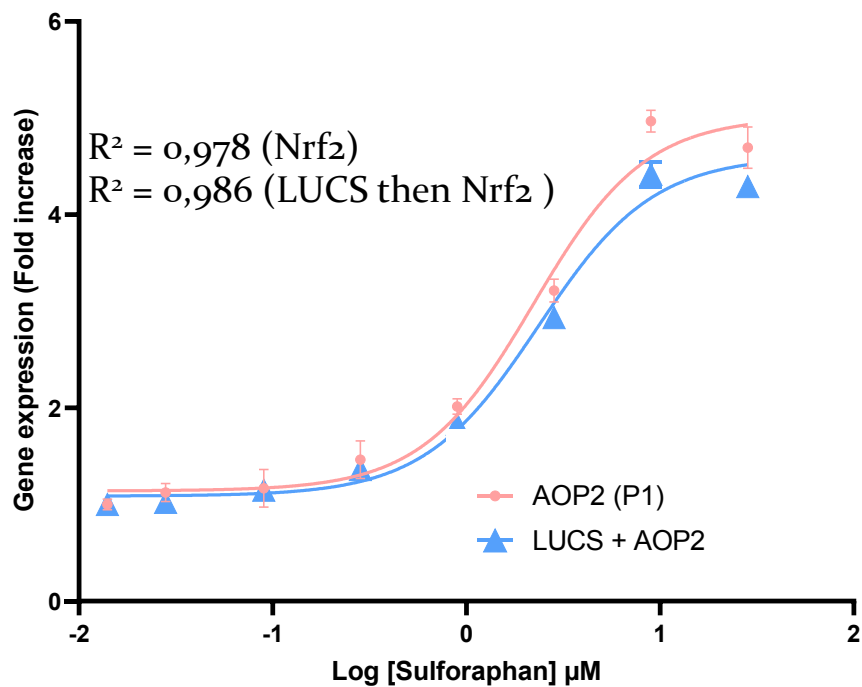# LUCS/Valitox assay at a glance

✓ LUCS is a fluorescence live cell assay

✓ First developed as an alternative to animal tests

✓ LUCS = VALITOX

✓ Based on a photoinduction process protected by patents delivered in Europe (EP2235505 & EP3044569)

✓ Measure of cell homeostasis status

✓ Different applications in public health

✓ Supported by two main publications

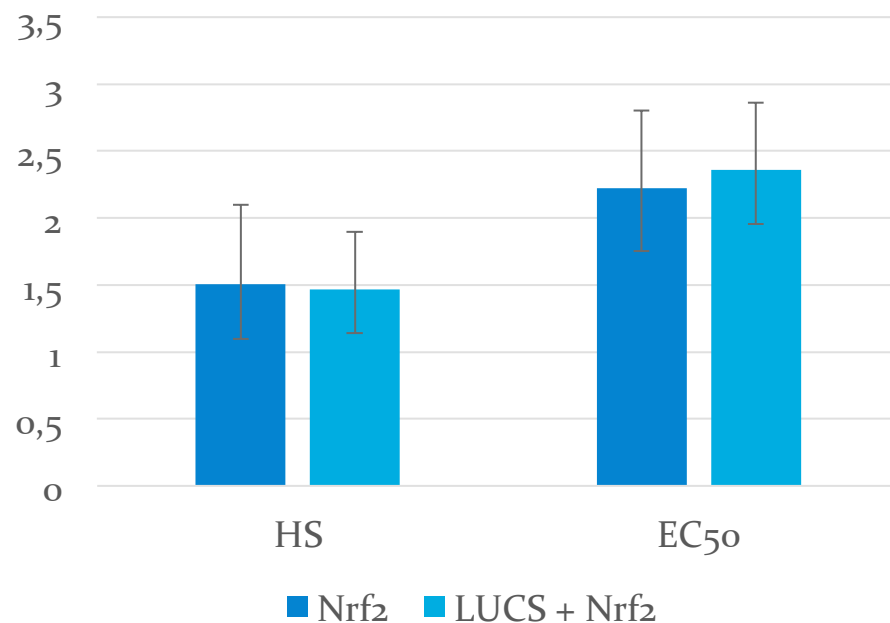✓ Submitted to EU ECVAM for regulatory applications

