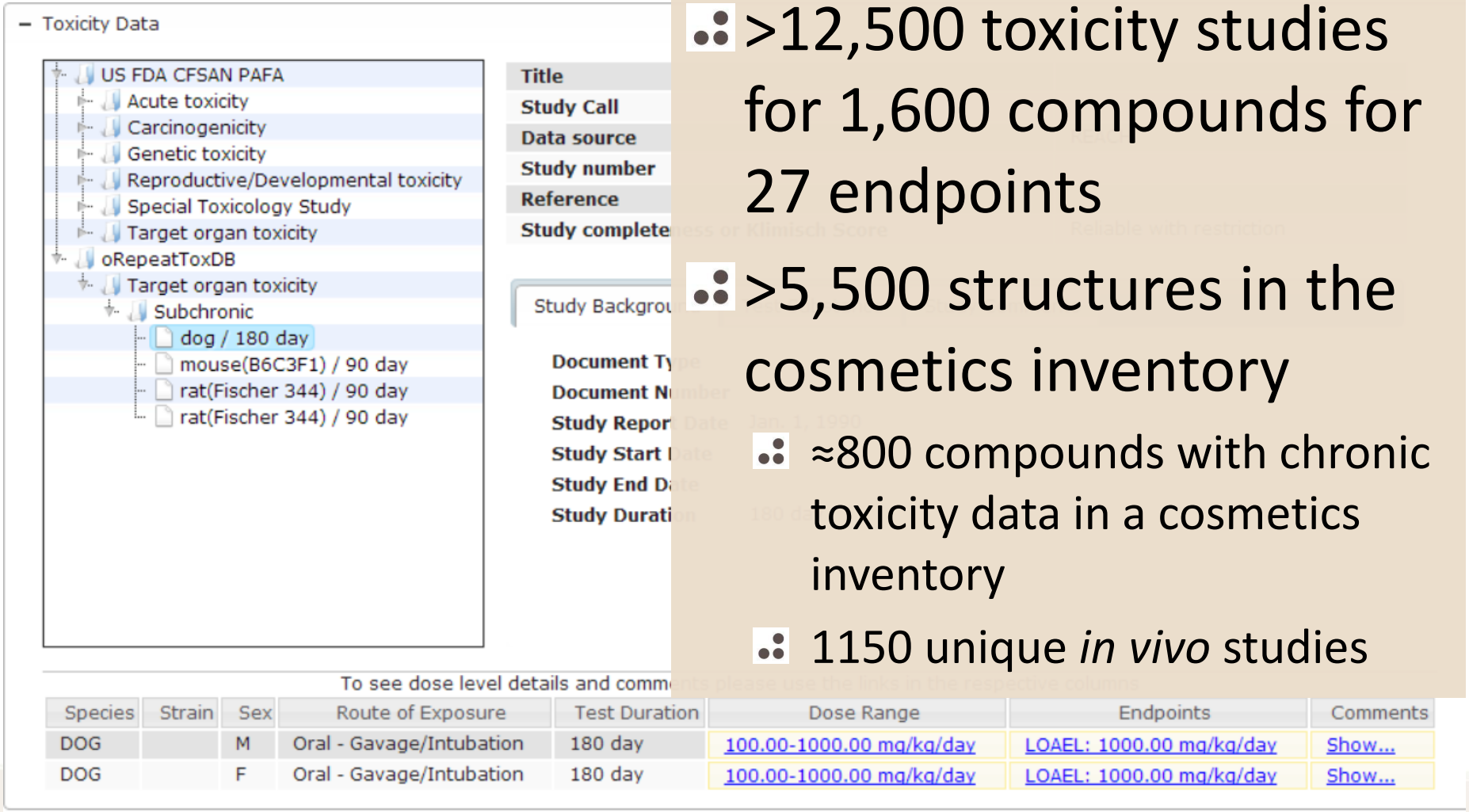


Stereochemistry	unassigned
Double Bond Geometry	unassigned
Structure Source	CAS
Structure Quality	High
Structure Representation	actual

+ IDs and Names
+ Compound Annotations
+ PAFA Chemical Information
+ Toxicity Data

- Open access
- Searchable
  - ➔ Chemistry
  - ➔ Toxicity
- Legacy assured

COSMOS Database: Historical *In Vivo* Data



- >12,500 toxicity studies for 1,600 compounds for 27 endpoints
- >5,500 structures in the cosmetics inventory
  - ≈800 compounds with chronic toxicity data in a cosmetics inventory
  - 1150 unique *in vivo* studies

COSMOS Database: Historical *In Vivo* Data

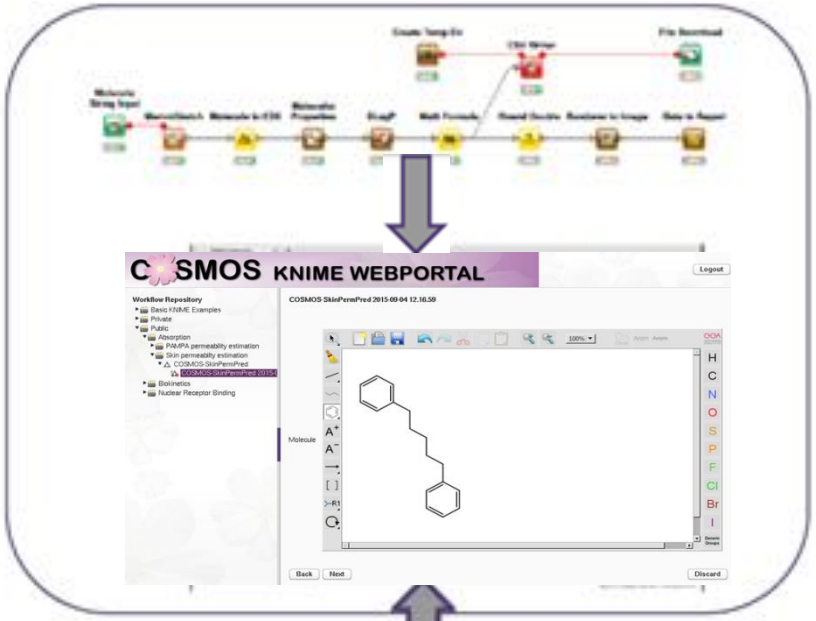
Treatment Groups

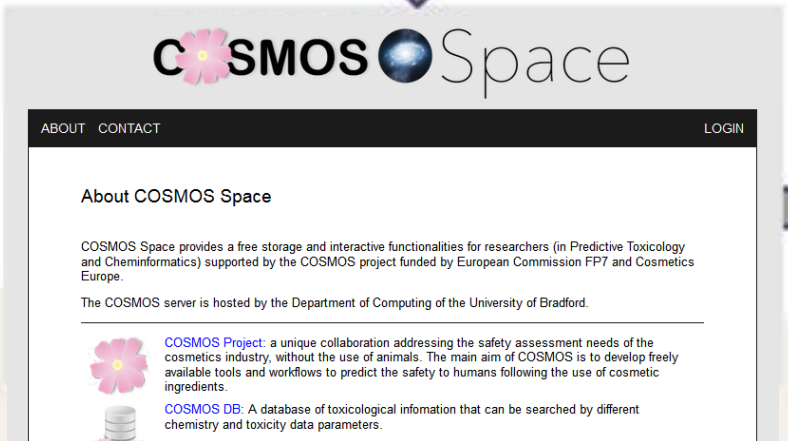
Dose	Finding Category	Assay	Site	Effect	Description	Treatment Related	Stat Sig	Comments
100.0 mg/kg/day		BODY WEIGHT		BODY WEIGHT GAIN	No Change			
		CLINICAL SIGNS		STOOL/URINE > FECES > SOFT STOOL	Increase	Treatment-related		
		ORGAN WEIGHT	KIDNEY	ABSOLUTE WEIGHT	No Change			
		ORGAN WEIGHT	KIDNEY	RELATIVE TO BODY WEIGHT	No Change			
1000.0 mg/kg/day		BODY WEIGHT		BODY WEIGHT GAIN	No Change			
		CLINICAL SIGNS		STOOL/URINE > FECES > SOFT STOOL	Increase	Treatment-related	Significant	
		ORGAN WEIGHT	KIDNEY	ABSOLUTE WEIGHT	No Change			
		ORGAN WEIGHT	KIDNEY	RELATIVE TO BODY WEIGHT	Increase	Treatment-related	Significant	

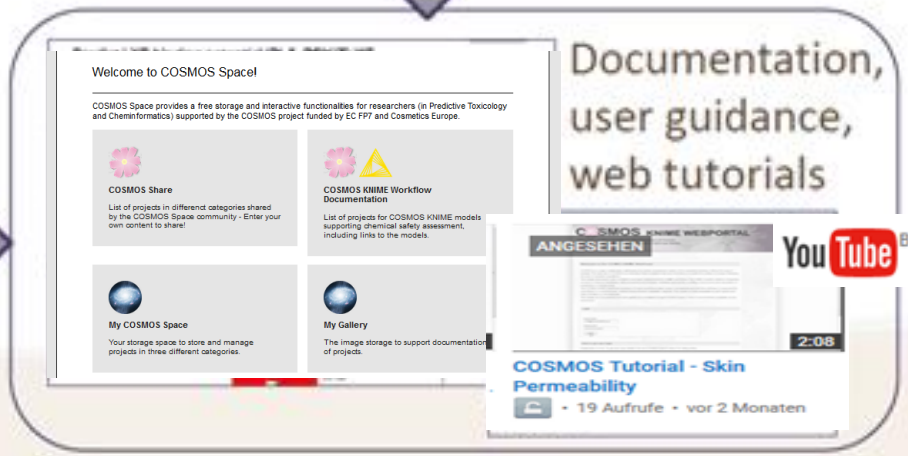
- Details for each dose
- Effects described

