

The European ONTOX project: goals and first results

*Ontology-driven and artificial intelligence-based
repeated dose toxicity testing of chemicals
for next generation risk assessment*

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GSR23 Workshop “*Present and future of AI, open science and transparency in regulatory science*”

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Parma-Italy

SCOPE



Goal

Development of an animal-free and human-relevant strategy for the prediction of chemical-induced toxicity



Focus

- Systemic repeated dose toxicity
- 6 case studies
 - ▶ Liver: steatosis and cholestasis
 - ▶ Kidney: tubular necrosis and crystallopathy
 - ▶ Brain: neural tube closure and cognitive function defects
- Drugs, cosmetics, biocides and food ingredients



Driving principles

- Replacement, reduction and refinement of animal experimentation
- 21st century toxicity testing
- Next generation risk assessment

CONCEPT

✓ Rationale

- Rely as much as possible on available data, models and methods
- Fill data gaps by means of targeted *in vitro* testing and *in silico* modeling

✓ Toolbox

- Human data and biological material
- *In vitro* methods and stem cell technology
- *In silico* methods and artificial intelligence

✓ Deliverable

6 new approach methodologies (NAMs) for each of the 6 case studies



Artificial intelligence system



Cell culture system



In vitro assays



In silico tools

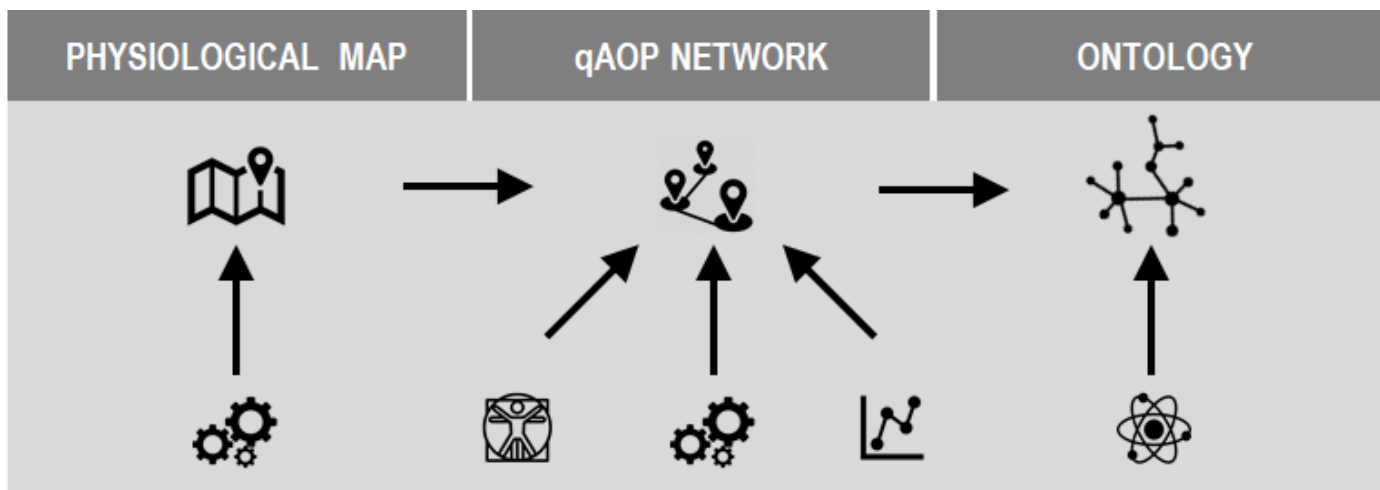
In vitro/in silico test battery

NAM

ARTIFICIAL INTELLIGENCE

✓ Collecting (big) data

- Type: biology, toxicology, chemistry and kinetics (ontology)
- Sources: safety dossiers, databases, papers, text books, other projects, ...