Features & Benefits of LUCS

- ✓ Fully performed on live cells

- ✓ Open to multiplexing

- ✓ Not limited in terms of cell models (universality)

- ✓ Very robust with a Z'=0,8

- ✓ Very high signal-to-noise ratio (nonspecific fluo easily removed)

- ✓ Very simple procedure (+ biosensor, fluo measure, light application, fluo measure)

- ✓ Adapted to optogenetics plate illuminators

- ✓ Antioxidant version AOP1: first live cell assay for the measure of intracellular free radical quenching

- ✓ Cost effective

- ✓ Adapted to HTS

Nrf2/LUCS Multiplex analysis

$R^2 = 0,978$ (Nrf2)
$R^2 = 0,986$ (LUCS then Nrf2)

Gene expression (Fold increase) vs Log [Sulforaphan] µM

- AOP2 (P1)
- LUCS + AOP2

Uniplex vs multiplex comparison
(error bars = 95%CI)

- Nrf2
- LUCS + Nrf2

	HS	EC50 (µM)
Nrf2	1.508	2.224
LUCS then Nrf2	1.466	2.356

COMITÉ SCIENTIFIQUE PRO ANIMA

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Application notes on industrial plate readers

Cell viability assessment by LUCS assay using EnSight™ multimode plate reader

Christophe FURGER[1], Camille GIRONDE[1], Mylène RIGAL[1], Wenli DEBORD[1], Carmen MARANGONI[2] & Brigitte MACHARD[2]

[1] Anti Oxidant Power – AOP, 78 allées Jean Jaurès, BP 71028, 31000 Toulouse, France
[2] PerkinElmer - ZA Courtabœuf, 12/14 Avenue de la Baltique, 91140 Villebon sur Yvette, France
Published 15/01/2022

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Abstract

LUCS (Light Up cell System) is a new viability assay based on the activation of an intracellular

very interesting property for cell biology: its fluorescence quantum yield remains very low (2×10^{-4}) in the culture medium due to free rotation of its two aromatic rings around the

MATERIAL & METHODS

CLARIOstar Multimode Reader
Extern light source (470 nm)
AOP "one-step" kits
Reagents :
- – Chloroquine for LUCS
- – Quercetin for AOP1

HepG2 cells, 75000 cells/well, 96 well plates

perimental protocol
ne different reagent concentrations (500-1.95 µM, 2X dilutions), in serum-free
edium. Cells were incubated for 24 h (LUCS) or 4h (AOP1) at 37°C in 5% CO_2 with each

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Plug-and-Play mode

- ✓ Perkin-Elmer (EnSight)
- ✓ Agilent (Cytation)

Move-in Move-out mode

- ✓ BMG (ClarioStar)
- ✓ Molecular Devices (SpectraMax i3x)
- ✓ Tecan (Spark Cyto)

