Test battery for the detection of induction of molecular initiating events of cholestatic liver toxicity induced by chemical compounds

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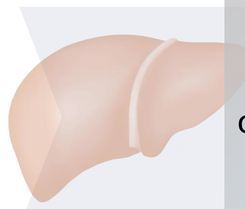
Introduction and aim



Problem

Cholestatic liver toxicity induced by chemical compounds remains a **challenge** to **predict**

Solution

Development of a set-up relying on a combination of ***in vitro***, ***in silico*** and **kinetic** modelling to…

…**classify** chemicals according to the level of **cholestatic liver toxicity concern**

Group	Test chemicals	EPAA reference list	Cholestatic potential
Azole antifungals	Fuberidazole	Included	Unknown
	Ipconazole	Included	Suspected
	Ketoconazole	Included	Inducer
PFAS	GenX	Not included	Unknown
	PFHpA	Included	Unknown
	PFOA	Included	Inducer
Mechanistic control	Sodium valproate	Not included	Negative

Integration and interpretation of component methods

In vitro modelling

What

Three **assays** detecting **MIEs** in a **AOP network** mechanistically describing cholestatic liver injury

Set-up

Human **HepaRG™** liver cell cultures exposed to the highest soluble **non-cytotoxic**-derived concentrations of test chemicals

MIE 1: Bile acid transporter activity

Basolateral efflux (1h exposure, CDFDA)
Basolateral uptake (1h exposure, CLF)

MIE 2: Transporter expression

qRT-PCR
6h exposure
BSEP, MRP2/3, OATP1B1/1B3, NTCP

MIE 3: Bile canaliculi dilatation

Phase-contrast microscopy
1-6h exposure
Average bile canaliculi area

Test chemicals	Basolateral bile acid transporter functionality		Gene expression of drug/bile acid transporters						Bile canalicular dilatation
	Efflux inhibition	Uptake inhibition	BSEP	MRP2	NTCP	MRP3	OATP1B1	OATP1B3	
Fuberidazole	N	N	-	-	-	-	N	-	N
Ipconazole	N	N	-	N	N	N	N	N	N
Ketoconazole	N	P	-	N	N	N	N	N	N
GenX	N	N	-	N	-	N	N	N	N
PFHpA	N	N	N	N	N	N	N	N	N
PFOA	P	N	P	P	P	P	P	P	N
Sodium valproate	N	N	N	N	N	N	N	N	N

Evaluation of concern:
- Low (L): all 3 read-outs are negative (N) and/or ambiguous (-)
- Medium (M): 1 read-out is positive (P)
- High (H): 2 or more read-outs are positive (P)

In silico modelling

What

A series of **QSAR** models to predict inhibitory/substrate effects on transporters

Set-up

Trained and validated on inhibition/binding data retrieved from **ChEMBL v33.**

Activity data were converted to **binary categorical labels** based on a **IC50** threshold of 100.000 nM

MT

Simultaneous modelling of multiple endpoints for BSEP, MRP2/3/4, OATP1B1/1B3

ML

Reinforce the predictions of MT model of **BSEP** (random forest) and **MRP2** (SMOTE-multilayer perceptron)

Target	BSEP			MRP2			MRP3		MRP4	OATP 1B1	OATP 1B3
Model	MT	MT	ML	MT	MT	ML	MT	MT	MT	MT	MT
Task	INH	SUB		INH	SUB		INH	SUB	INH		
Fuberidazole	N	N	N	N	N	N	N	N		N	N
Ipconazole		N		N		P	N	N	N		
Ketoconazole	P			N		N	N	N	N	P	N
GenX	N	N		N		N	N		N	N	N
PFHpA	N			N		N	N		N	N	N
PFOA			N	N		N	N		N	N	N
Sodium valproate	N		N	N		N	N		N	N	N

Evaluation of concern:
- Low (L): No positive (P) predictions
- Medium (M): 1 positive (P) prediction
- High (H): 2 or more positive (P) predictions

Kinetic modelling

What

Prediction of systemic availability relying on a **HT-PBK** modelling approach

Set-up

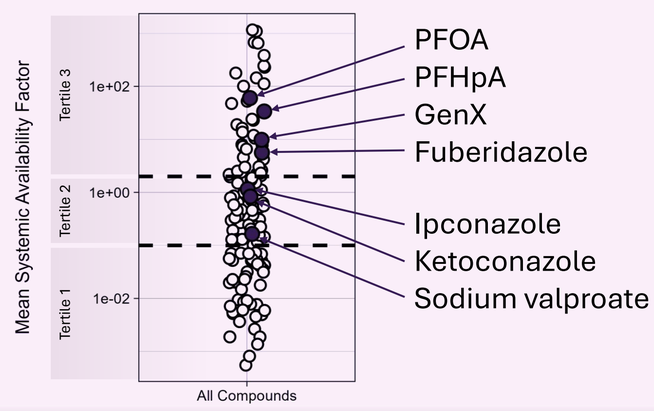
Open-source software **PK-Sim** and the corresponding **R package**

HT-PBK

Prediction of **ADME** properties (lipophilicity, clearance and fraction unbound) of **139 chemicals** included in the EPAA reference list

Further details

Outlined in the separate case study report "**ONTOX HT-PBK**"



Evaluation of concern: Tertiles

Test chemicals	Kinetic concern
Fuberidazole	H
Ipconazole	M
Ketoconazole	M
GenX	H
PFHpA	H
PFOA	H
Sodium valproate	M

Test chemicals	*In vitro* concern	*In silico* concern
Fuberidazole	L	L
Ipconazole	L	M
Ketoconazole	M	H
GenX	L	L
PFHpA	L	L
PFOA	H	L
Sodium valproate	L	L

Overall activity concern
L
M
H
L
L
M
L

Outcome

Activity (toxicodynamics)	Potential systemic availability (toxicokinetics) H	M	L
H		Ketoconazole	
M	PFOA	Ipconazole	
L	Fuberidazole, GenX, PFHpA	Sodium valproate	

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Abbreviations: ADME, absorption/distribution/metabolism/excretion; AOP, adverse outcome pathway; BSEP, bile salt export pump; CDFDA, 5(6)-carboxy-2',7'-dichlorofluorescein; CLF, cholyl-L-lysyl-fluorescein; GenX, ammonium salt of the hexafluorpropylene oxide dimer acid; HT-PBK, high-throughput physiologically-based kinetic; MIE, molecular initiating events; ML, Machine learning models; MRP, multidrug resistance-associated protein; MT, Multi-tasking model; NTCP, sodium-taurocholate co-transporting polypeptide; OATP, organic anion transporting polypeptide; PFAS, per- and polyfluorinated substances; PFHpA, perfluoroheptaenoic acid; PFOA, perfluorooctanoic acid; qPCR, quantitative reverse transcriptase polymerase chain reaction; QSAR, quantitative structure-activity relationship.