VALIDATION STUDY OF SKINETHIC™ HCE EIT

For Identifying Chemicals Not Requiring Classification

For Serious Eye Damage/Eye Irritation

N. Alépée

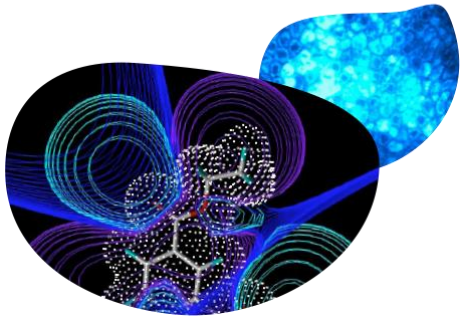
OECD Meeting

9-10 November 2015

ADVANCED RESEARCH

For the upcoming ESAC Working Group meeting

April 29,2016



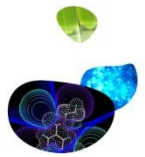
EYE IRRITATION TEST VALIDATION STUDY (EIT)

Objective:
Assess the reliability and relevance of SkinEthic™ HCE EIT to identify and discriminate chemicals not requiring classification for serious eye damage/eye irritation (No Category) from chemicals requiring classification (Category 2/Category 1) according to UN GHS / EU CLP

This methods is not intended to differentiate between UN GHS Cat 1 (serious eye damage) and UN GHS Cat 2(A/B) (eye irritation).

Ultimate aim:
Use in combination with other *in vitro* methods in a testing strategy such as the Bottom-up/Top-down approach (Scott *et al.*, 2010) in view of replacing the *in vivo* Draize eye test.

Timeframe:
From March 2014 to July 2015



MECHANISTIC COVERAGE: DEPTH OF INJURY

- All chemicals classified for serious eye damage/eye irritation induce some level of ocular injury/cytotoxicity, which always starts at the epithelium level

- It is the extent of injury rather than the mechanism behind its induction that determines the outcome, i.e. the classification

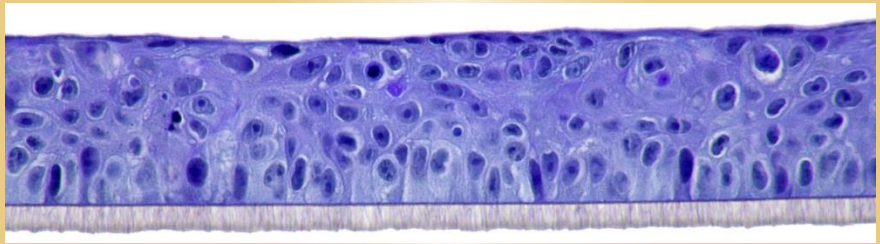
- By measuring cytotoxicity in a cornea-like epithelium after chemical exposure, the SkinEthic™ HCE EIT should be able to identify all types of classified chemicals

Maurer et al. (2002). Reg. Tox. Pharmac. ***36****, 106-117*
Jester, *et al.* (2001). Toxicol. in Vitro 15, 115–130.

SkinEthic™ Human Corneal Epithelium (HCE)

- Consists of a human corneal epithelium constructed with human immortalized corneal epithelial cells.

- Multilayered epithelium resembling to the *in vivo* epithelium with similar thickness, morphology and histology.



MILESTONES SINCE 2006

2006 — **Development of a short exposure time SkinEthic™ HCE protocol**
Van Goethem et al. (2006) Toxicology In vitro, 20:1-17

2007 — **Development of a long exposure time protocol for cosmetics specificity**
Cotovio et al. (2010). Toxicol In Vitro, 24:523-37

2008 — **Cosmetics Europe working group**
SOP and Transfer formalized
Ring studies conducted (Procter & Gamble, Janssen & Janssen, Henkel, Beiersdorf)

2009 – 2012 — **EURL-ECVAM Validation study (EIVS)**
Conducted by EURL-ECVAM and Cosmetics Europe
Within and between laboratory reproducibility demonstrated (> 90%)
Performance: not all of the acceptance criteria were met

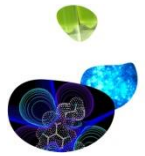
New classification EU CLP / UN GHS

2013 — **L'Oréal optimisation**
Establishment of 2 protocols for the liquid and solid chemicals

2014 – 2015 — **Prospective multicentre study (EIT)**
L'Oréal coordination
Transfer to VITO and Charles River Laboratories (naive laboratories)
Within and between laboratory reproducibility on 60 liquid and 60 solid chemicals
Relevance on 200 chemicals