Computational Models to Predict Effects from Chemical Structure

SEURAT-1

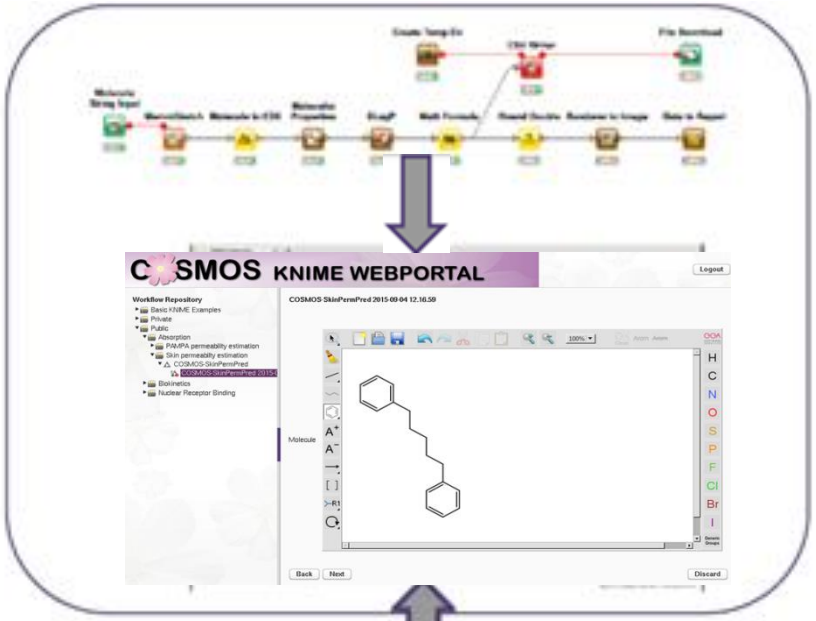


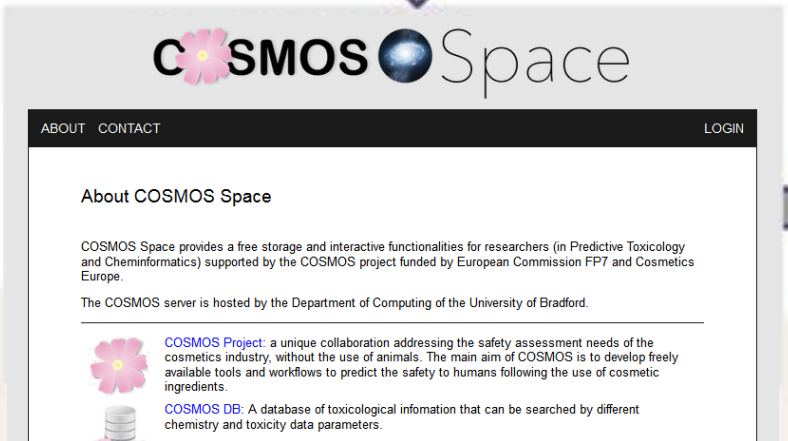


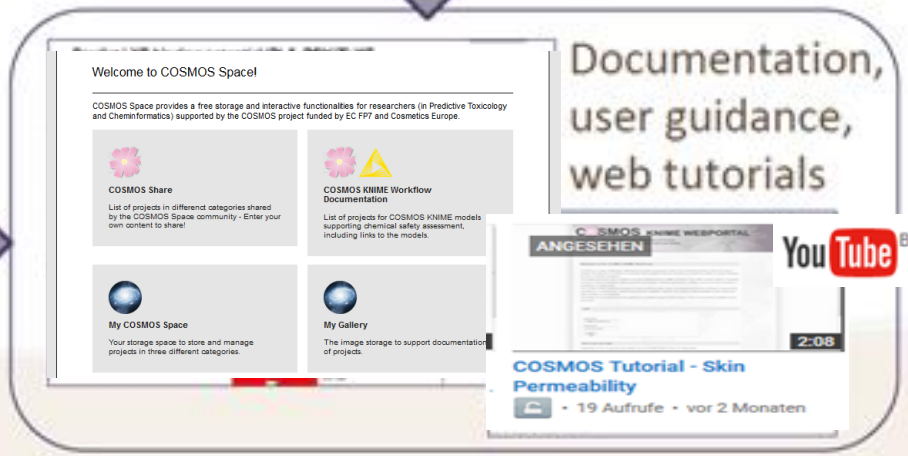


Computational Models to Predict Effects from Chemical Structure

SEURAT-1



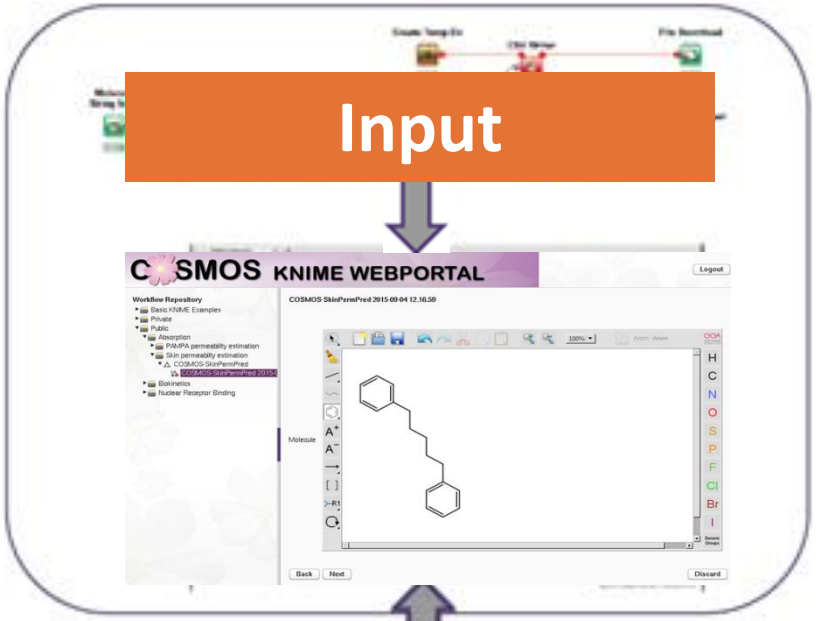


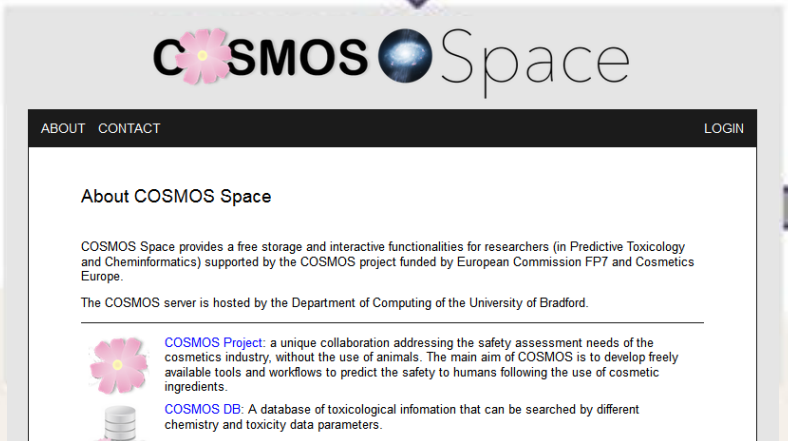


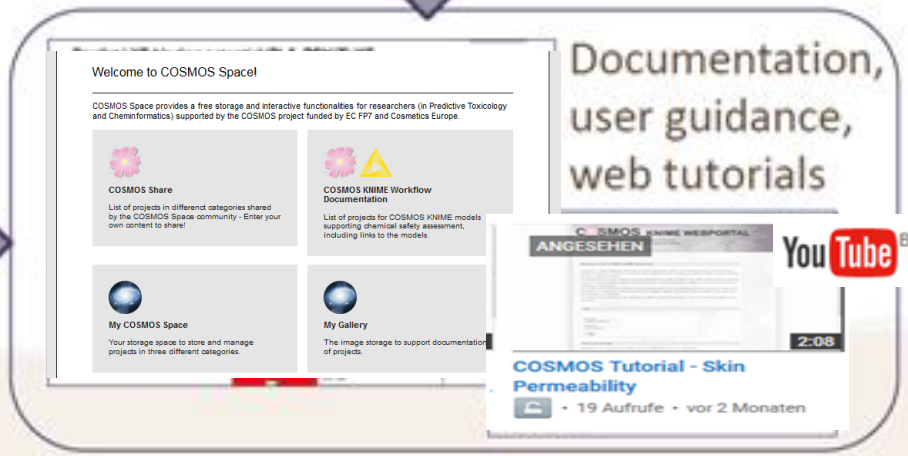
Computational Models to Predict Effects from Chemical Structure

SEURAT-1

Web Access







Computational Models to Predict Effects from Chemical Structure

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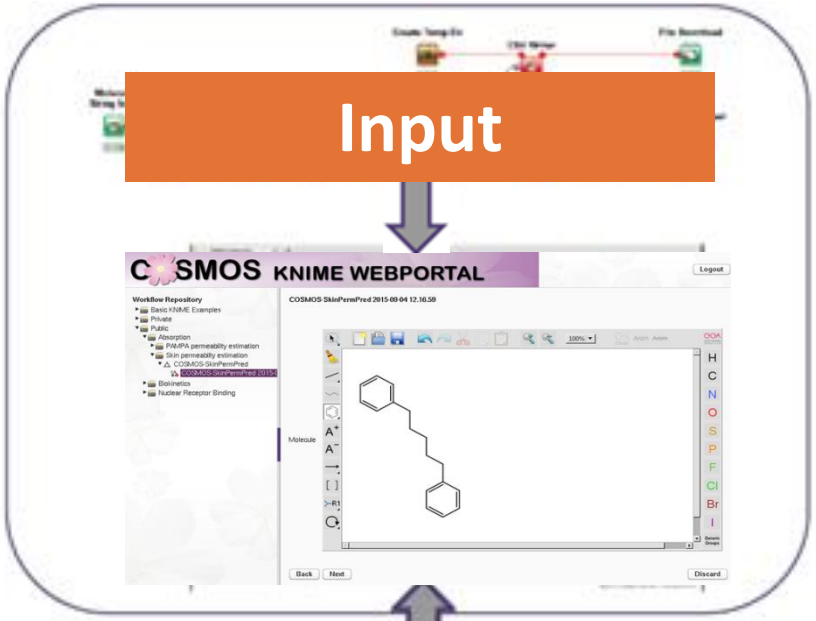
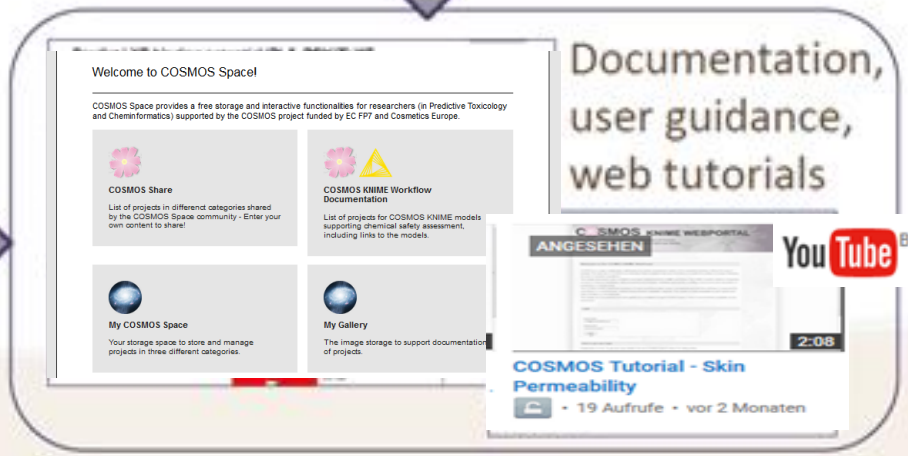


Workspace

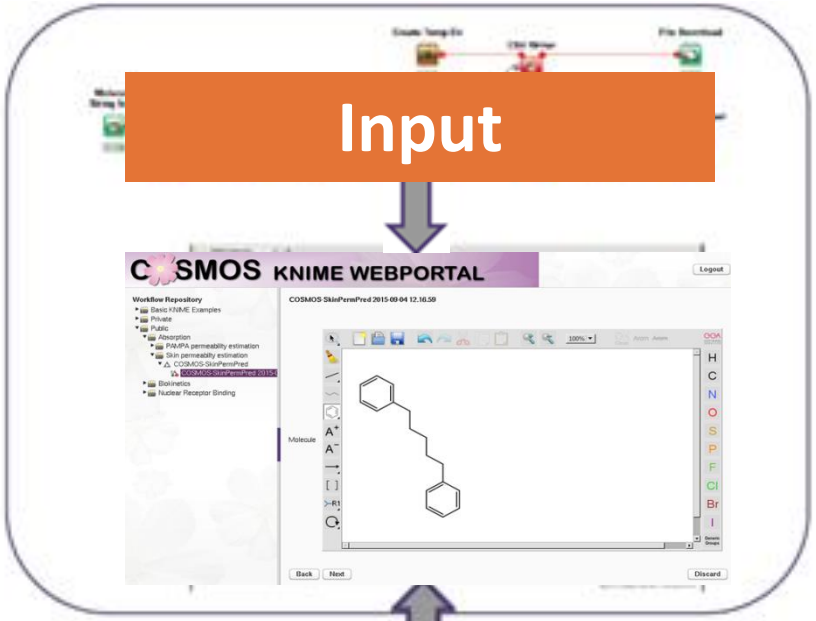


Computational Models to Predict Effects from Chemical Structure

SEURAT-1

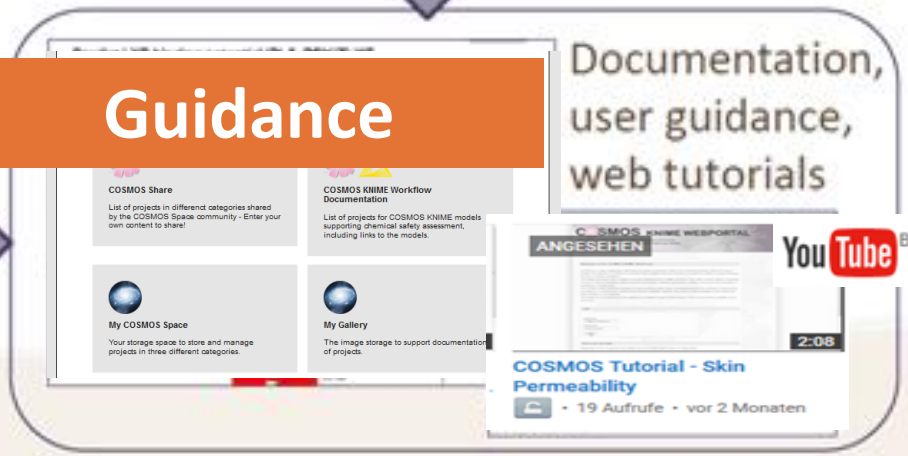
Web Access

Input



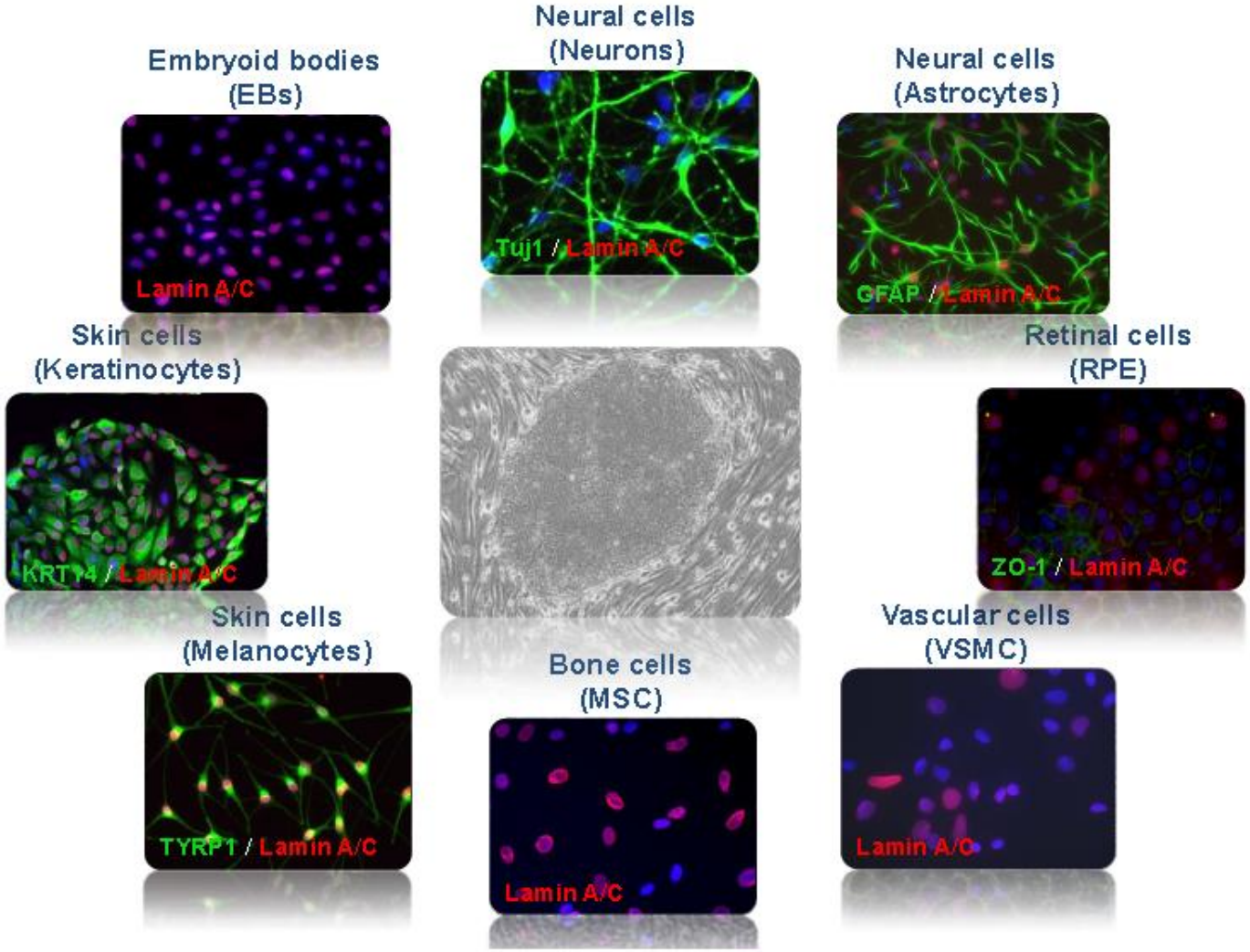
Workspace

Guidance

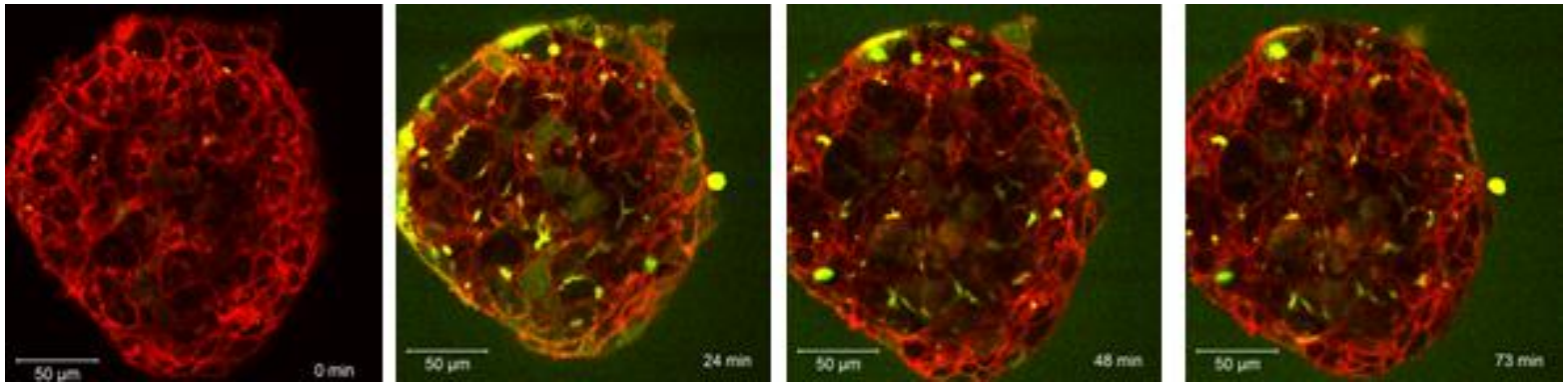


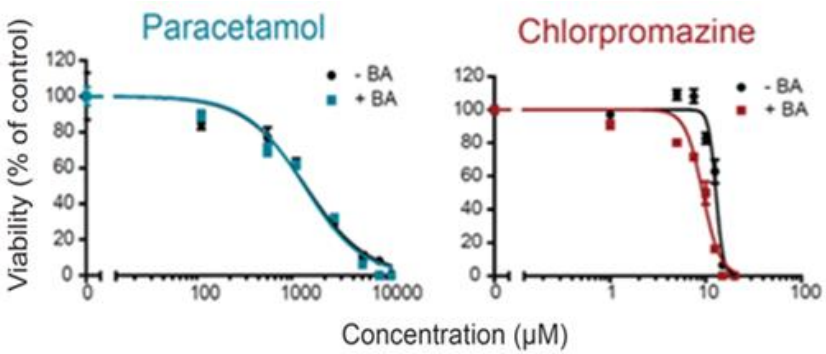
Documentation, user guidance, web tutorials