Consumable kits & HTS robotic platforms

- ✓ Kits of consumables for HTS applications

- ✓ SOP validated

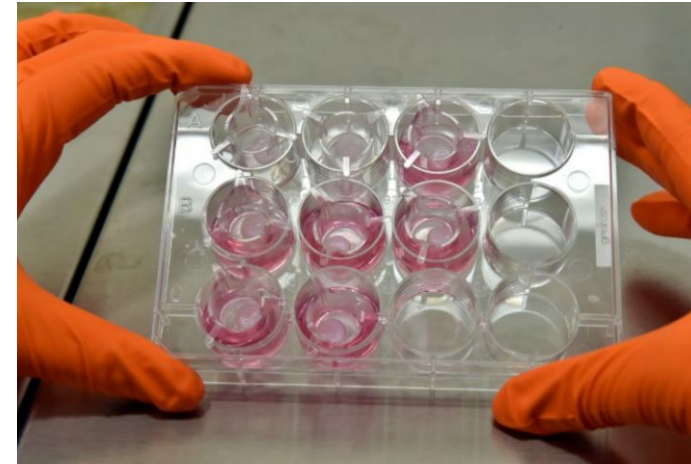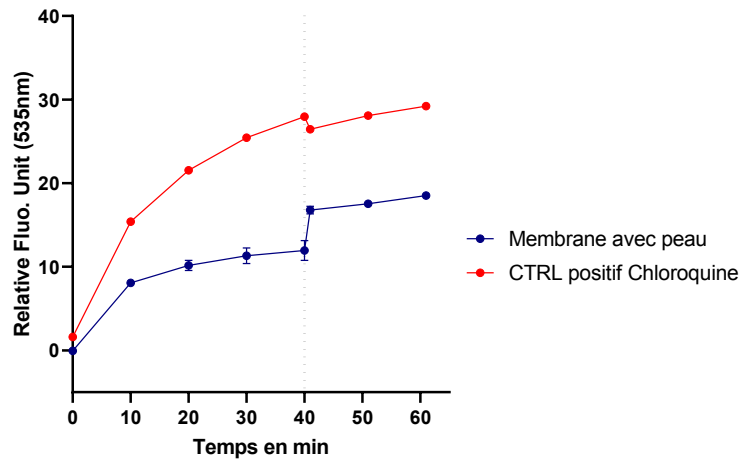- ✓ International distributor envisaged

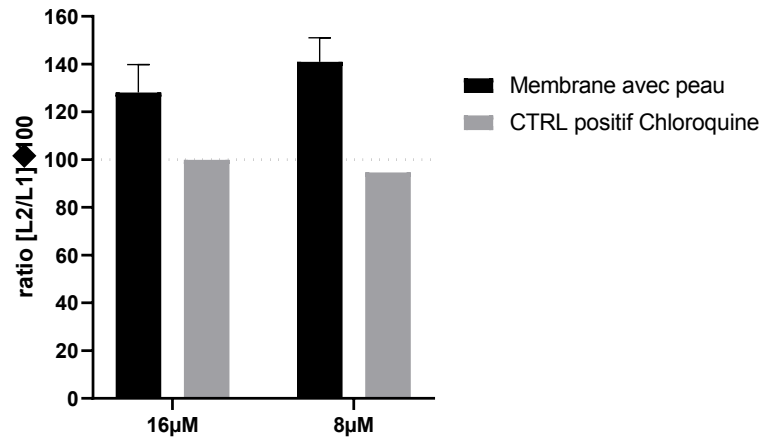*470 nm 96/384 well plate LED light applicator (Teleopto)*

Application on skin models

Case of skin models (derm/collagen + mature bioprinted epiderm) POIETIS (2)

191205_Test Poietis peau complète 8µM (RawData-Blanc)

Legend:
- Membrane avec peau
- CTRL positif Chloroquine

X-axis: Temps en min
Y-axis: Relative Fluo. Unit (535nm)

191205_Ratio

Legend:
- Membrane avec peau
- CTRL positif Chloroquine

X-axis: 16µM, 8µM
Y-axis: ratio [L2/L1]◆100

THE FUTURE OF MEDICINE IS HERE

BIO X™

In vitro tests for regulatory purposes in EU (situation Year 2020)

Type of targeted toxicity	Available in vitro assays	Partial substitution	Total substitution
Ocular irritation/corrosion	3*	X	
Acute toxicity	none		
Genotoxicity	5	X	
Repeated doses	none		
Skin absorption/irritation/corrosion	4	X	
Skin sensitization	3	X	
Phototoxicity	1		X
Endocrine disrupters	5	X	
Reprotoxicity	none		
Carcinogenicity	none		
Ecotoxicity (poisson, oiseaux)	none		

* + 2 ex-vivo tests: ICE and BCOP

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Use of LUCS (Light-Up-Cell System) as an alternative live cell method to predict human acute oral toxicity