SPSF submission

TST submission (TM 2015-03)



EIVS - EIT
QUICK FLOW CHARTS

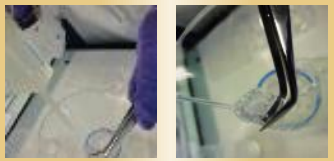
EIVS PROTOCOL

Receipt :

Short Exposure :

Exposure: 10 min

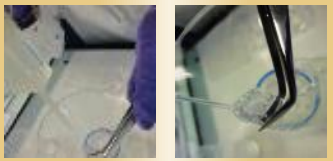
Rinse



Long Exposure

Exposure: 60 min

Rinse



Post incubation: 16h

Viability assessment :

EIT PROTOCOL

Receipt :

Liquid:

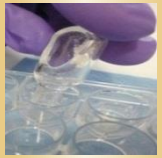
Exposure: 30 min

Rinse

Post Soak: 30 min

Solid:

Exposure: 4 hours

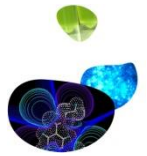


Rinse

Post Soak: 30 min

Post incubation: 18h

Viability assessment :



SKINETHIC™ HCE EITL
EYE IRRITATION TEST LIQUID & PREDICTION MODEL

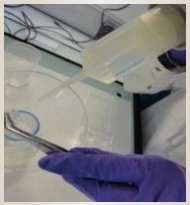
1 - Tissue Receipt

Transfert to medium
Incubate overnight
(37C, 5% CO_2, ≥ 95% humidity)

2 - Tissue Treatment

Topical application, with controls and test chemicals **(30µL)**
Exposure : 30 min
(37°C, 5% CO_2, ≥ 95% humidity)

3 - Rinse

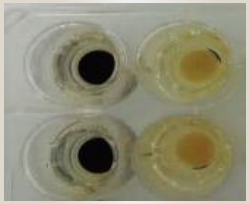


PBS-
(20mL: 10mL/ jet)

4 – Post Soak

1,5 mL medium
30 min
(37°C, 5% CO_2, ≥ 95% humidity)

5 - Viability



Transfer tissues into
MTT solution (1mg/mL)
Incubate for 3 hours
(37°C, 5% CO_2, ≥ 95% humidity)

6 - Extraction

Place the inserts on
(750µL+750µL) isopropanol
Incubate 2 hours at least, RT

7 - Viability assesment

Read OD at 570 nm and/or analyse by HPLC/UPLC spectrophotometry

PREDICTION MODEL

In vitro result	Classification (Prediction)
Mean tissue viability > **60**%	Not classified (No category)
Mean tissue viability ≤ **60**%	Classified (Category 1 / Category 2)

SkinEthic™ HCE EIT test method is not intended to differentiate between UN GHS Category 1 (serious eye damage) and UN GHS Category 2 (eye irritation).

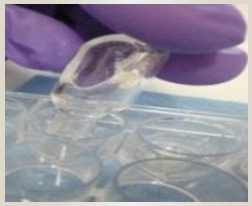
SKINETHIC™ HCE EITS

EYE IRRITATION TEST SOLID & PREDICTION MODEL

1 - Tissue Receipt

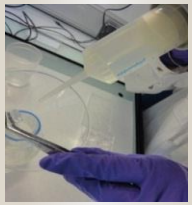
Transfert to medium
Incubate overnight
(37C, 5% CO_2, ≥ 95% humidity)

2 - Tissue Treatment



Topical application, with controls & test chemicals **(30µL PBS- + 30mg)**
Exposure : 4 hours
(37°C, 5% CO_2, ≥ 95% humidity)

3 - Rinse

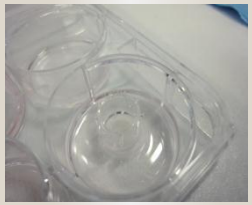


PBS-
(24mL: 2mL/ jet)

4 – Post Soak

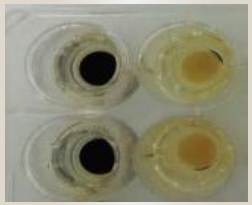
4mL medium
30 min **RT**

5 – Post incubation Pediod



Incubate for 18h
(37°C, 5%CO_2, ≥ 95% humidity)