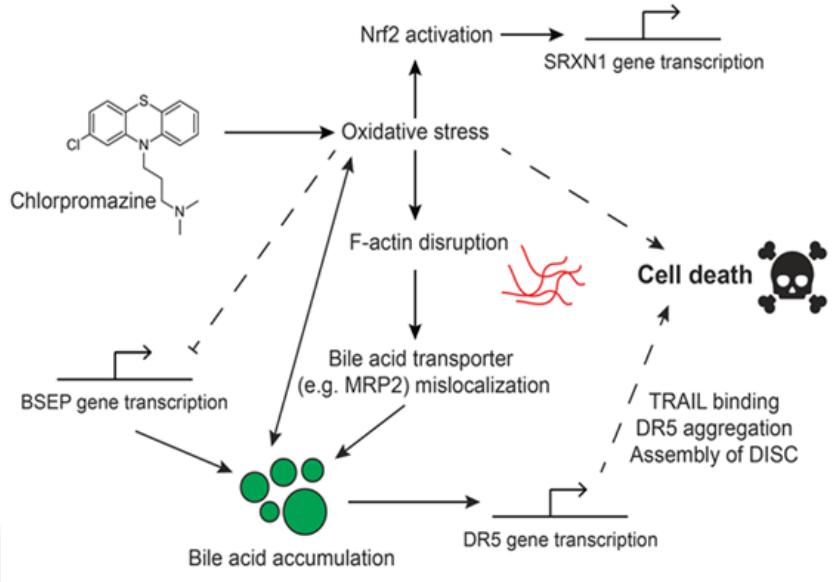
Pluripotent Stem Cells have Provided a Variety of Tissue Cultures



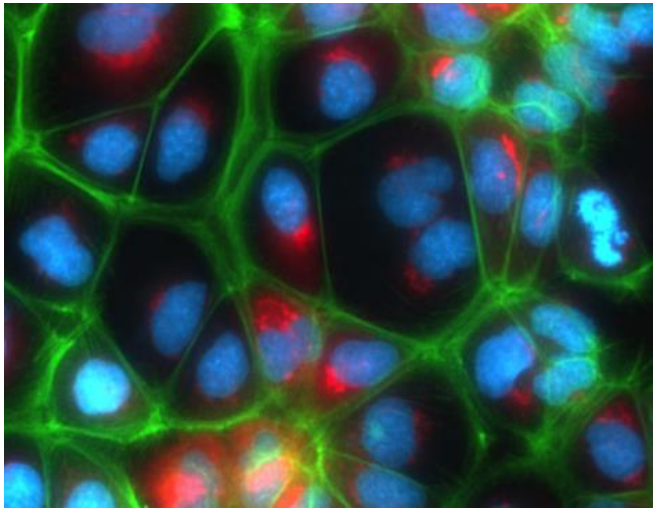
Liver Cells in Spheroids: Detection of the Restriction of Bile Flow

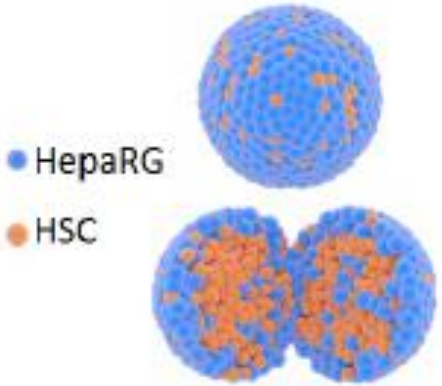


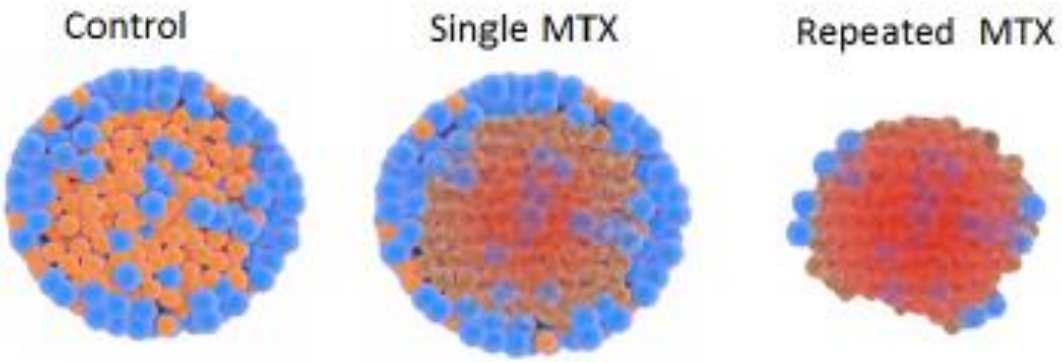




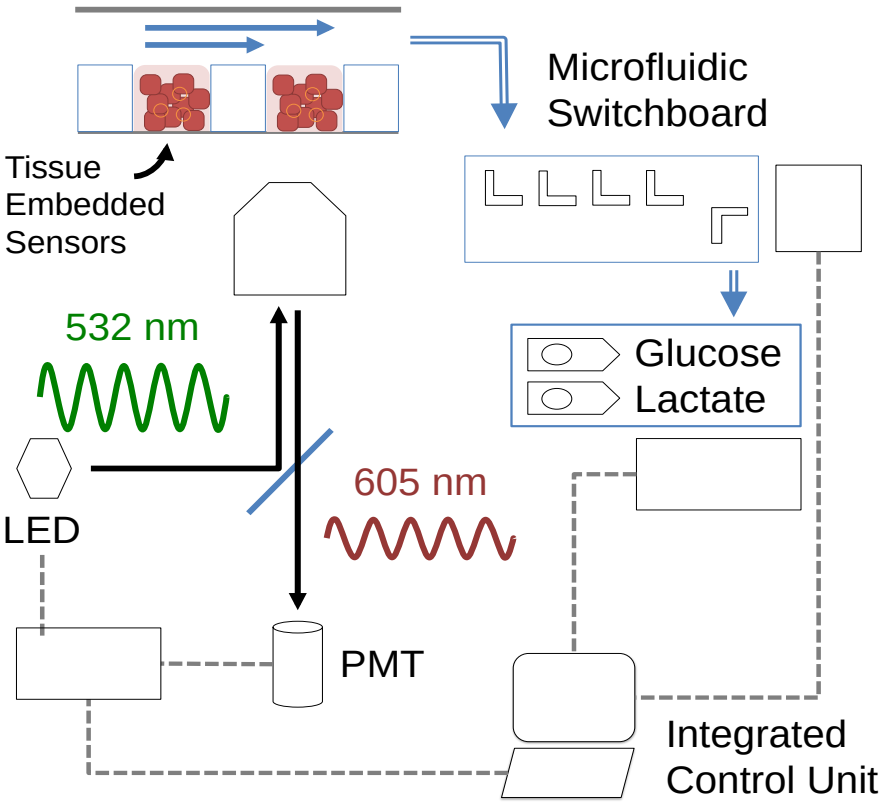
Diverse Human Cells in Spheroids: Ability to Identify Fibrosis

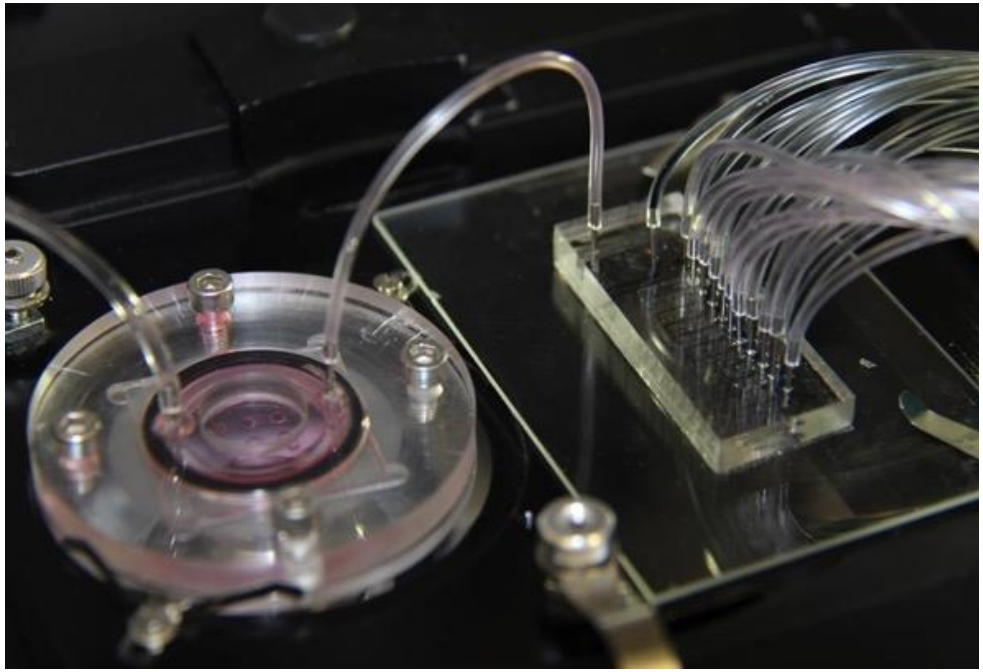




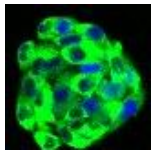


Bioreactor: Liver on a Chip

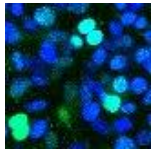




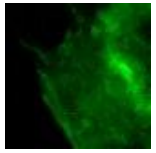
Engineered Cell Lines Identify Liver Toxicity



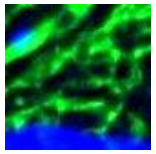
Oxidative Stress Reporter



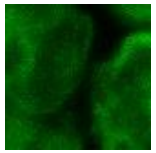
DNA Damage Reporter



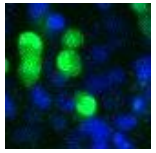
Cytoskeletal Integrity Reporter



Mitochondrial Morphological Reporter



ER Morphological Reporter

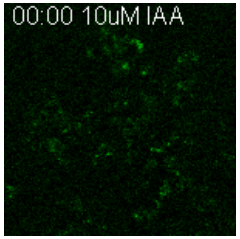


Unfolded Protein Stress Response Reporter

… and more (~50 reporter cell lines)

