

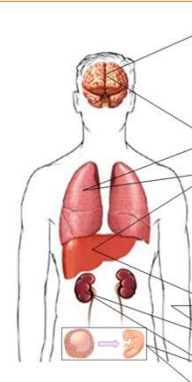
EU-ToxRisk test methods comparison in CSy (WP7) Comparison of cell-based viability tests

Authors: see attached publication

Coordinated by: UKN

Regulatory context: background data on test robustness, uncertainties and performance

Case Study Testing Approach



No.	Test method	Test system	V-readout	F-readout	Partner
1	UKN5	peripheral neurons	calcein	neurite area	UKN
2	UKN4	LUHMES cells	calcein	neurite area	UKN
3	UKN3b	LUHMES cells	calcein	neurite area	UKN
4	UKN3a	LUHMES cells	calcein	neurite area	UKN
5	hiPSC neuro	hiPSC-derived neurons	ATP	-	BIOT
6	SH-SY5Y prolif	SH-SY5Y cells	ATP	-	BIOT
7	SH-SY5Y neuro	SH-SY5Y cells	ATP	Ca ²⁺ signaling	Swetox
8	PBEC	bronchial epithelial cells	LDH	proliferation	LUMC
9	PBEC-ALI	bronchial epithelial cells	LDH	proliferation	LUMC
10	InSphero 3d	liver microtissues	ATP	-	InSphero
11	InSphero 2d	liver microtissues	ATP	-	InSphero
12	PHH	primary human hepatocytes	resazurin	morphology	IFADO
13	HepG2	HepG2 cells	resazurin	morphology	IFADO
14	HepG2-CHOP	HepG2 (GFP-reporter CHOP)	PI	GFP reporter	UL
15	HepG2-P21	HepG2 (GFP-reporter P21)	PI	GFP reporter	UL
16	HepG2-SRXN1	HepG2 (GFP-reporter SRXN1)	PI	GFP reporter	UL
17	iPSC-Hep	iPSC-derived hepatocytes	resazurin	LDH	KUL
18	HEK 293	HEK 293 cells	resazurin	LDH	UKN
19	U-2-OS	U2OS cells	PI	luciferase	BDS
20	RPTEC	RPTEC/TERT1	calcein	lactate	VUA
21	iPSC ren	iPSC-derived kidney cells	calcein	lactate	VUA
22	FET	zebrafish embryo	live fish	malformations	UHEI
23	UKN2	neural crest cells	calcein	migration	UKN

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IN VITRO SYSTEMS

The EU-ToxRisk method documentation, data processing and chemical testing pipeline for the regulatory use of new approach methods

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Case Study Toxicodynamics NAM Toolbox

compound name	abbreviation	structure	toxicity profile
Clofibrate	CF		negative
Ibuprofen	Ibu		negative
Sulfisoxazole	Sux		negative
Tolbutamide	Tol		negative
Colchicine	Col		neuro
Mercury chloride	Hg	HgCl ₂	DART, kidney
MPP+	MPP		neuro, liver, kidney
Paraquat	PQ		neuro, lung, kidney
Rotenone	Rot		neuro, DART
Taxol	Tax		neuro
Tebuconazole	Teb		liver, DART
Acrylamide	Acy		neuro, DART
Valproic acid	VPA		liver, DART

SOPs → EURL ADM → Test method description → Edelweiss Connect

EMBL-EBI BioStudies

Functional endpoints vs cytotoxicity

Compound	mMNC	LUKN4	LUHMES	LUHMES	LUHMES	PerTox	PBEC	RPTEC/TERT1	SH-SY5Y
Ratio (viability/migration)	6.0	1.5	1.6	1.3	8.2	18.5	16.2	5.9	1.5
Ratio (viability/reaction)	1.0	5.5	1.5	3.4	2.4	27.0	14.3	1.0	1.5
Ratio (viability/neurite area)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Ratio (viability/proliferation)	1.1	7082.2	1.1	3.7	31.6	0.6	0.9	1.0	0.3
Ratio (viability/replication)	1.2	0.9	1.5	2.3	1.5	NA	NA	0.4	0.7
Ratio (viability/lactate)	2.2	1.0	1.1	0.6	1.2	1.0	0.8	NA	1.1
Ratio (viability/ROS)	1.0	1.0	1.0	1.0	1.0	16.4	5.6	1.0	2.2
Ratio (viability/ATP)	1.0	52.9	1.8	1.0	1.0	61.8	87.5	1.5	1.6
Ratio (viability/ATP)	1.0	1.0	1.0	1.0	1.0	NA	NA	1.9	28.1
Ratio (viability/ATP)	5.5	1.0	1.3	1.5	9.1	8.4	11.5	0.5	1.6
Ratio (viability/ATP)	5.0	0.5	0.8	0.8	1.0	1.5	1.5	NA	1.0
Ratio (viability/ATP)	1.0	5.5	1.2	1.5	1.0	5.0	2.4	4.3	6.8
Ratio (viability/ATP)	4.4	153.4	11.8	9.5	79.4	37.5	600.0	5.5	2.9
Ratio (viability/ATP)	1.0	1.0	1.0	1.0	1.0	1.2	1.1	1.0	1.0
Ratio (viability/ATP)	31.6	11.7	4.8	367.6	79.8	NA	NA	1.0	1.6
Ratio (viability/ATP)	1.0	7.1	8.7	6.0	1.9	1.0	0.5	1.9	0.7
Ratio (viability/ATP)	1.0	1.0	1.0	1.0	1.0	7.7	4.2	1.0	1.0
Ratio (viability/ATP)	1.0	1.0	1.0	1.2	1.0	NA	NA	1.0	1.0
Ratio (viability/ATP)	1.0	1.0	1.5	1.0	0.9	12.2	8.9	1.0	0.5

Fold higher sensitivity of functional endpoint

- ratio = 3 (green)
- ratio > 3 (orange)

Case Study Battery Evaluation