6 - Viability



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Mean tissue viability > **50**%	Not classified (No category)
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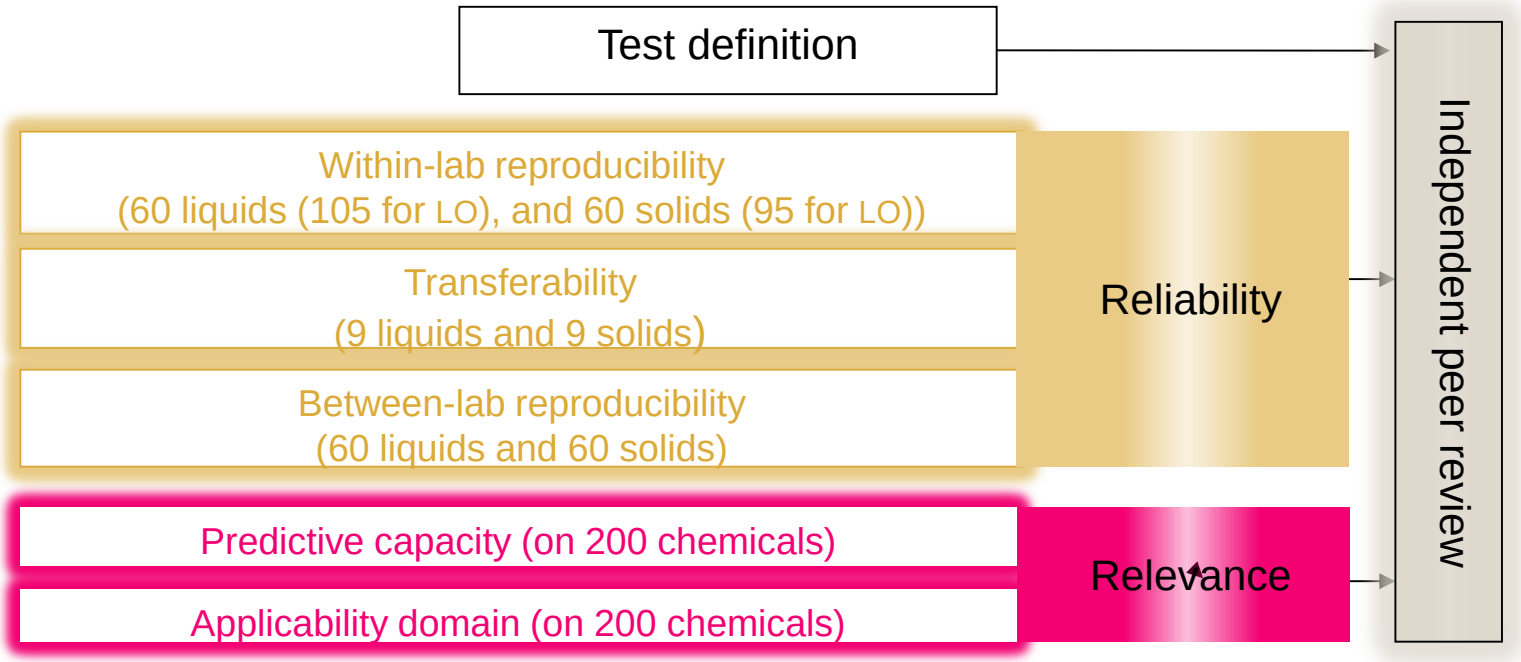
SKINETHIC™ HCE EIT PROCESS & VALIDATION



- **Independent coding and distribution of chemicals** (Random coding of the chemicals with different codes being used for each laboratory and each study supported by independent coordinator).
- All chemicals tested in each laboratory in **3 independent qualified runs** performed with **different tissue batches**
- **Limited re-testing** allowed to complete dataset.
- **Two tissue replicates used per chemical and controls** (supported by independent statistical evaluation).

STRUCTURE OF THE STUDY

Study Coordination Management

L'Oréal responsible for
- Study coordination
- Study goal and project plan
- Test chemicals selection
- Final reports and publications

Product Coordination

Study Products Coordinator, VitroScreen
- Liaison with suppliers
- Liaison with the Study Data Coordinator
- Chemical acquisition, coding and distribution
- Point of contact for chemicals and follow-up during the experimental phase
- Decoding, reception and check of sealed envelopes

Lead Laboratory

L'Oréal
- SOP
- Training
- Testing

Participating Laboratories

Charles River Laboratories · VITO
- Transfer
- Testing

Data Coordination

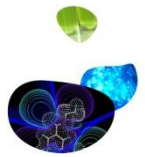
Study Data Coordinator, Adriaens Consulting BVBA
- Data storage, reporting and archiving
- Collaboration with the Study Product Coordinator
- Supervision of the laboratories and follow-up of the experimental phase
- Clarification of any data related issues once testing is completed
- Statistical analysis of the study data
- Reporting of the study results obtained