Image acquisition

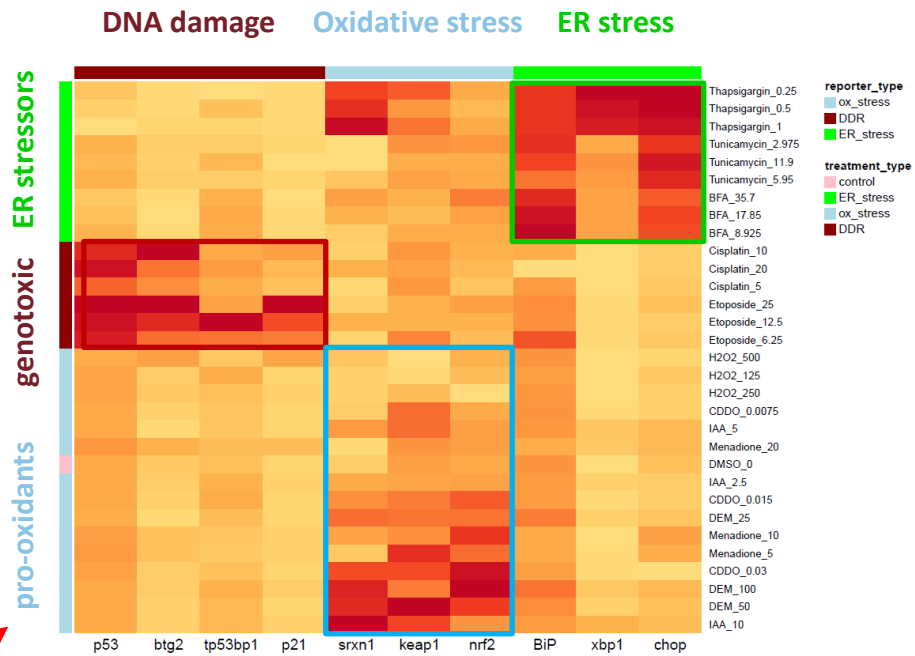
384 well plates



e.g.: Nrf2-GFP

Analysis & statistics

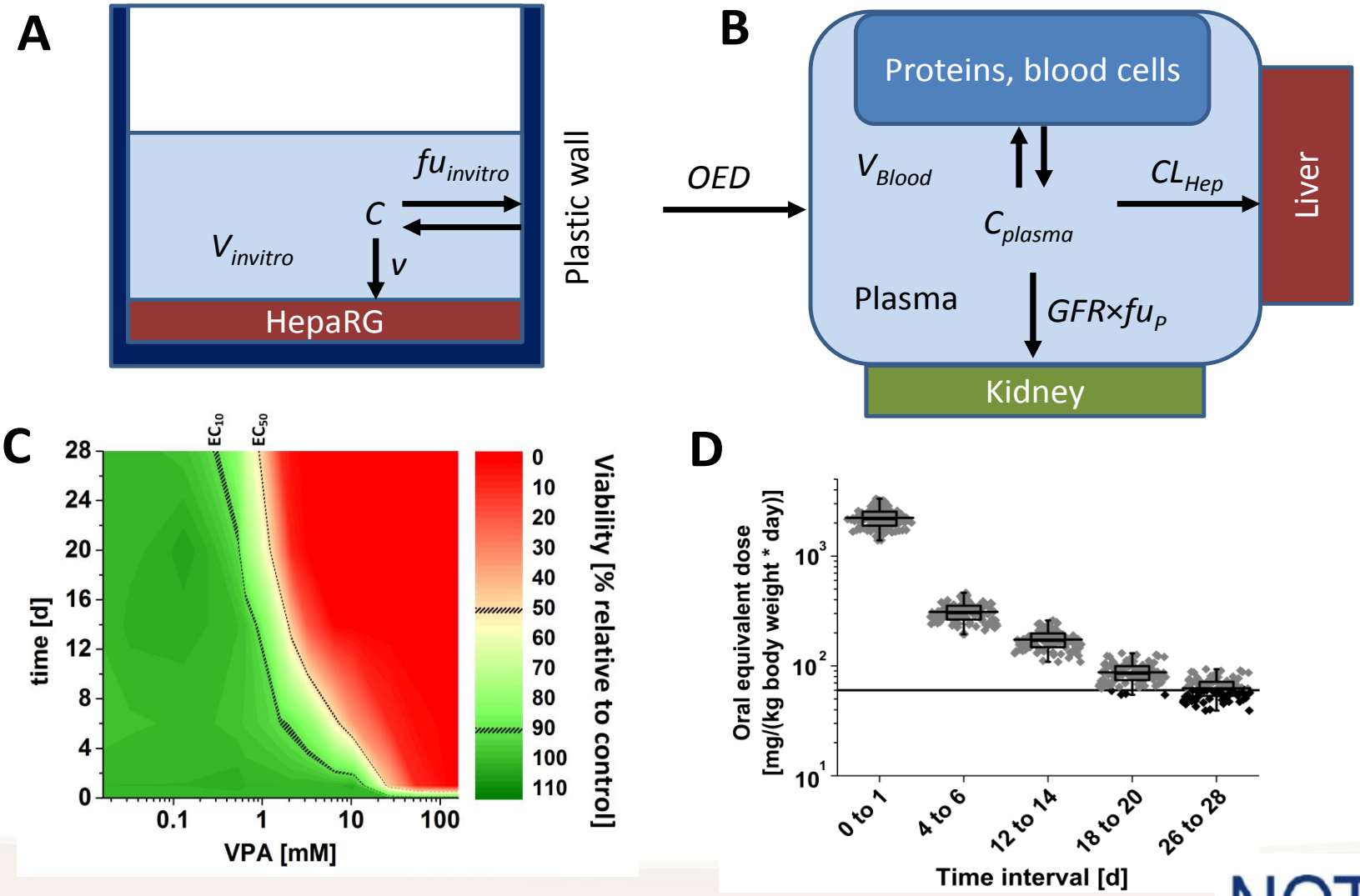
	ER	DNA	..
cell line 1			
cell line 2			
cell line 3			
cell line 4			
cell line 5			
cell line 6			
cell line 7			
cell line 8			
cell line ..			



Safety assessment: classification of compounds into type & severity of toxicity

	TP53	TP53BP1	HSPA5	CHOP	SRXN1	KEAP1	CYCS	LC3	etc
Compound 1	1	1	0	0	1	1	0	0	
Compound 2	0	0	0	0	0	0	0	0	
Compound 3	0	0	0	0	0	0	1	1	
Compound .	0	0	0	1	0	0	1	1	
Compound .	1	1	1	1	0				
Compound N	1	1	0	1	0				

Estimation of Oral Equivalent Doses from *in Vitro* Data



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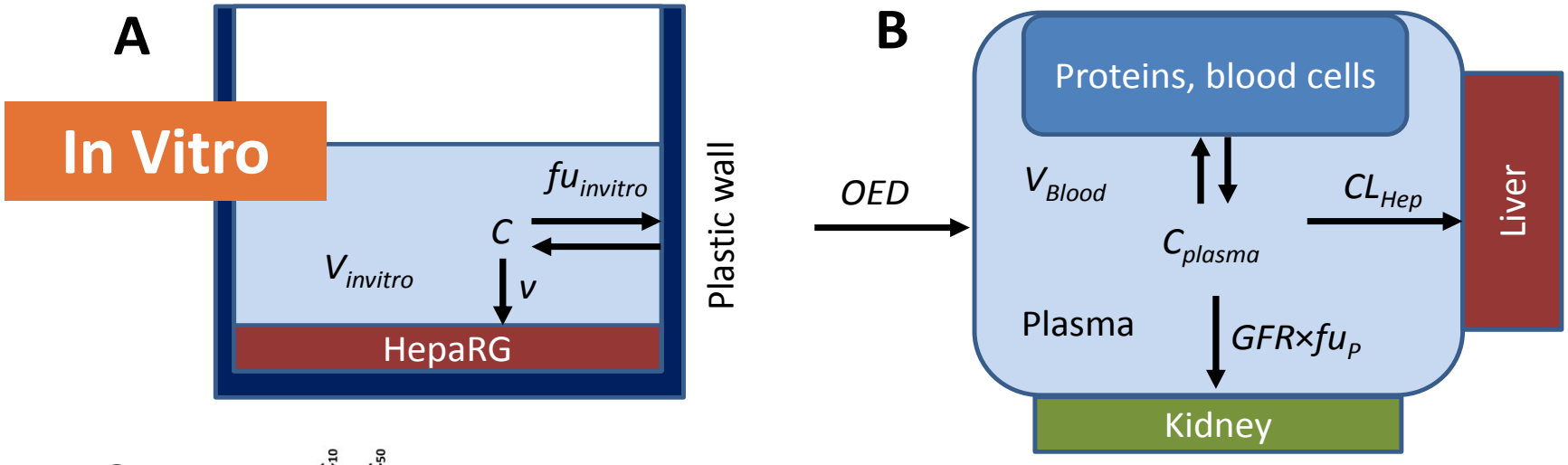
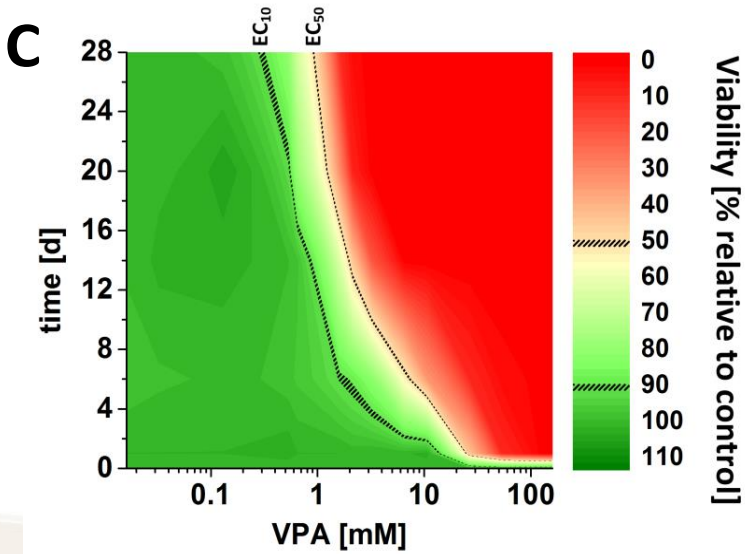
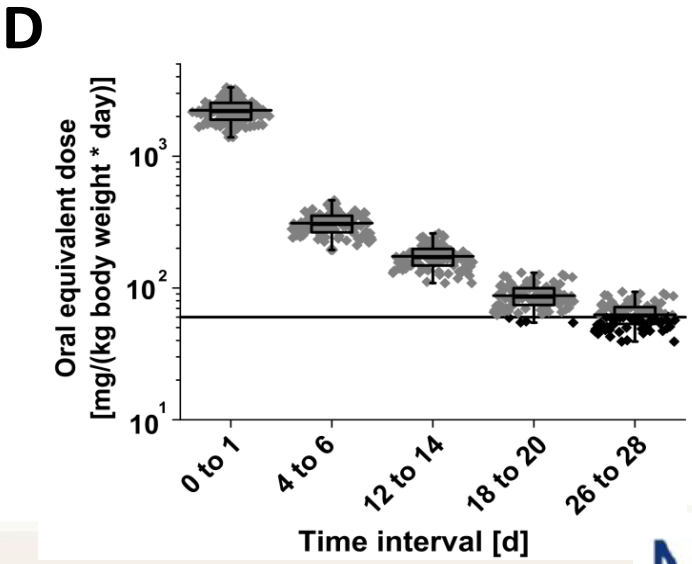
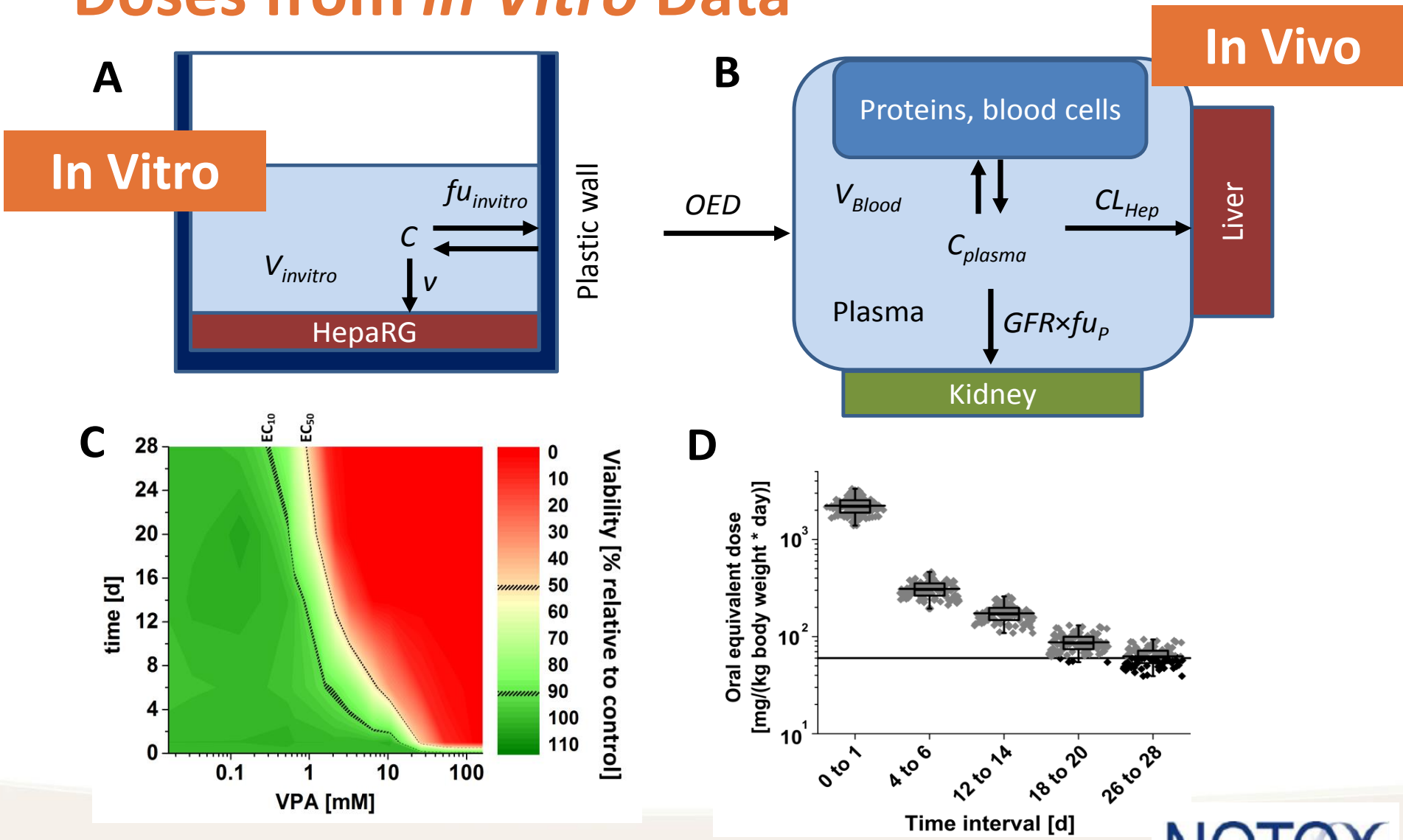






Estimation of Oral Equivalent Doses from *in Vitro* Data

SEURAT-1



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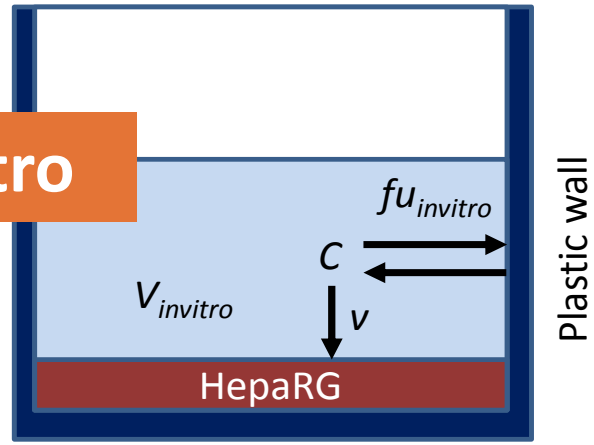
Estimation of Oral Equivalent Doses from *in Vitro* Data