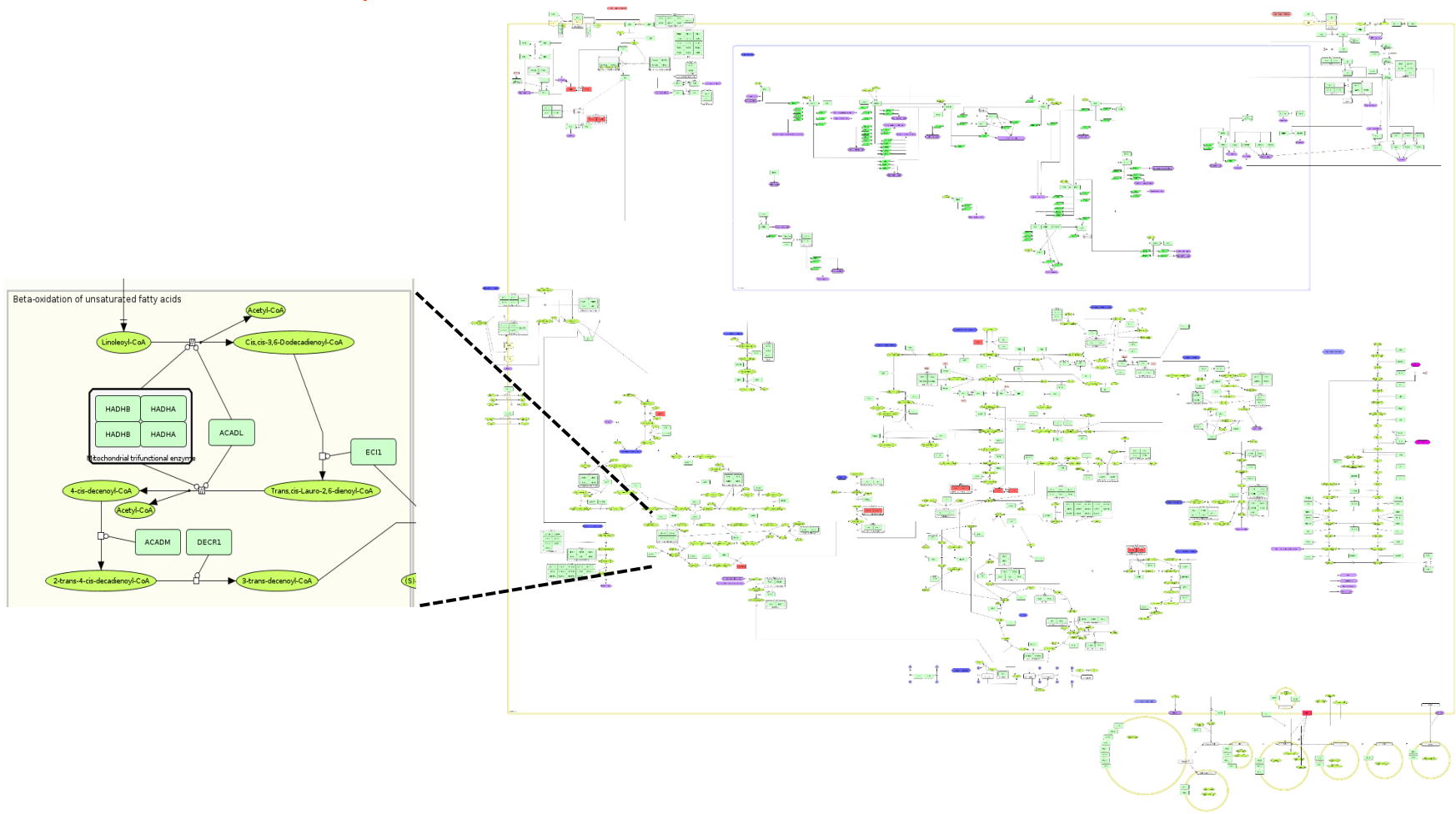


✓ Making sense out of the data

- Goal: create a blueprint of toxicological effects
- Technique: machine learning (advanced read-across structure-activity relationship)