SKINETHIC™ HCE EIT
CHEMICALS SELECTION

120 chemicals (+ 80 extended set) selected eligible to fulfill the following criteria:

- Availability of high quality *in vivo* Draize reference data
- 50±2.5% split for classified (GHS Cat 1, 2A, 2B) vs. not-classified (GHS No Cat)
- As close as possible to a 50% split between GHS Cat 1 and GHS Cat 2 chemicals
- Good representation of GHS Cat 2A and GHS Cat 2B chemicals
- Good representation of all ocular effects driving classification i.e., severity and/or persistence of corneal, iris and conjunctiva effects
- 50±2.5% split for physical form (solids vs. liquids)
- All chemicals available from commercial sources
- Least possible number of chemicals already tested in the test method
- Diverse structural and chemical classes

…. several colour interfering chemicals (1 liquid and 7 solids), MTT reducers (7 liquids and 12 solids) and MTT reducing colorants (1 liquid and 10 solids).

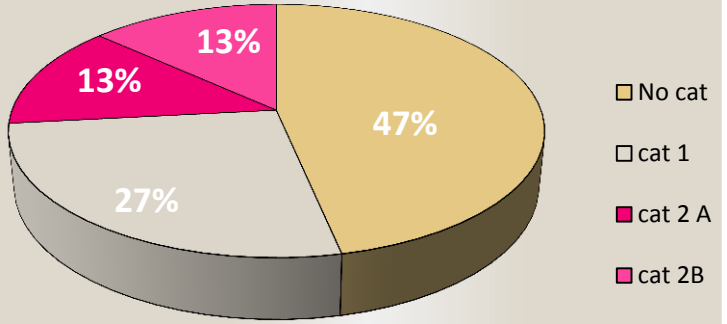


SKINETHIC™ HCE EIT
CHEMICALS SELECTION

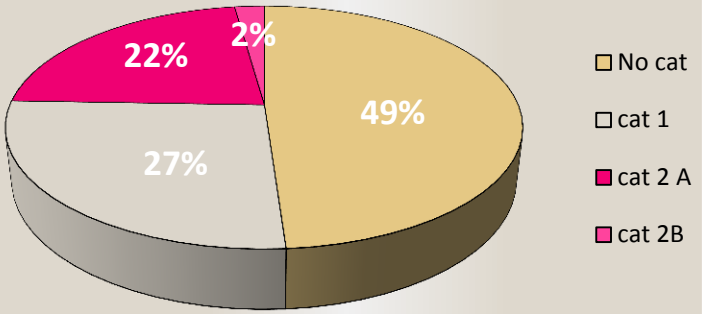
	Cat 1		Cat 2A		Cat 2B		No Cat	
	Liquid	Solid	Liquid	Solid	Liquid	Solid	Liquid	Solid
Total	27	24	19	10	9	8	50	53
UN GHS Categories	51		29		17		103	
Classified vs Not Classified	97						103	
Overall	200 (105 Liquids + 95 Solids)							