SEURAT-1

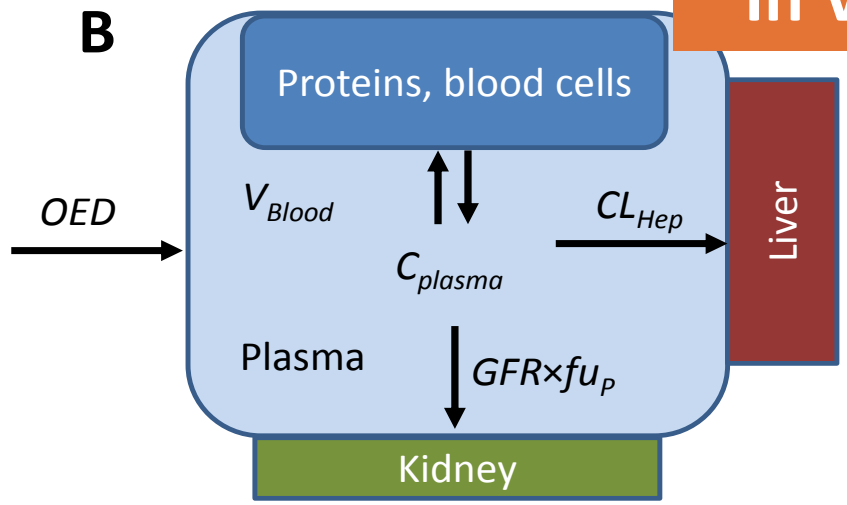
In Vitro

A

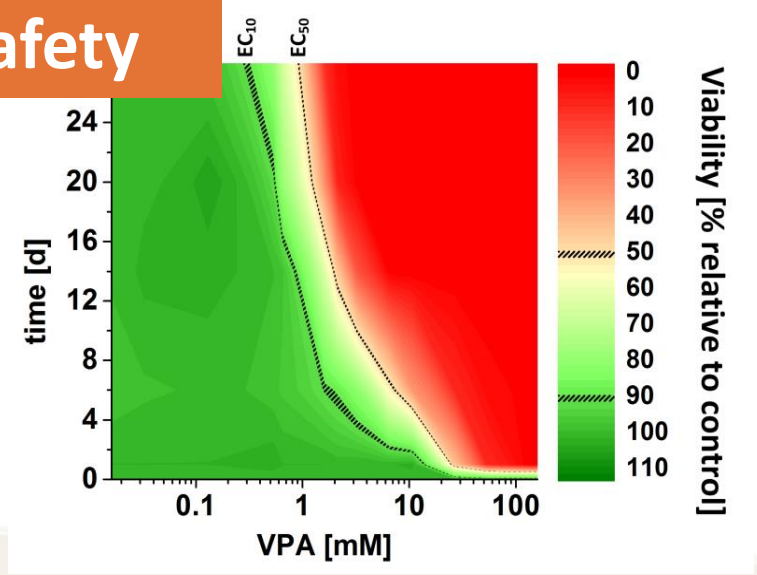


In Vivo

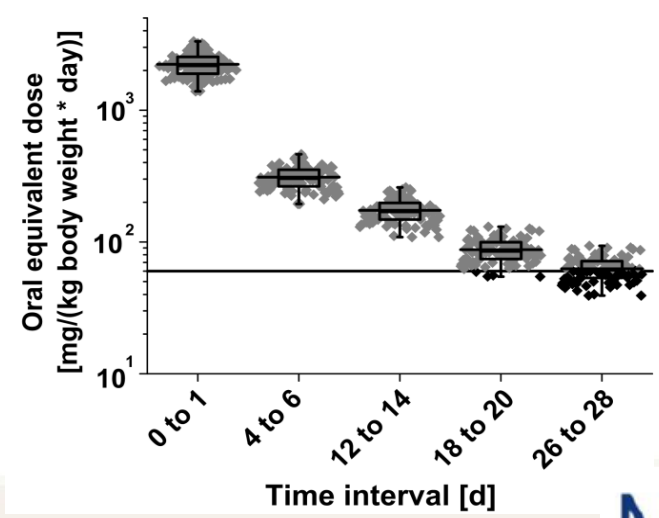
B



Safety



D



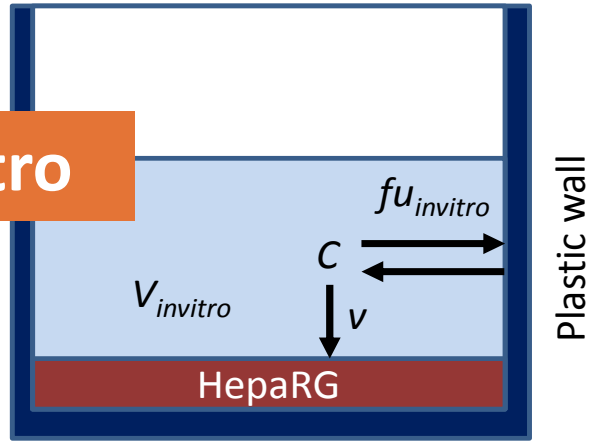
NOTOX

Estimation of Oral Equivalent Doses from *in Vitro* Data

SEURAT-1

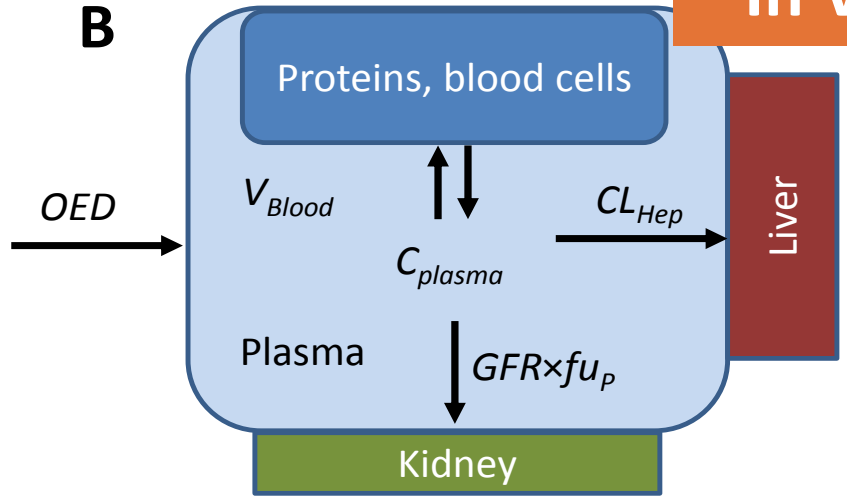
In Vitro

A

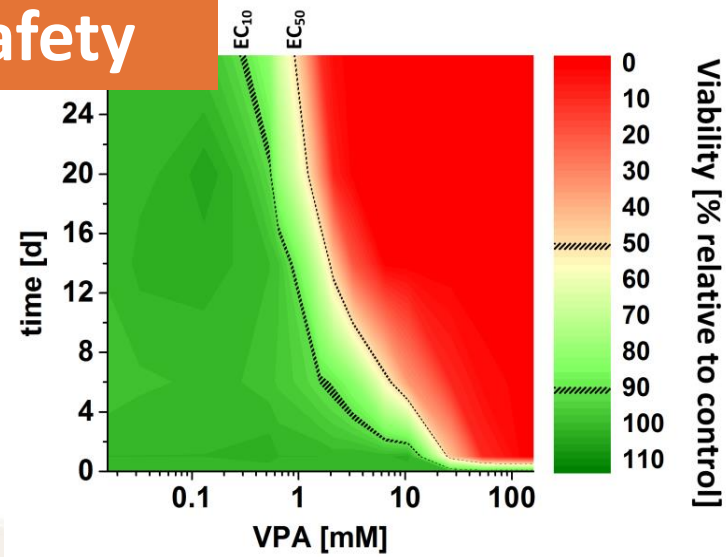


In Vivo

B

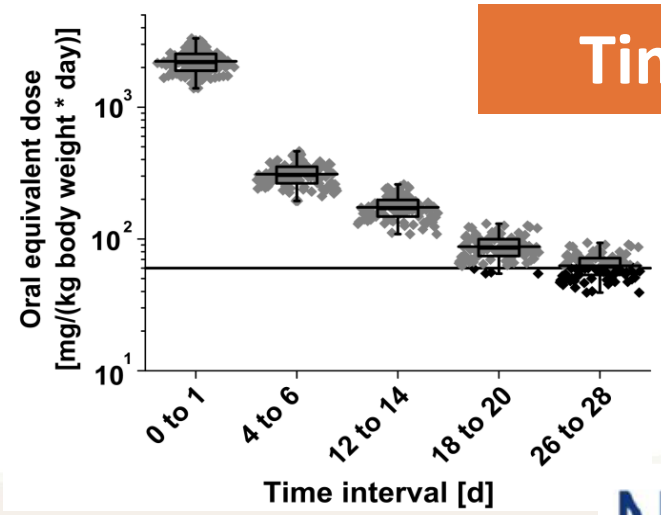


Safety



D

Time

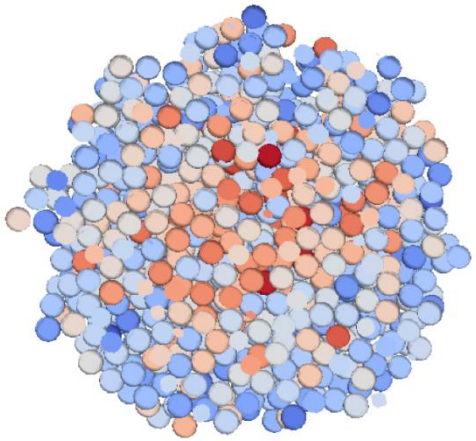


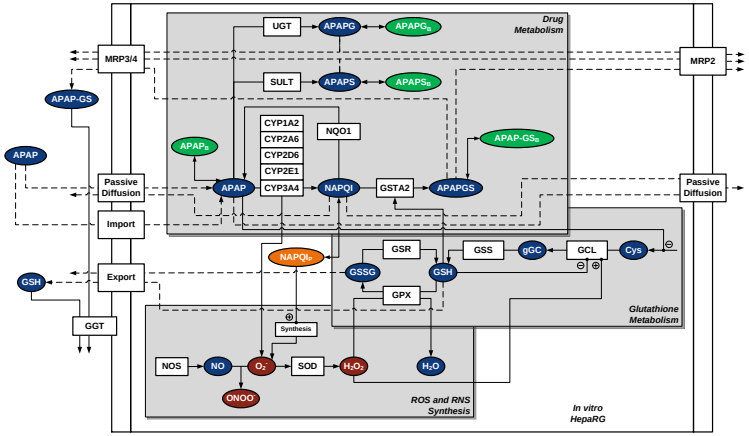
NOTOX

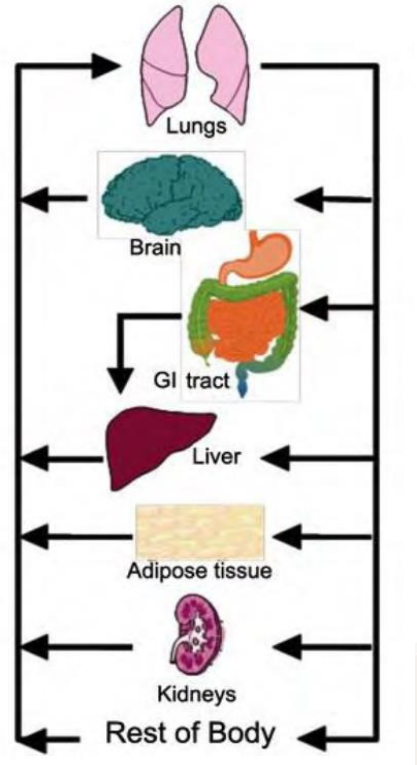
Computational Models Incorporating the Complexity of the Liver

SEURAT-1

Multicellular Model (spheroid, monolayer) + Intracellular Model → Human Model







Making Data, Experimental Protocols and Knowledge Available

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DATA WAREHOUSE

The ToxBank Data Warehouse will establish a centralised compilation of data for systemic toxicity.

Data generated under the research program and additional public data will be uploaded and integrated whenever possible into computerised models capable of predicting repeated-dose toxicity.

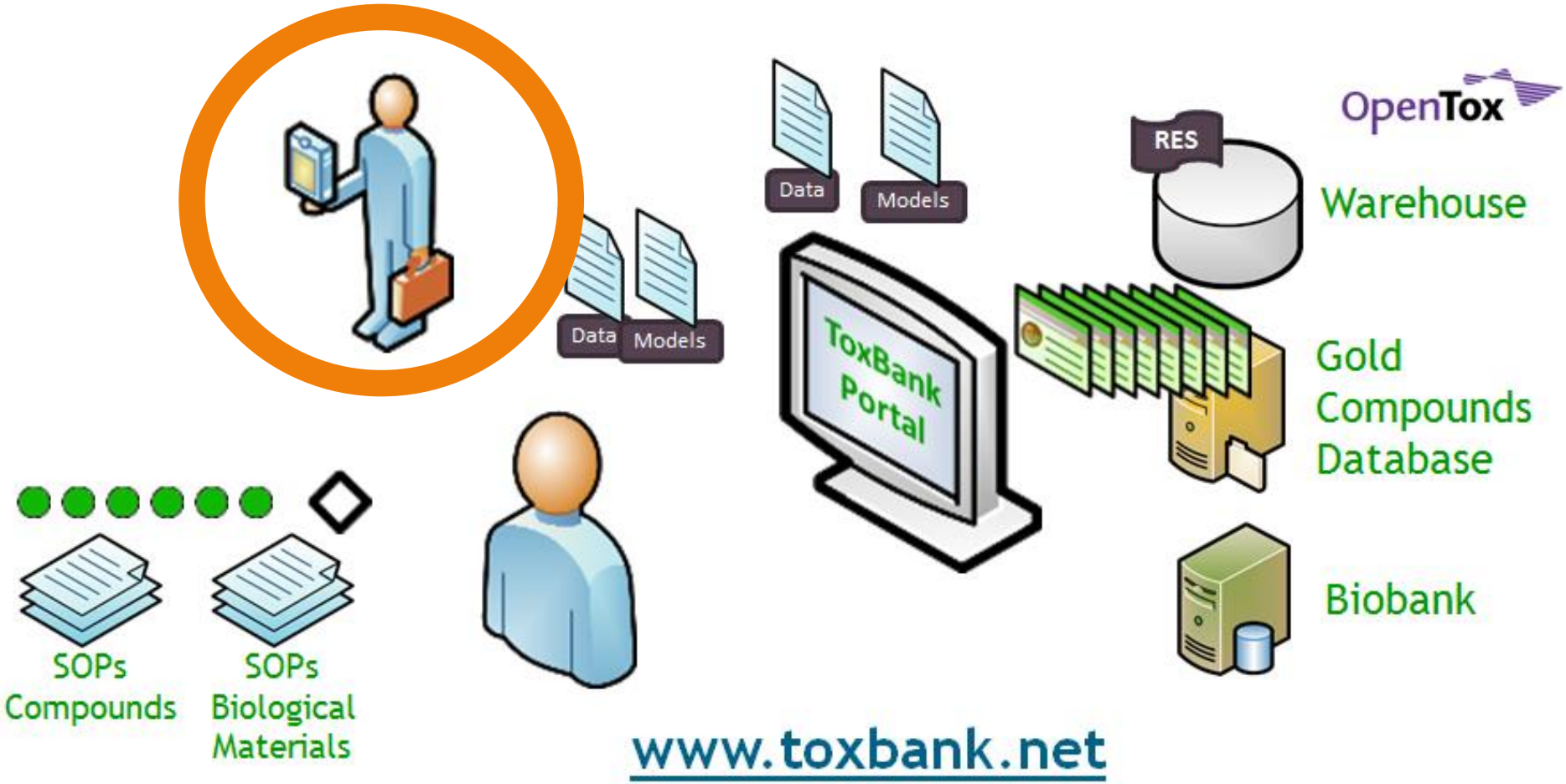
The ToxBank data warehouse will use the ISA-TAB file format for interchange, storage and query of any investigation data, including dose-response and omics results.