C. Gironde, C. Dufour, C. Furger*

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## ARTICLE INFO

ABSTRACT

LUCS (Light-Up Cell System) is a new live cell test that allows assessment of a cell's homeostasis and its alteration by a toxic agent. To evaluate the effectiveness of LUCS as an alternative test method for acute oral toxicity, we compared EC_{50}s determined in HepG2 cells treated with 53 chemicals selected from the ACuteTox EU database with corresponding human blood LC_{50}s derived from human acute poisoning cases. Linear regression analysis showed that LUCS results predict human data to 69 %. Rodent oral LD_{50}s and LUCS EC_{50}s were then correlated to human LC_{50}s using shared data sets. Linear regression analyses comparing LUCS and animal data clearly showed that LUCS always predicts human toxicity better than animal data do.

These successful prediction values prompted us to simplify the LUCS test, adapting it to regulatory and high throughput applications, resulting in a new protocol with consistent dose-response profiles and EC_{50}s.

This study demonstrates that the LUCS test method could be relevant for assessing human acute oral toxicity with a simplified protocol adapted to commercially available fluorescence readers. We suggest that this new alternative method can be used for acute systemic toxicity testing in combination with other tests under European REACH and other regulations, wherever pertinent alternative methods are still lacking.

Human acute oral toxicity
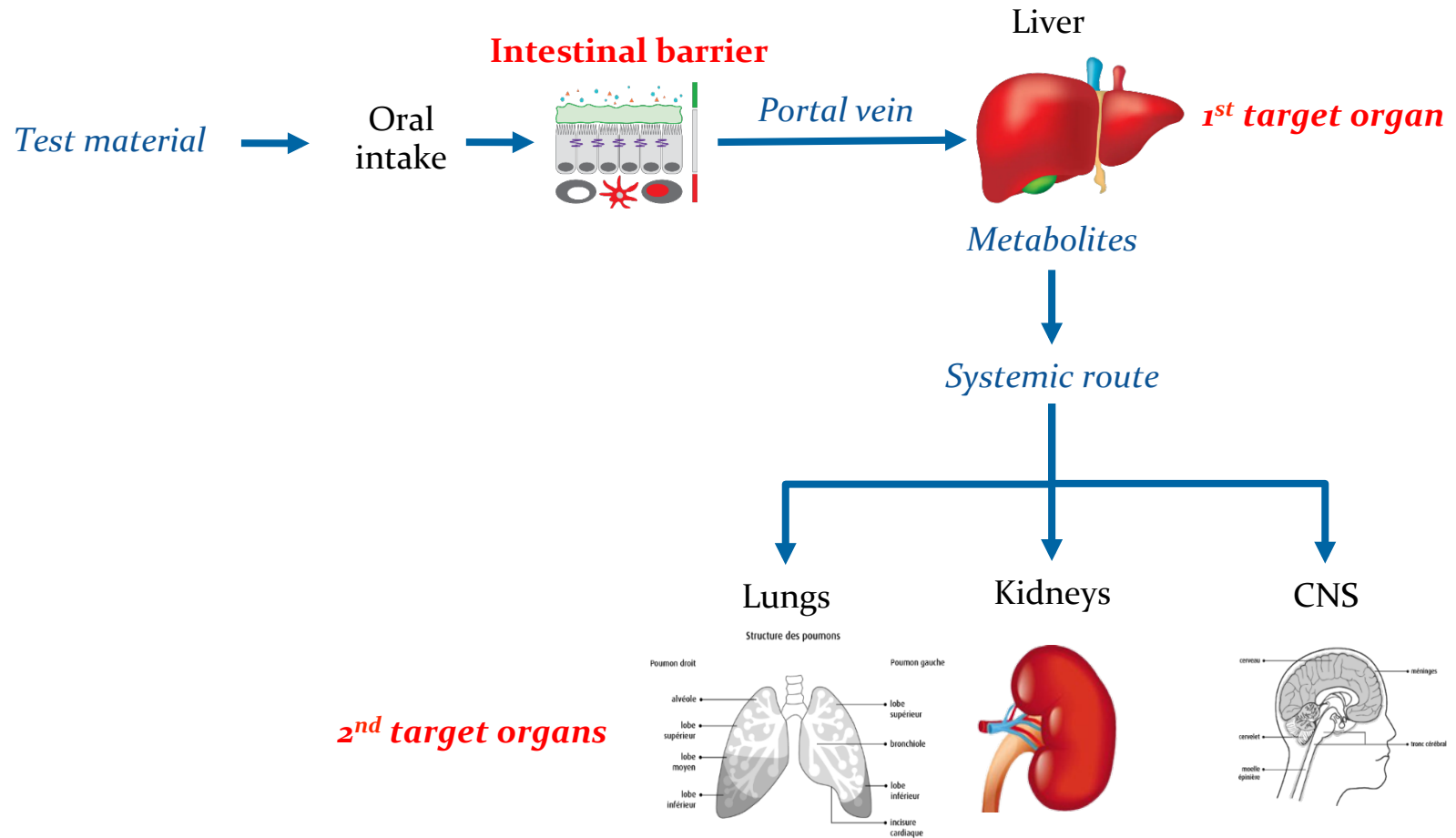
LUCS EC$_{50}$s vs Human LC$_{50}$s
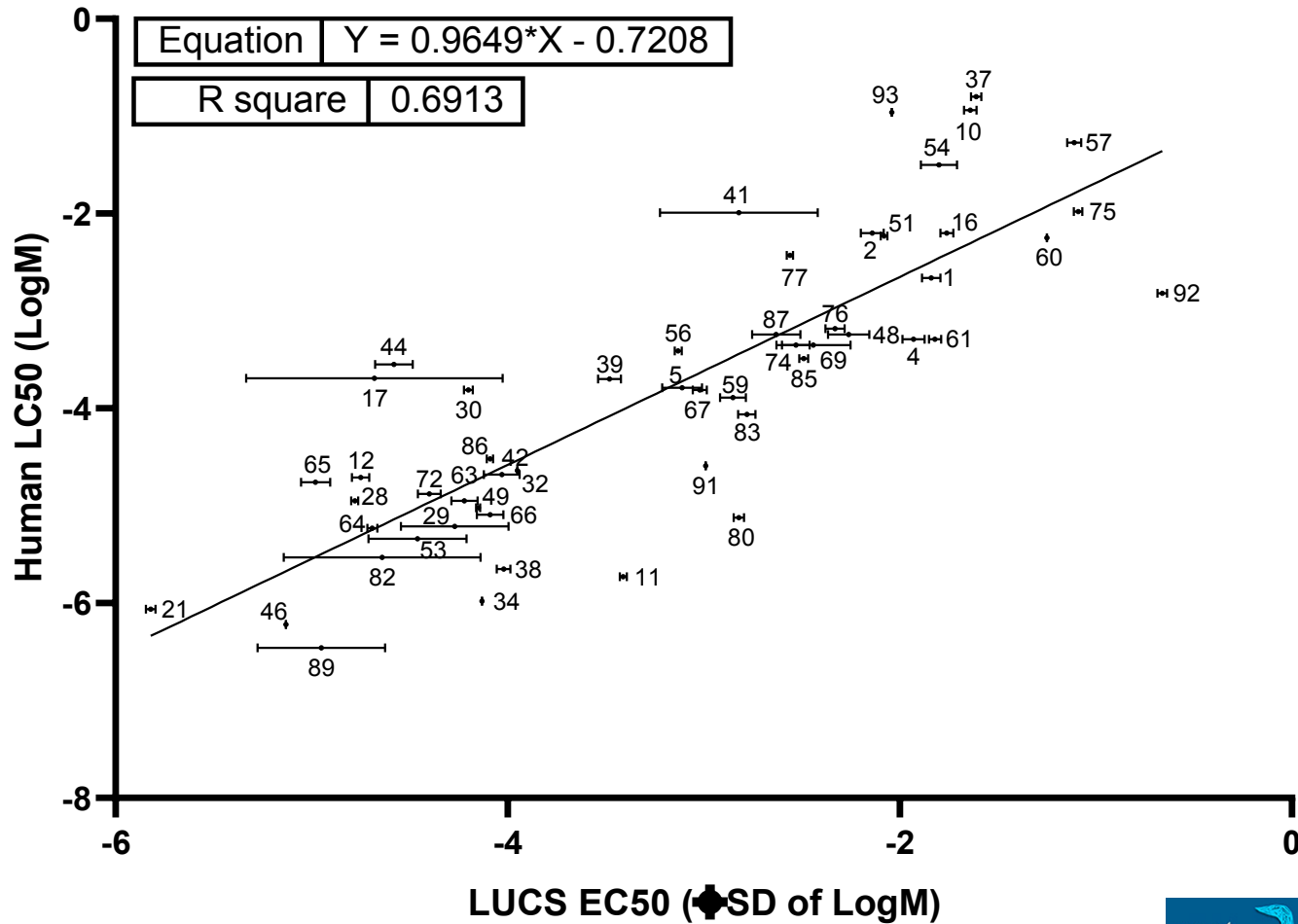
LUCS EC_{50}s and Animal LD_{50}s vs Human LD_{50}s