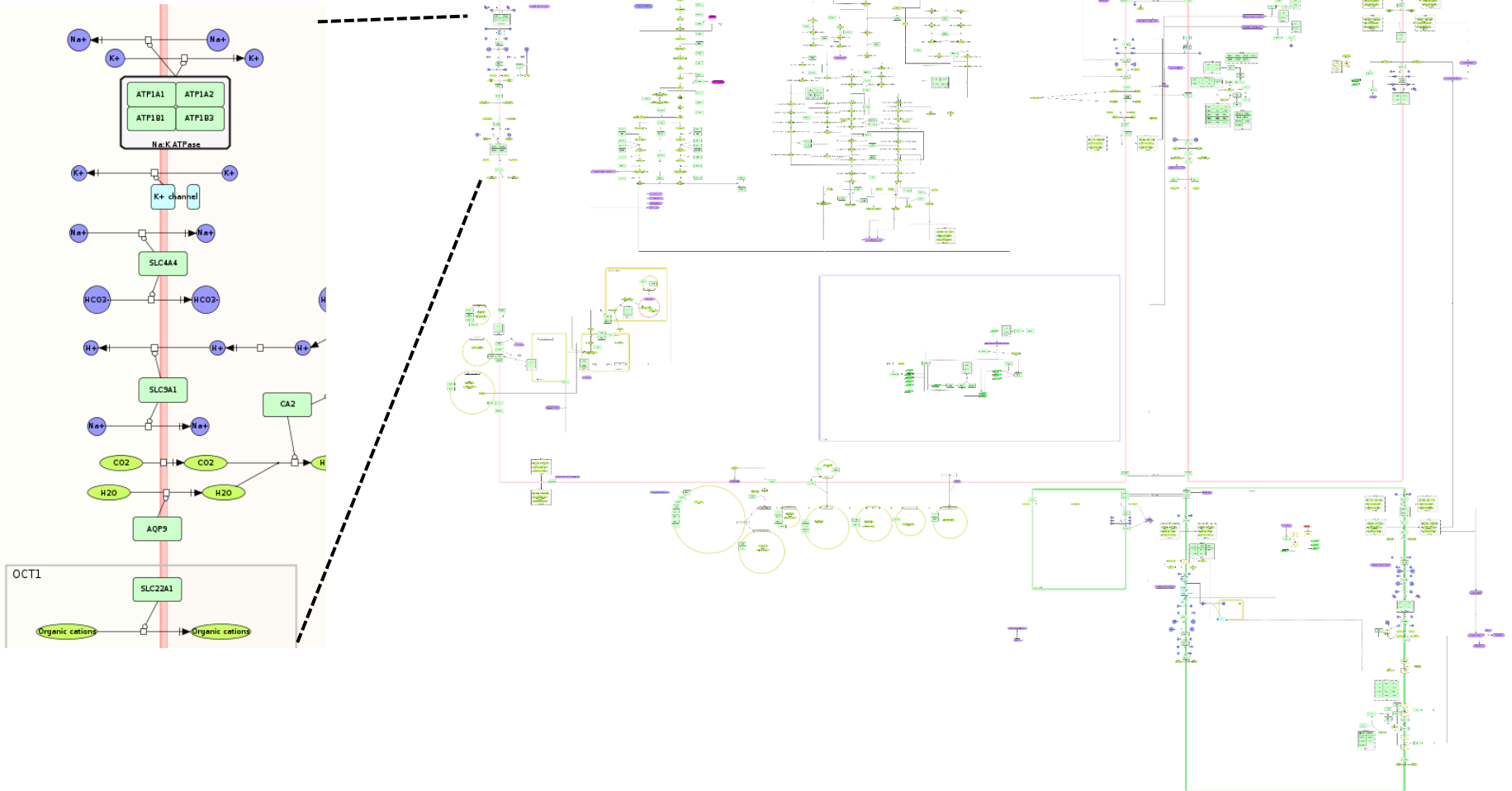
PHYSIOLOGICAL MAPS

✓ Liver lipid metabolism



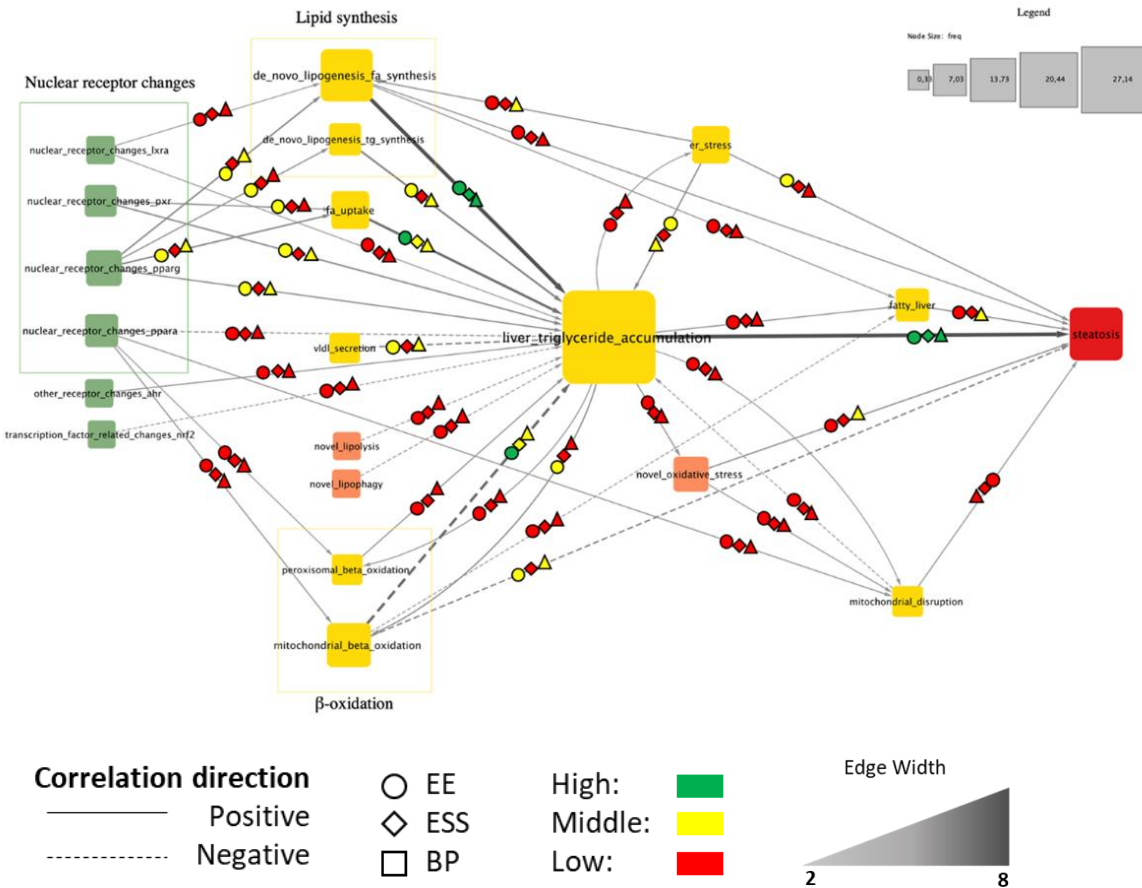
PHYSIOLOGICAL MAPS

✓ Liver bile acid metabolism



AOP NETWORKS

✓ Liver steatosis



SysRev-assisted literature search

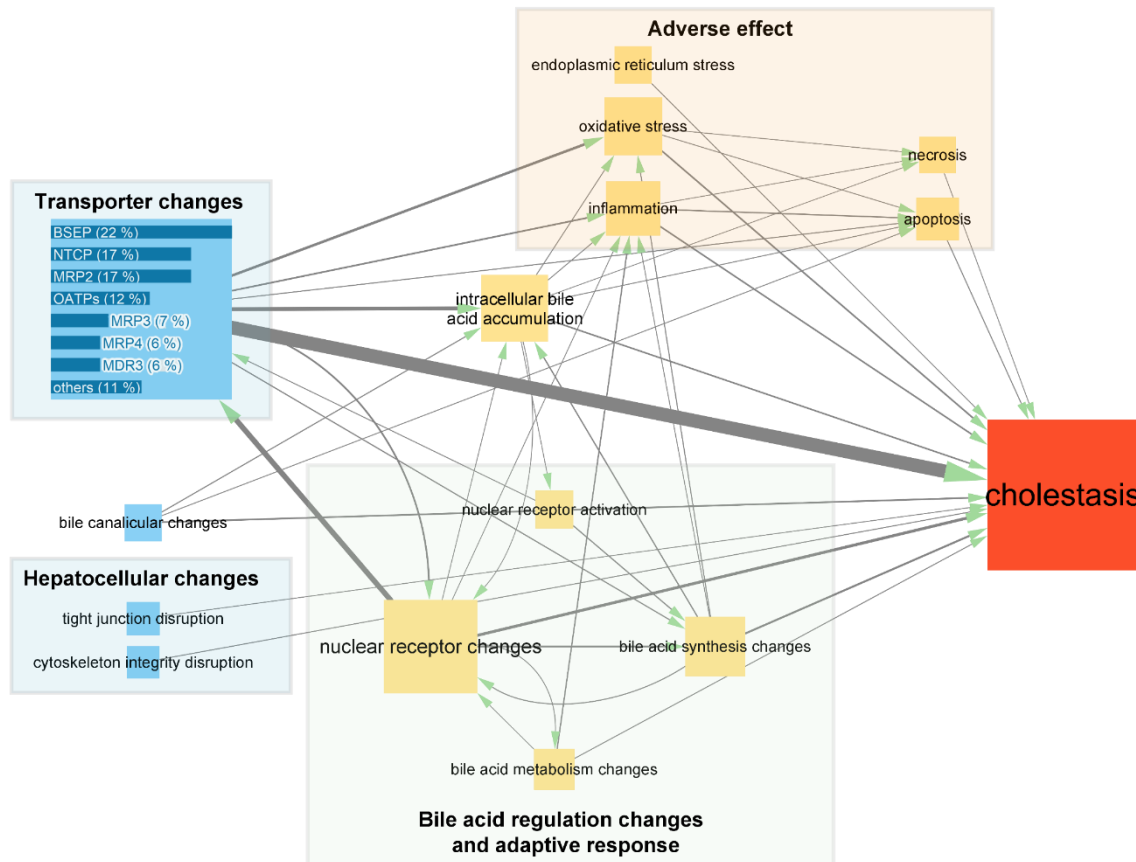
Step	Tool	Result
Literature search	PubMed	12478 articles
Title and abstract screening	Manual 10% SysRev 90%	1626 articles
Full-text screening and data extraction	SysRev	178 articles
AOP evaluation	The tailored Bradford-Hill Criteria and the OECD guidelines	Biological plausibility Essentiality Empirical evidence
Number of unique KEs	Manual	107 KEs
Number of unique KERs	Manual	231 KERs
Network virtualization	Cytoscape 3.9.1	

Abbreviations: KE(s), key event(s); KER(s), key event relationship(s); OECD, Organization for Economic Cooperation and Development



AOP NETWORKS

✓ Cholestasis



SysRev-assisted literature search

Step	Tool	Result
Literature search	PubMed	6572 articles
Title and abstract screening	Manual 10% SysRev 90%	544 articles
Full-text screening and data extraction	SysRev	150 articles
AOP evaluation	The tailored Bradford-Hill Criteria and the OECD guidelines	Biological plausibility Essentiality Empirical evidence
Number of unique KEs	Manual	97 KEs
Number of unique KERs	Manual	456 KERs
Network virtualization	Cytoscape 3.9.1	

Abbreviations: KE(s), key event(s); KER(s), key event relationship(s); OECD, Organization for Economic Cooperation and Development