RESOURCES LIST

- DATA WAREHOUSE
- COMPOUND WIKI
- BIO WIKI

Management and Sharing Knowledge

Management and Sharing Knowledge



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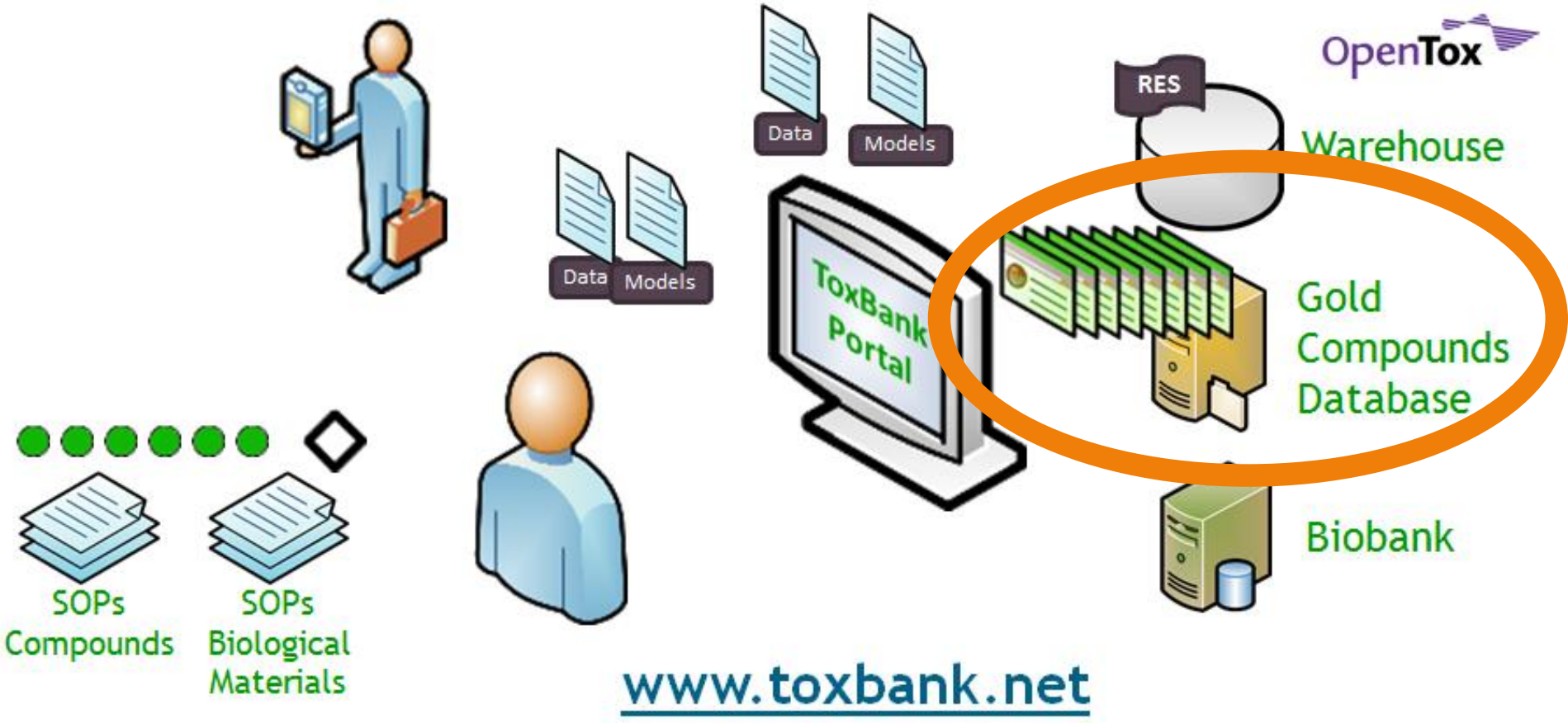

Management and Sharing Knowledge

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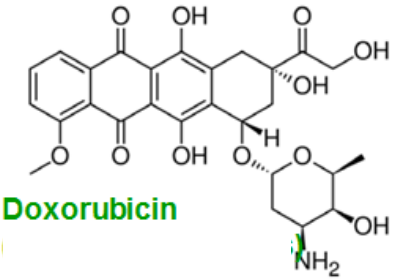
Legacy

ToxBank Enabling Integration and Analysis



Doxorubicin

A

ToxBank Data Warehouse (data curation and retrieval)

Transcriptomics profiles

Protocols and SOPs, upload investigation data in ISA-TAB format

Differentially expressed genes (R/Bioconductor)

B

C

Connectivity Map (MCF7, PC-3 cell lines; $p < 0.01$)

1. Doxorubicin (0.999) *
2. H-7 (0.999) *
3. Mitoxantrone (0.998) *
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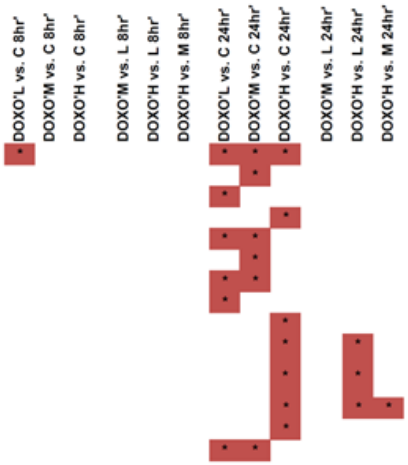
20. Famprofazone (0.643)

*= topoisomerase II inhibitor (Mantra 2.0)

Pathway meta-analysis using KEGG pathways (InCroMap) software)



Pathways

1. Cell cycle
2. p53 signaling pathway
3. Oocyte meiosis
4. TNF signaling pathway
5. DNA replication
6. Mismatch repair
7. Fanconi anemia pathway
8. Viral carcinogenesis
9. Rheumatoid arthritis
10. Influenza A
11. Chagas disease (American trypanosomiasis)
12. Hepatitis B
13. Herpes simplex infection
14. Pyrimidine metabolism

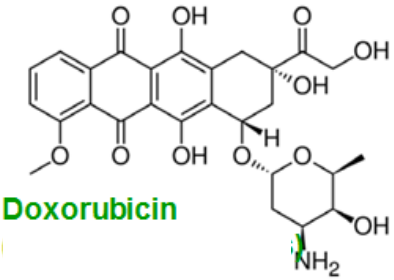
D

Comparative Toxicogenomics Database (q-value < 0.01)

Disease Name	Disease ID
1. Cardiovascular Diseases	MESH:D002318
2. Digestive System Diseases	MESH:D004066
3. Neoplasms	MESH:D009369
4. Neoplasms by Histologic Type	MESH:D009370

Significance: *=FDR q-value < 0.05
Doses: C=Control, L=Low, M=Middle, H=High; Time: 8hr=8 hours, 24hr=24 hours

ToxBank Enabling Integration and Analysis



Doxorubicin

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Transcriptomics

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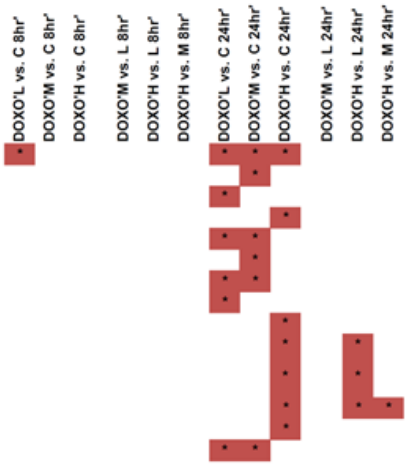

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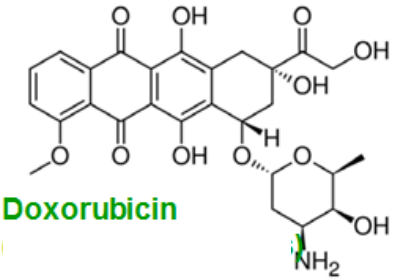

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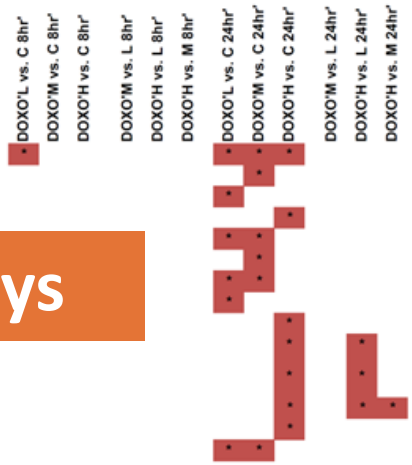

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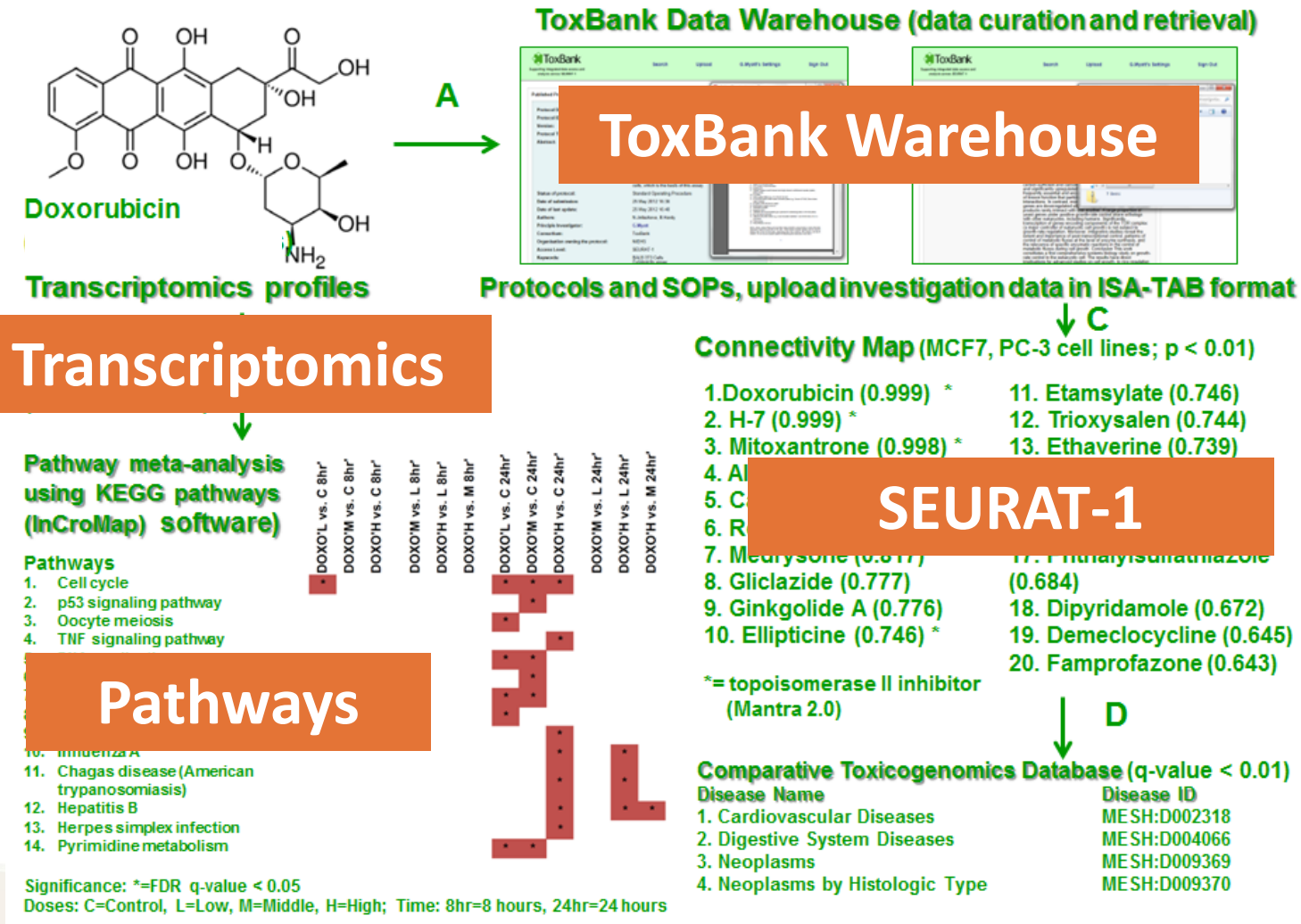