Comparison	n	Test	R² (test vs human)	Slope	Intercept
LUCS & rat[b] data	37	LUCS (HepG2 cells)	0.670	0.960	- 0.698
		Rat	0.504	0.971	- 1.089
LUCS & rat[c] data	35	LUCS (HepG2 cells)	0.695	1.042	- 0.414
		Rat	0.579	1.138	- 0.651
LUCS & mouse[c] data	30	LUCS (HepG2 cells)	0.753	1.140	- 0.200
		Mouse	0.537	1.279	- 0.324

b) Kinsner-Ovaskainen A., Prieto P., Stanzel S., Kopp-Schneider A. (2013)
c) Hoffmann S., Kinsner-Ovaskainen A., Prieto P., Mangelsdorf I., Bieler C., Cole T. (2010)

✓ LUCS always predicts human toxicity better than animal-based assays do

Main comments from EURL ECVAM for our pre-submission TM2021-01 (15 march 2022)

Claims	ECVAM position
Relevance to measure cell homeostasis	OK
Within Lab Reproducibility (WLR)	CV values seem to indicate good WLR but raw data were not provided
Between Lab Reproducibility (BLR)	Limited set of data – Difficult to conclude
Suitability to HTS	OK
Prediction model	Provided but not clear how it can be used in regulatory context
Positive impact on the 3Rs	Information provided is not enough to make any judgement
Metabolic competence addressed using Upcyte cells in relation to OECD GD 129	The lack of metabolic competence (bio-activation and detoxification) could apparently be addressed by applying LUCS to Upcyte cells. However, not enough evidence is provided to make any judgement
Multiplexing (use prior to OECD TG 442D)	A clear comparison with other methods is not provided - Waiting for the publication
Use in OECD GD 129	Our interpretation is that the LUCS test method may be a similar method to the 3T3 NRU
Integrating LUCS in defined approaches (DA)	Yes, good correlations, R2 and regression slope values, good base for the claim but not enough data is provided to make any judgement

Conclusion

Applicability of LUCS/Valitox as an alternative method to animal testing to various contexts:

- ✓ Can be applied by big pharma companies for their upstream research (validated for HTS, low cost, open to multiplexing, adapted to robotic platforms, excellent Z', very informative with EC_{50} determination)

- ✓ Can be applied by nutraceutics and food/dietary/nutritional supplement companies either for cytotoxicity or antioxidant studies

- ✓ Regulatory purposes : the winning strategy has yet to be found !

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Valitox – Next steps

1. Organotypic cultures
2. hiPSCs
3. Organs-on-Chips

Human induced Pluripotent Stem Cells (hiPSC)

Somatic cells

Reprogrammation

Differentiation

Patient or donnor

Tests

Neurons, hair cells, blood cells, hepatocytes, cardiomyocytes, endothelial cells,...