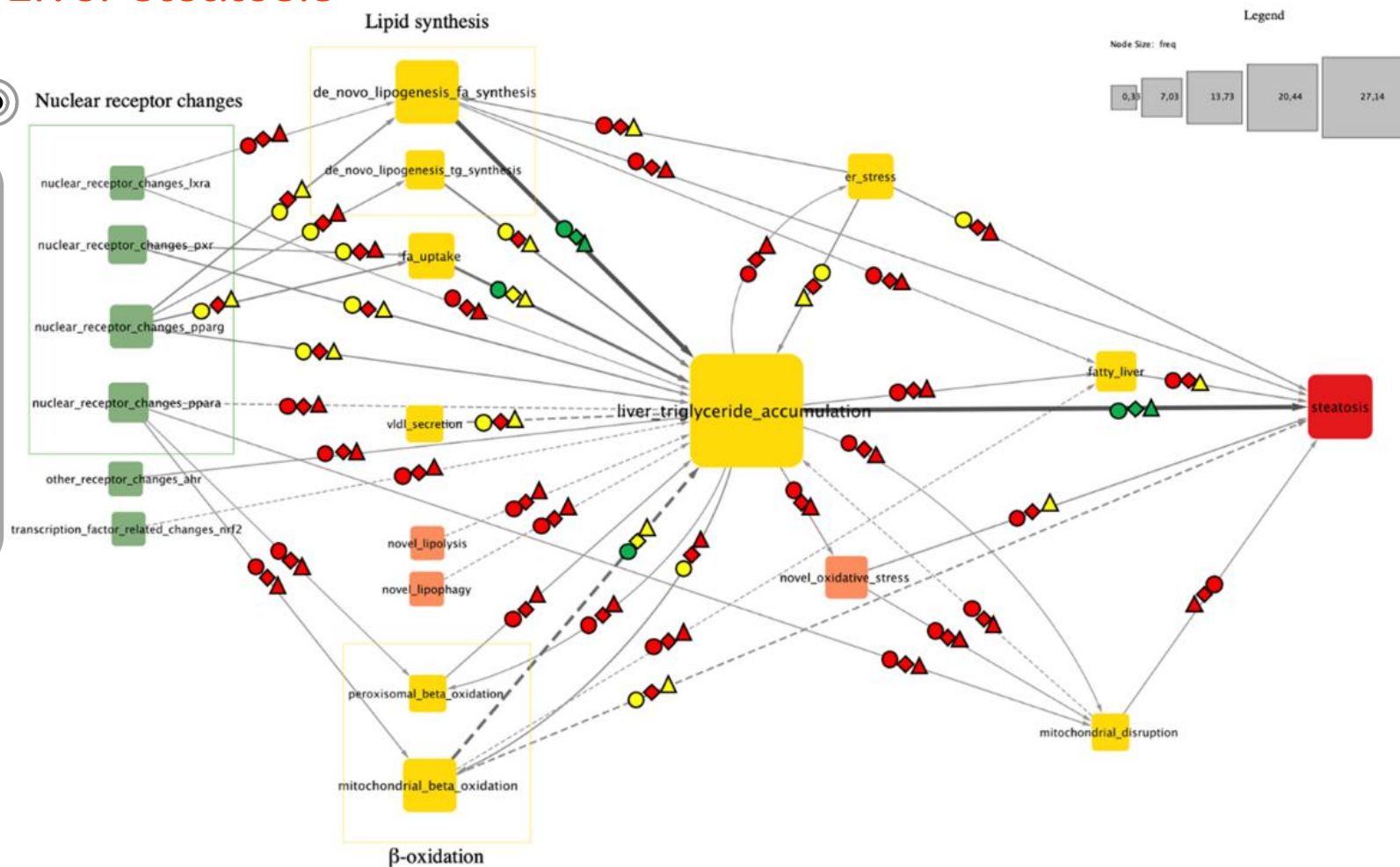


ONTOLOGIES

☑ Liver steatosis



Physico-chemical properties of chemicals triggering the AOP

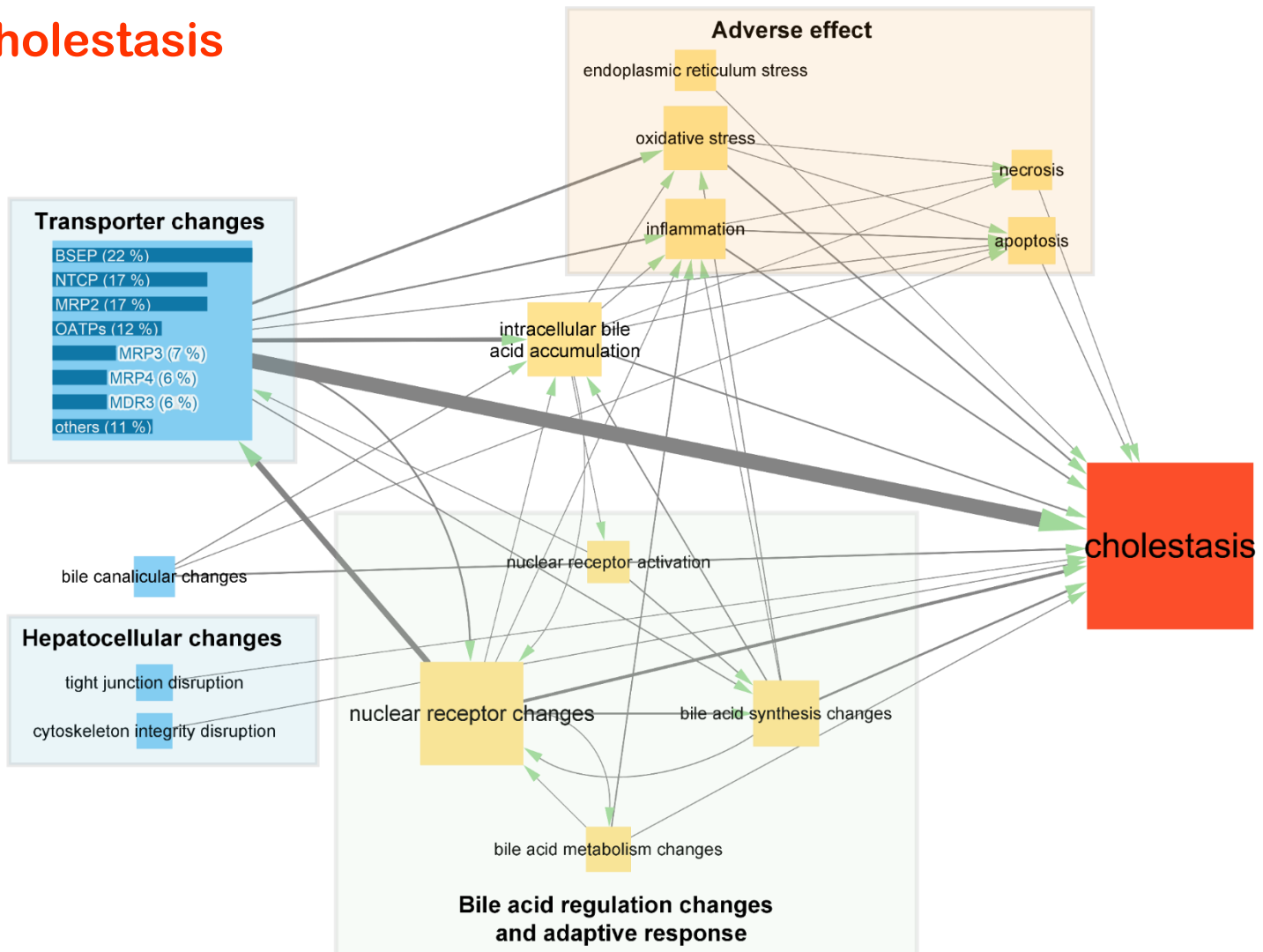


ONTOLOGIES

☑ Cholestasis



Physico-chemical properties of chemicals triggering the AOP



ONTOLOGY-BASED TOXICITY SCREENING

☑ Tiered testing approach for hazard identification

● Tier 1

- ▶ Physico-chemical profiling
- ▶ Read-across/quantitative structure-activity relationships

IN SILICO

● Tier 2

- ▶ Holistic approach (AOP network)
- ▶ Transcriptomics

IN VITRO

● Tier 3

- ▶ Atomistic approach (key event)
- ▶ Functional assays

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ONTOLOGY-BASED TOXICITY SCREENING

✓ Tier 2

● Liver steatosis

Gene symbol	Gene name	MIE/KE
<i>NR1H3</i>	Liver X Receptor-Alpha	Modulations in nuclear receptors
<i>NR1C1</i>	Peroxisome Proliferator Activated Receptor Alpha	Modulations in nuclear receptors
<i>NR1C3</i>	Peroxisome Proliferator Activated Receptor Gamma	Modulations in nuclear receptors
<i>AHR</i>	Aryl Hydrocarbon Receptor	Modulations in receptors
<i>FASN</i>	Fatty Acid Synthase	Fatty acid synthesis
<i>SCD</i>	Stearoyl-CoA Desaturase	Fatty acid synthesis
<i>DGAT2</i>	Diacylglycerol O-Acyltransferase 2	Triglyceride synthesis
<i>ACOX1</i>	Acyl-CoA Oxidase 1	Peroxisomal fatty acid beta-oxidation
<i>CPT1</i>	Carnitine Palmitoyltransferase 1	Mitochondrial fatty acid beta-oxidation
<i>CD36</i>	cluster of differentiation 36	Fatty acid uptake
<i>APOB</i>	Apolipoprotein B	Very-low-density lipoprotein secretion