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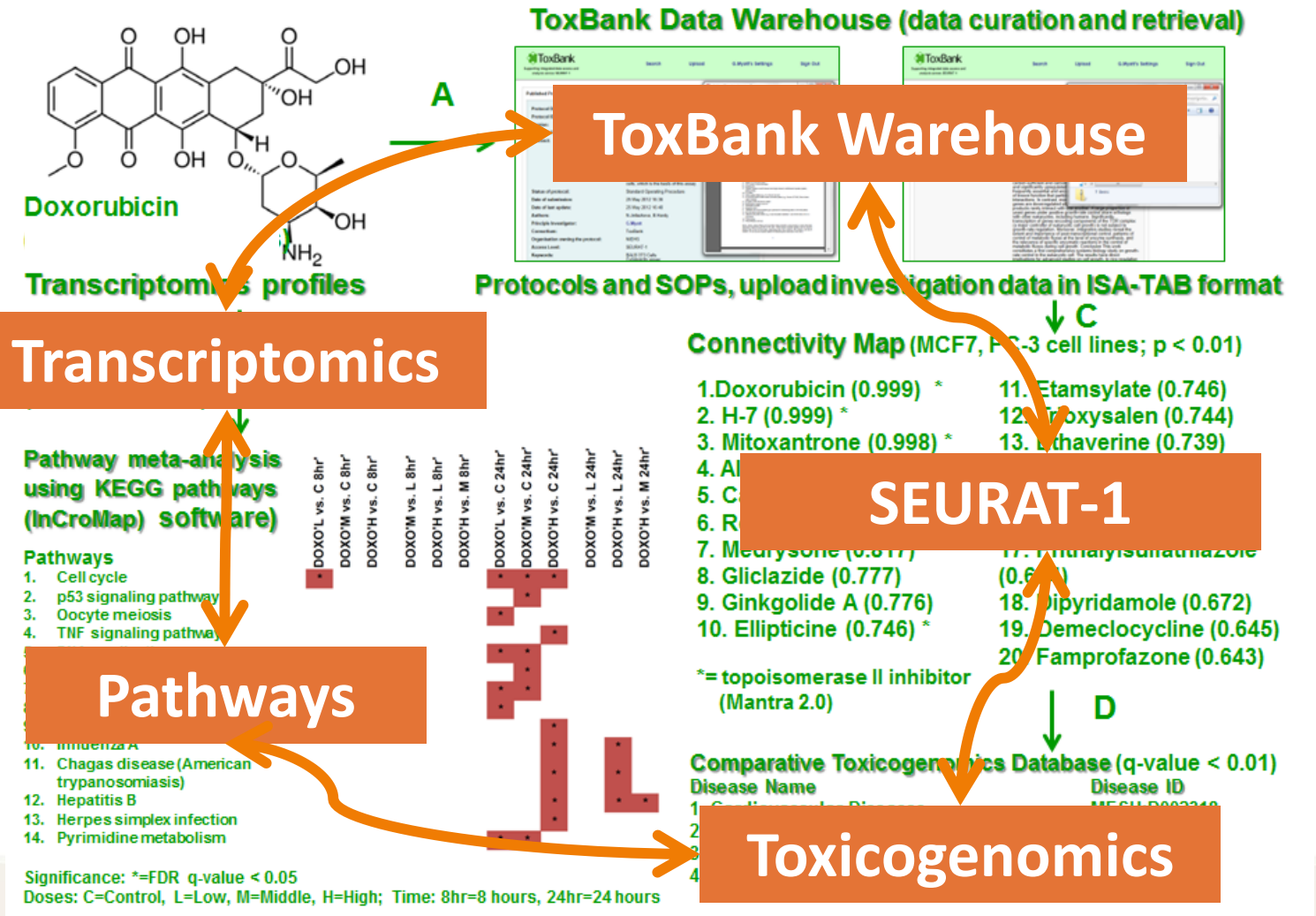
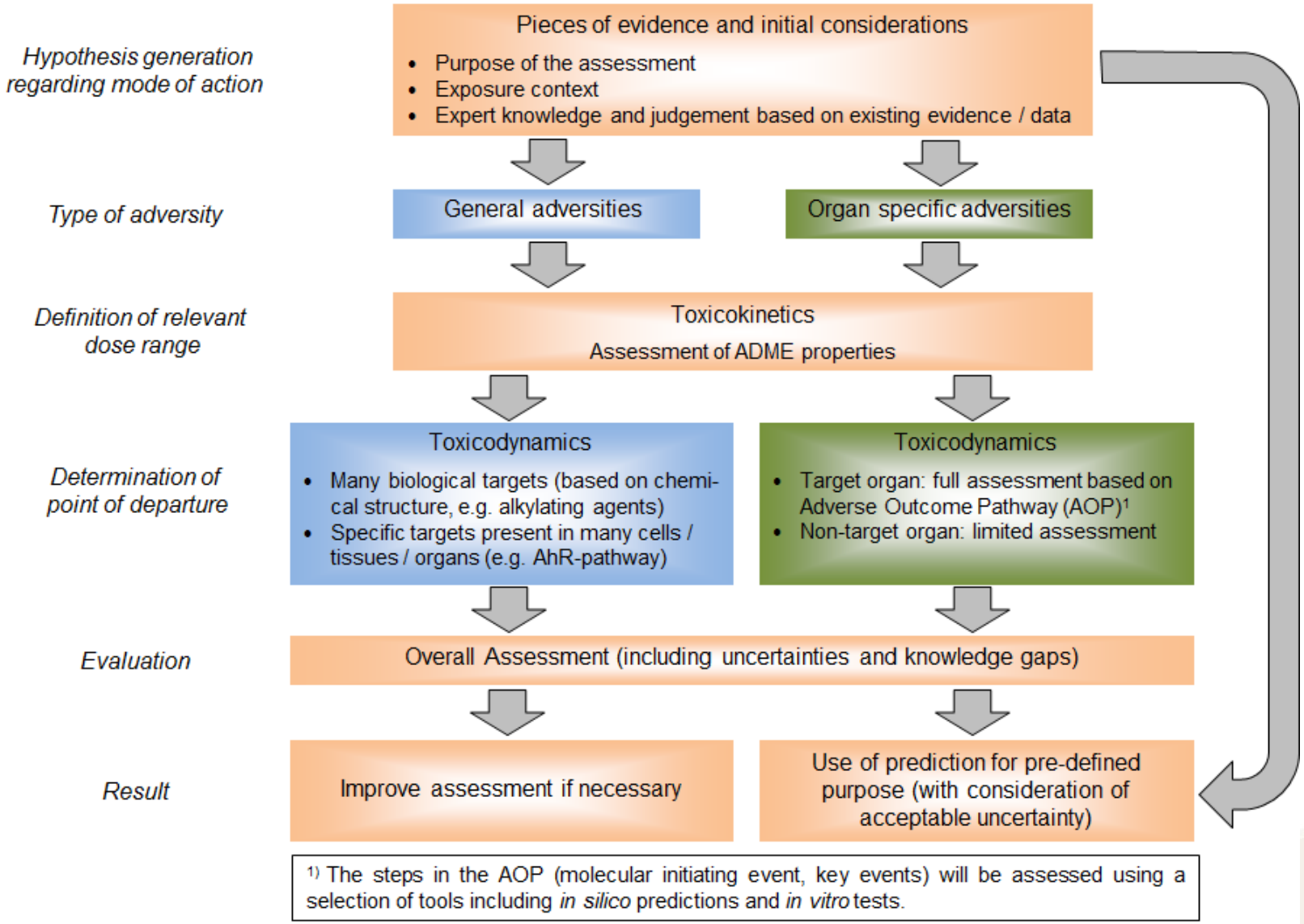


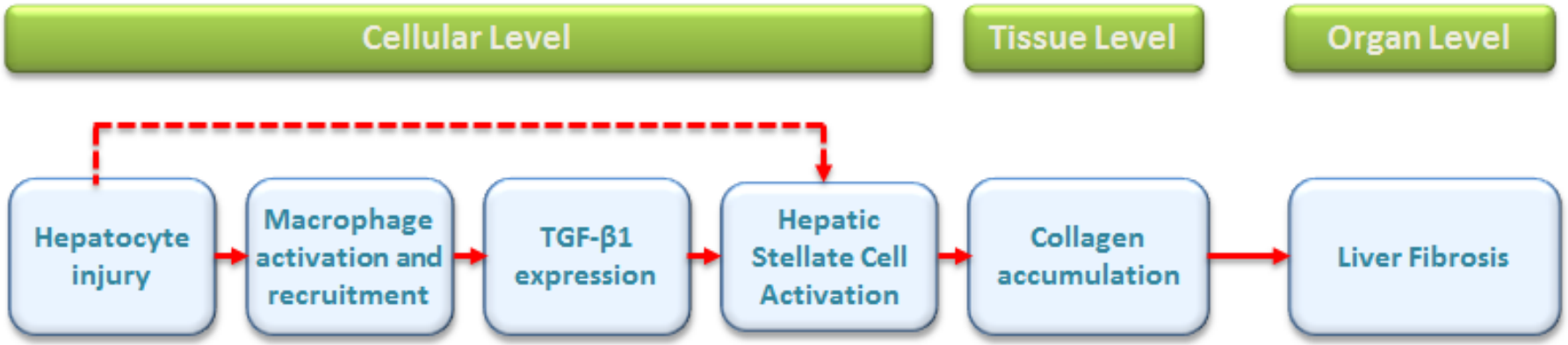
Knowledge Driven Integration: SEURAT-1 Conceptual Framework



A Framework to Organise Biological Knowledge Relevant to Adverse Effects

Integration

Systematic Presentation



Direction

Collaboration

Identification

A Framework to Organise Biological Knowledge Relevant to Adverse Effects

SEURAT-1

Integration

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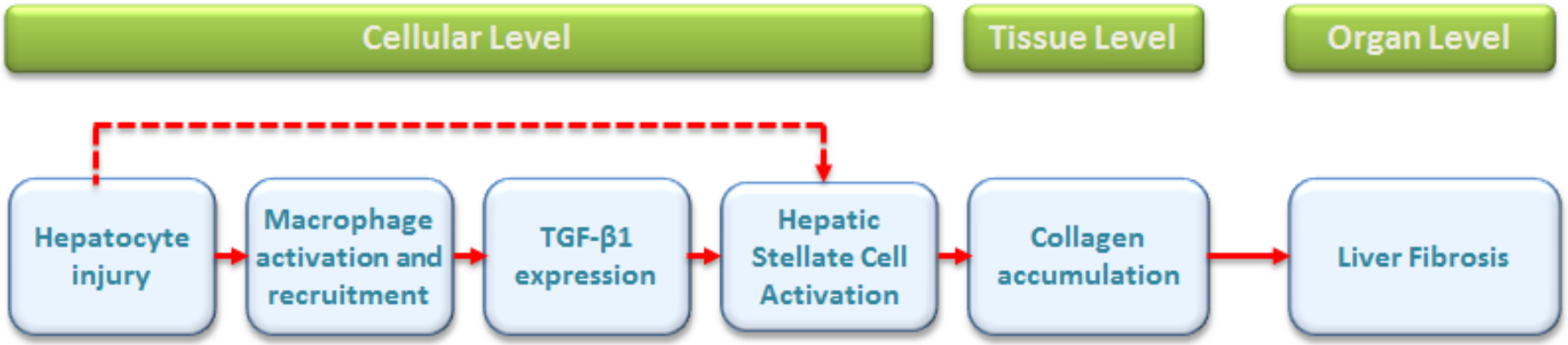

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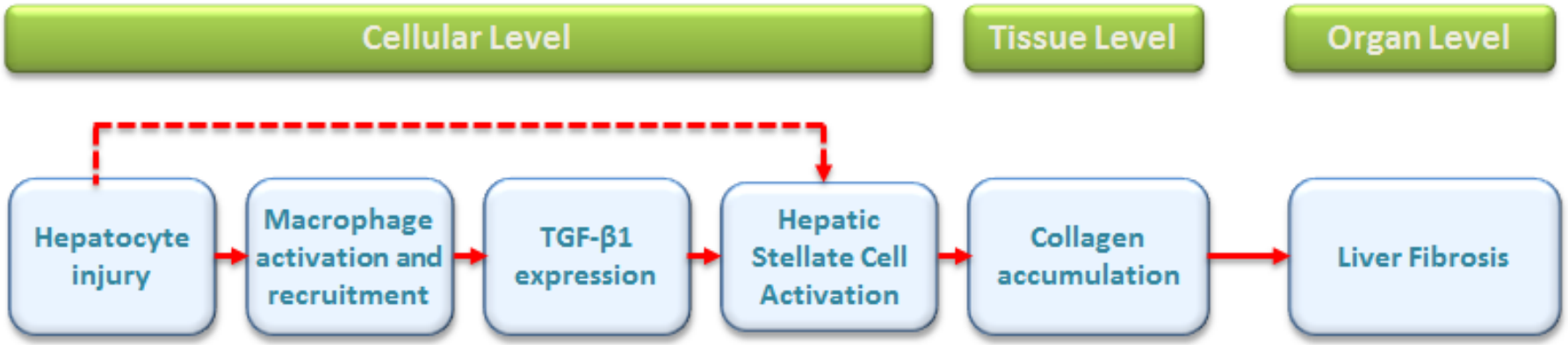

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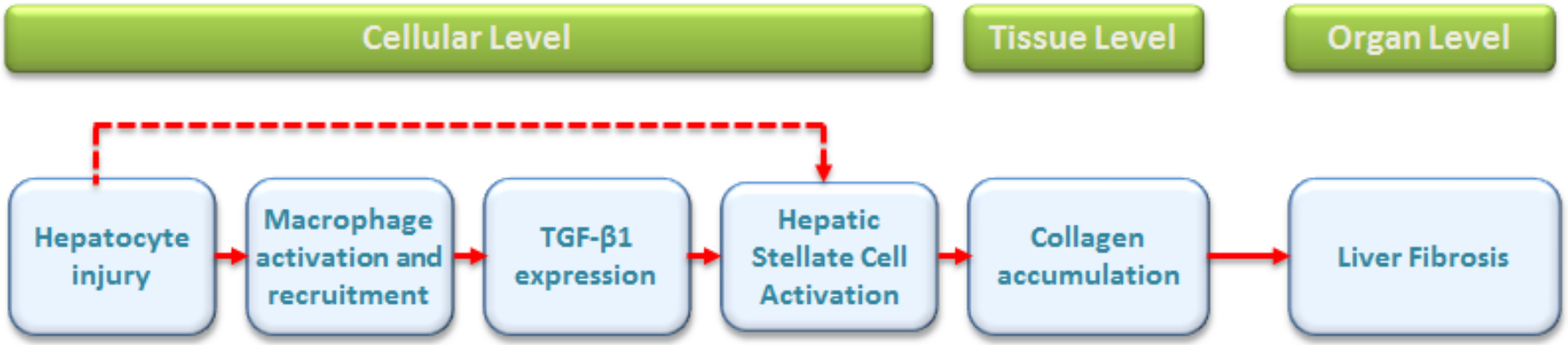

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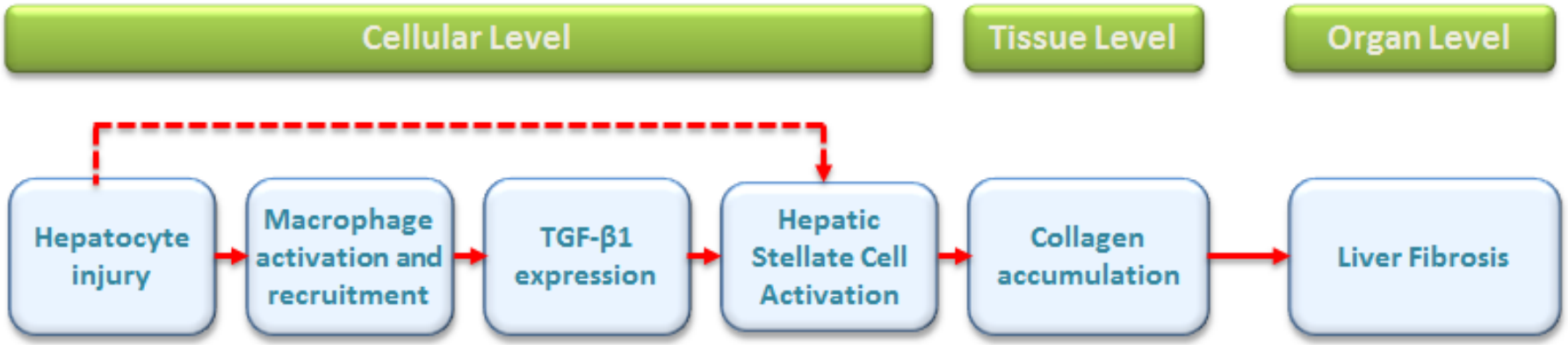

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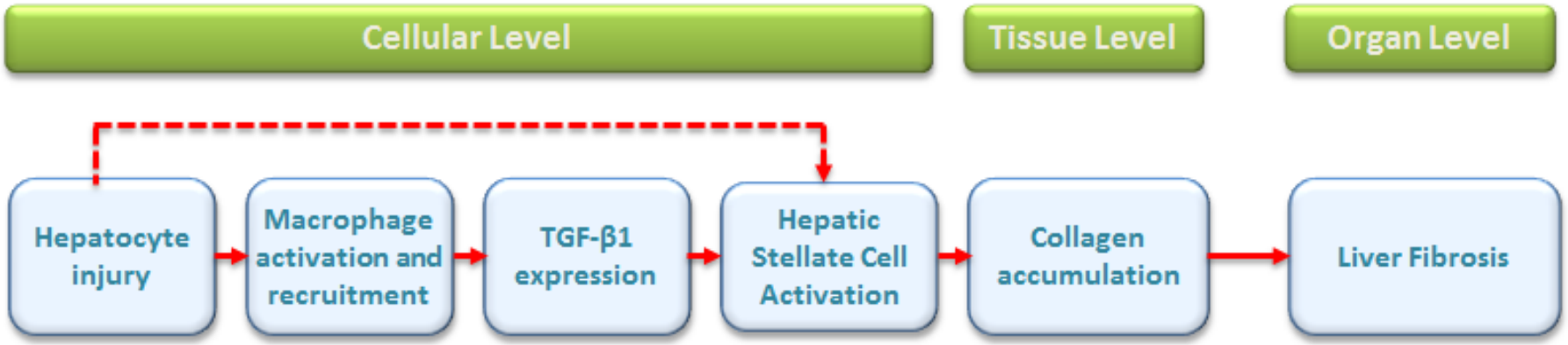

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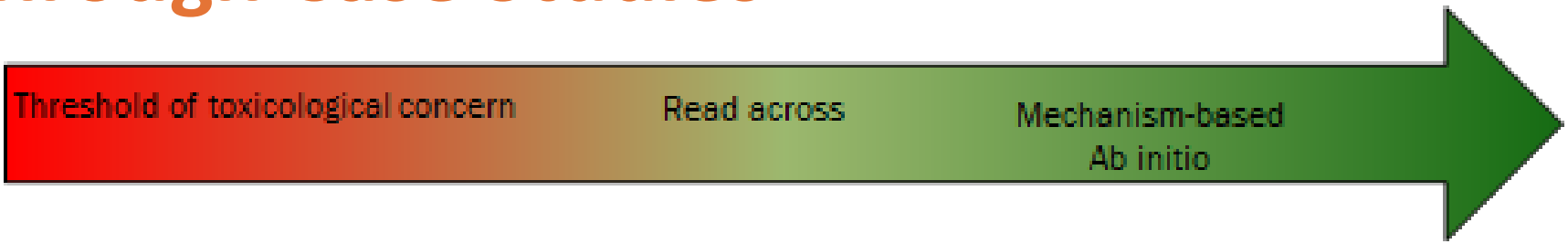
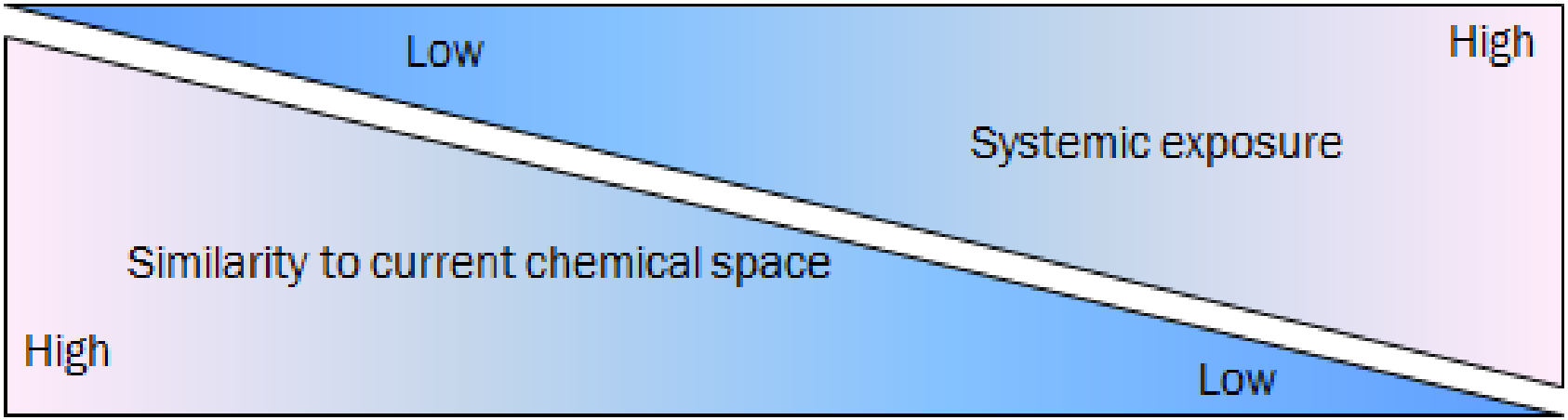
Application of Technologies Through Case Studies





NOW:
Low freedom to operate

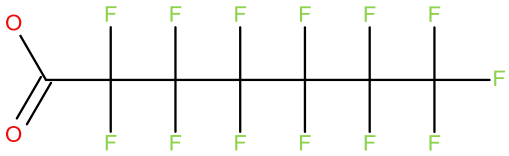
AMBITION:
High freedom to operate

Underpinned by international scientific co-operation
and regulatory acceptance

Case Study: Threshold of Toxicological Concern (TTC)

- TTC aims to identify a safe concentration of a compound
- Pre-SEURAT-1, TTC was not determined on cosmetics-relevant compounds
- Expert review of chronic toxicity data expanded the dataset and provided new knowledge
- Route-to-route extrapolation scheme developed
- Analysis confirms precautionary nature of TTC values

Case Study: Enhancing Read-Across



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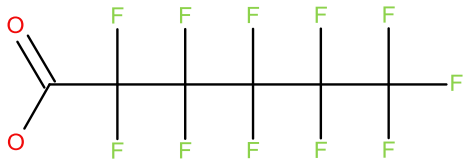

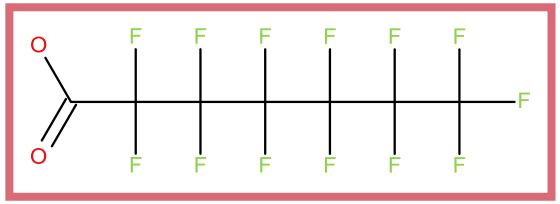

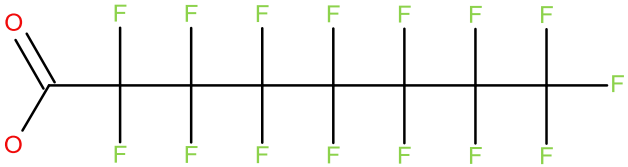

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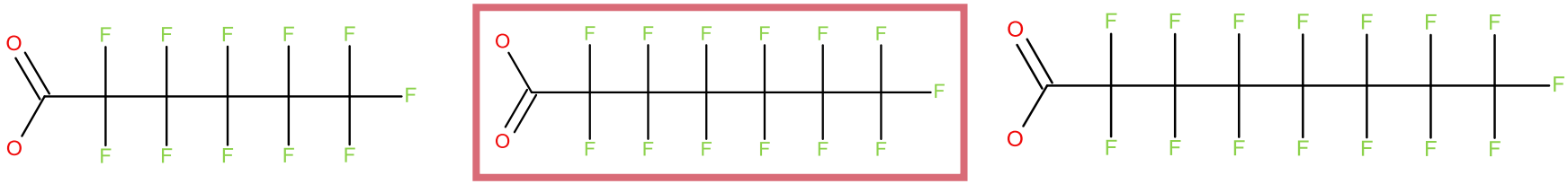
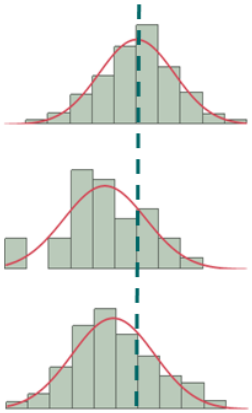
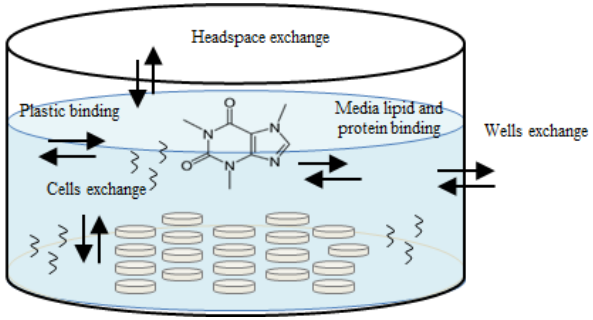


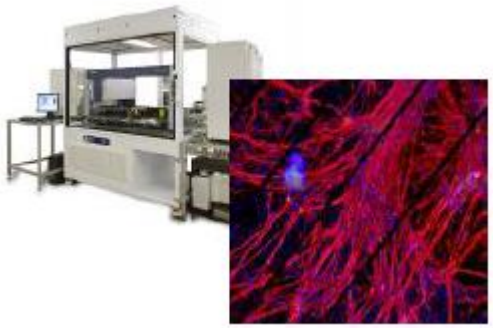
- Four scenarios developed
- Reporting format proposed
- Extensive data collection and review
- Support from SEURAT-1 and ToxCast Technologies
- Review, analysis, improvement
- Link to ECHA Topical Workshop April 2016

Case Study: *Ab Initio* Risk Assessment

- Demonstrating SEURAT-1 TTC, *in silico* and *in vitro* technologies as risk assessment tools







- Applying SEURAT-1 conceptual framework to a real compound in a real safety assessment for a product
- Understanding limitations through review and analysis

Realisation – From Novel Technologies to 21st Century Risk Assessment

- Threshold of Toxicological Concern presented at the Scientific Committee of Consumer Safety (SCCS)
- Read-across scenarios apply methods
  - ➔ Presented at OECD
  - ➔ Application of ECHA RAAF to case studies
- AOPs contributing to the OECD Working Group and AOP-Wiki
- Direct application of technologies to an *ab initio* risk assessment
- Provided basis for international collaboration
  - ➔ US EPA's ToxCast and Tox21
- Informing future research needs and projects

SEURAT-1: Achievements

- Technologies
  - From *in silico* to *in vitro*
- Integration
  - Applying the Conceptual Framework through Adverse Outcome Pathways
- Realisation
  - Gaining international acceptance

Acknowledgements

SEURAT-1

NOTOX

HeMiBio

COSMOS

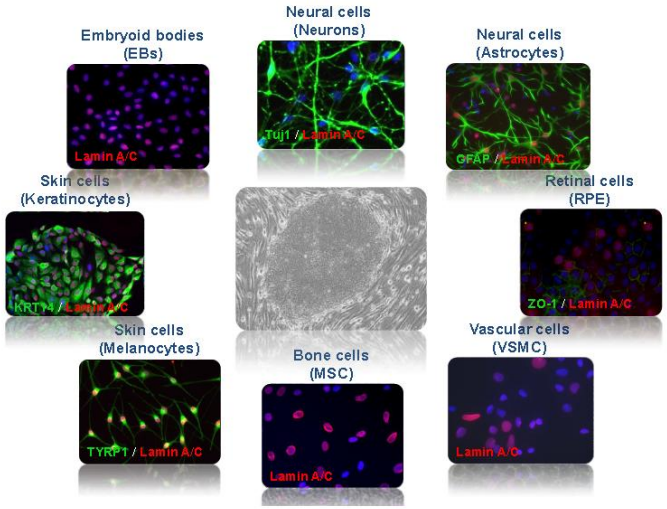
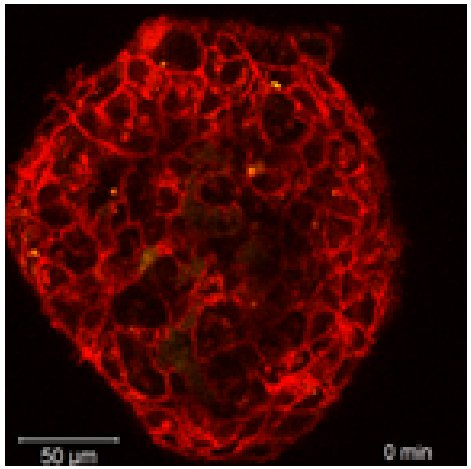
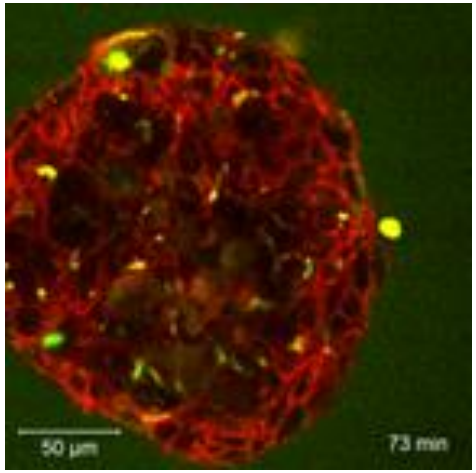
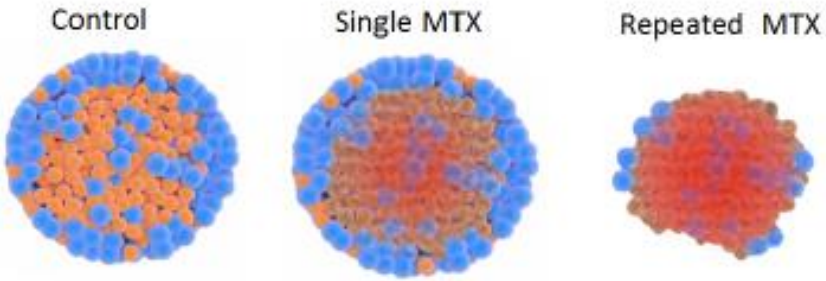
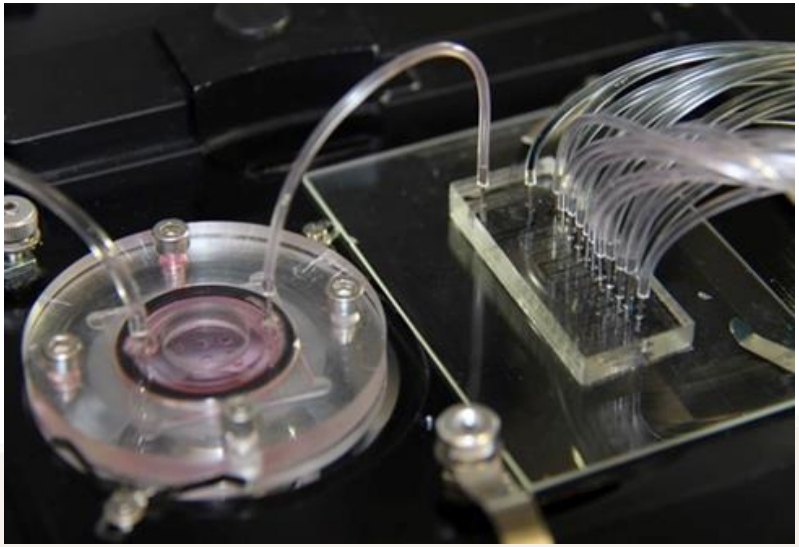
SEP

COACH (secretariat)

ToxBank

DETECTIVE

Detection of Endpoints and Biomarkers for Repeated Dose Toxicity Using In Vitro Systems

ScrTox

Increasing Complexity in Cellular Systems to Predict Different Effects