ONTOLOGY-BASED TOXICITY SCREENING

✓ Tier 2

● Cholestasis

Gene symbol	Gene name	General function
ALAS1	Aminolevulinic synthase 1	Adaptive response
LMAN1	Lectin, Mannose Binding 1	Endoplasmic reticulum stress
MMP3	Matrix Metalloproteinase 3	Extracellular matrix remodeling
NDUFA4L2	NDUFA4 mitochondrial complex associated like 2	Oxidative stress; apoptosis
PMP22	Peripheral Myelin Protein 22	Apoptosis
PPDPF	Pancreatic Progenitor Cell Differentiation And Proliferation	Apoptosis
SEMA6C	Semaphorin 6C	Apoptosis
SLC16A3	Solute Carrier Family 16 Member 3	Bile flow disruption
SLC9A3R2	NHERF Family PDZ Scaffold Protein 2	Bile acid synthesis
TM4SF1	Transmembrane 4 L Six Family Member 1	Apoptosis
TMPRSS11D	Transmembrane Serine Protease 11D	Inflammation
TSKU	Tsukushi, Small Leucine Rich Proteoglycan	Adaptive response; bile acid synthesis
VSIG10L	V-Set And Immunoglobulin Domain Containing 10 Like	Oxidative stress; autophagy



ONTOLOGY-BASED TOXICITY SCREENING

✓ Tier 3

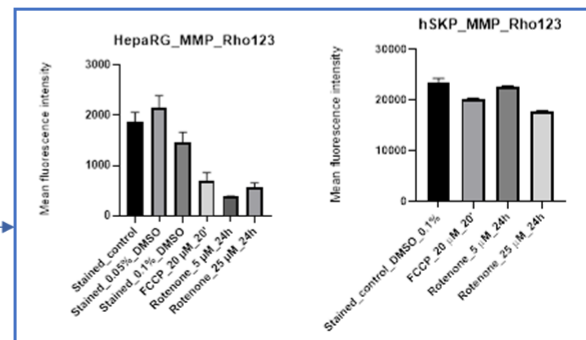
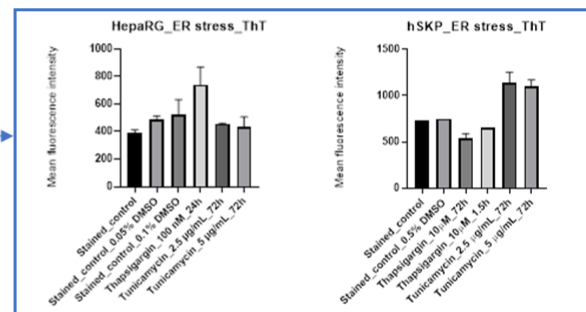
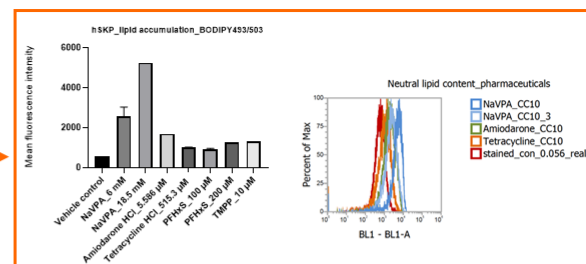
● Liver steatosis

The selection of key events and the corresponding assays for detecting chemical-induced liver steatosis.

Key Event	Assay	
	Tier 2	Tier 3
Intrahepatic lipid accumulation	NA	Flow cytometric quantification of neutral lipid content using BODIPY493/503
Nuclear receptor changes	Quantitative PCR analysis	Reporter gene assay
Mitochondrial beta-oxidation	Quantitative PCR analysis	Flow cytometric quantification of mitochondrial membrane potential using Rhodamine123 and Seahorse XF Cell Mito Stress test
Fatty acid-uptake	Quantitative PCR analysis	Quantitative fatty acid uptake assay using BODIPY™ FL C16
De novo lipogenesis	Quantitative PCR analysis	Quantitative Western blot analysis
VLDL-secretion	Quantitative PCR analysis	Quantitative Western blot analysis
Oxidative stress	NA	Flow cytometric quantification ROS using DHCf-DA
Endoplasmic reticulum stress	NA	Flow cytometric quantification of protein aggregation with thioflavin
Mitochondrial impairment	NA	Flow cytometric quantification of mitochondrial membrane potential using Rhodamine123

Abbreviations: ER, endoplasmic reticulum; hSKP, human skin precursor-derived hepatic progenitor cells; MMP, mitochondrial membrane potential; NAVPA, sodium valproic acid; PFHxS, perfluorohexanesulfonic acid; Rho, Rhodamine; ThT, thioflavin T; TMPP, tricesyl phosphate

Disease-specific key events, Generic key events



Anouk Verhoeven



ONTOLOGY-BASED TOXICITY SCREENING

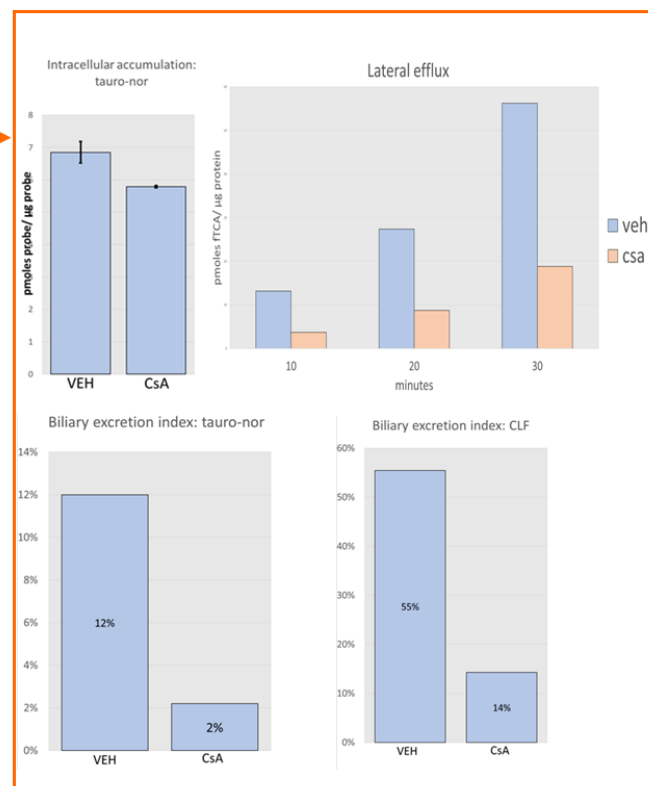
✓ Tier 3

● Cholestasis

The selection of key events and the corresponding assays for detecting chemical-induced liver cholestasis.

Key Event	Assay	
	Tier 2	Tier 3
Transporter changes	Quantitative PCR analysis	Quantitative transporter assays with fluorescent probes: tauro-nor, CDFDA, CLF and C6-NBD-PC
Nuclear receptor changes	Quantitative PCR analysis	NA
Inflammation	Quantitative PCR analysis	Quantitative ELISA assay of IL-1b
Oxidative stress	NA	Flow cytometric quantification ROS using DHCF-DA
Endoplasmic reticulum stress	NA	Flow cytometric quantification of protein aggregation with thioflavin
Mitochondrial impairment	NA	Flow cytometric quantification of mitochondrial membrane potential using Rhodamine123

Abbreviations: CLF, cholineyl-lysyl-fluorescein; CsA, Cyclosporin A; VEH, vehicle control
Disease-specific key events, Generic key events

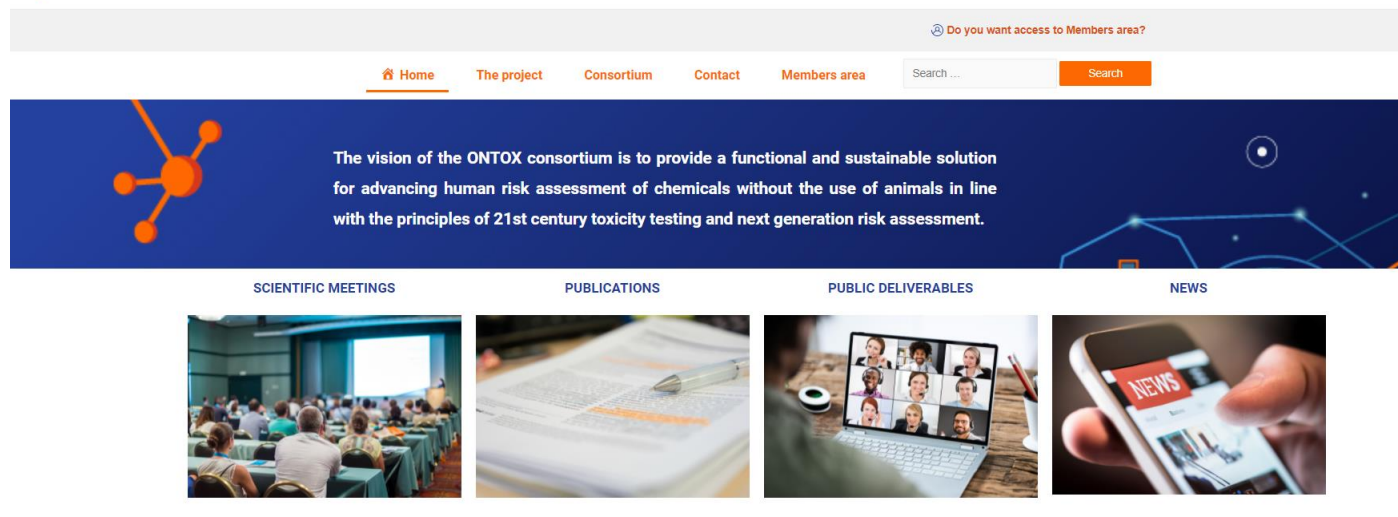


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