SKINETHIC™ HCE EITL
UN GHS CLASS REPARTITION

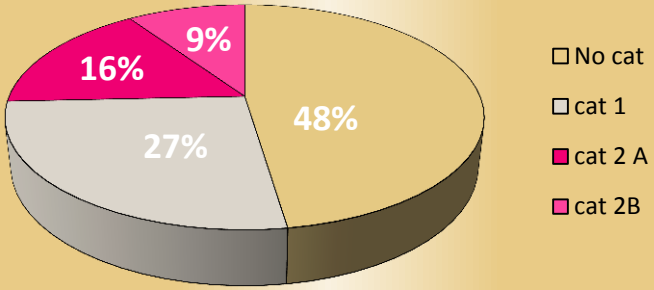
EITL – 60 CHEMICALS



EXTENDED DATASET – 45 CHEMICALS

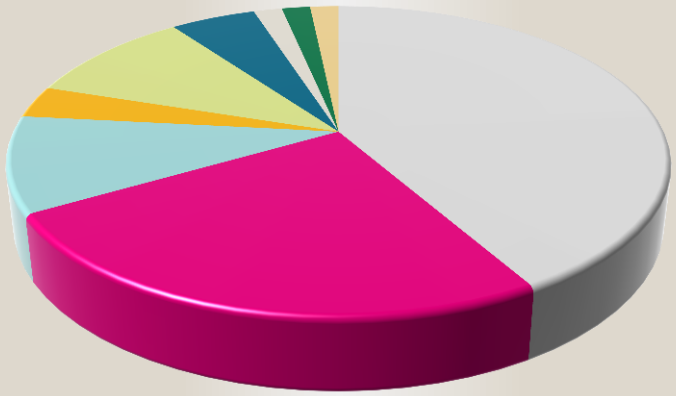


OVERALL UN GHS CLASS REPARTITION - 105 CHEMICALS

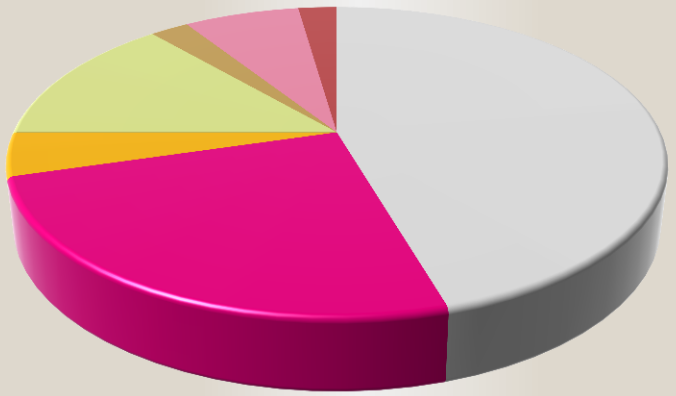


EITL
IN VIVO SOURCES REPARTITION

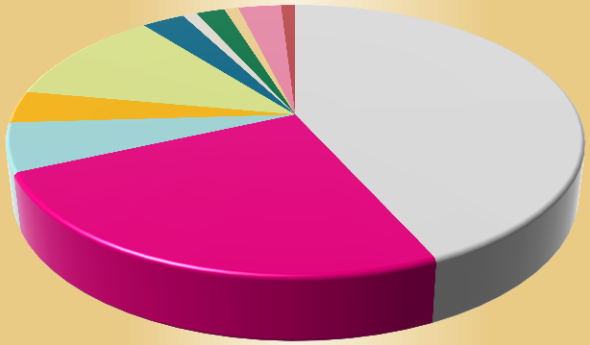
EITL – 60 CHEMICALS



EXTENDED SET - 45 CHEMICALS



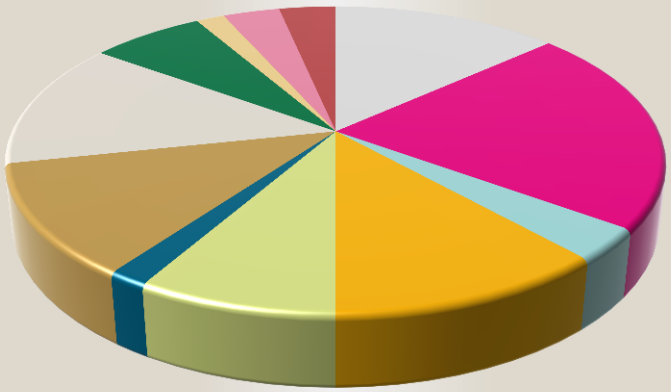
OVERALL – 105 CHEMICALS



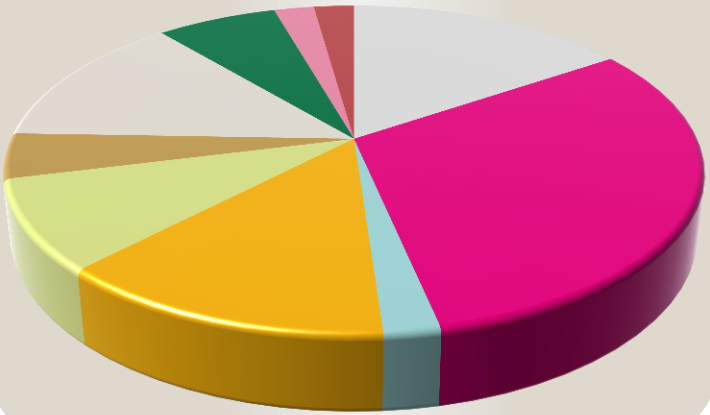
- ECETOC
- NICEATM
- EURL ECVAM
- LNS
- ZEBET
- ECETOC + NICEATM
- NICEATM + LNS
- ECETOC + LNS
- NICEATM + EURL ECVAM
- ECETOC + ZEBET
- ECETOC + LNS + NICEATM

EITL
FUNCTIONAL GROUP

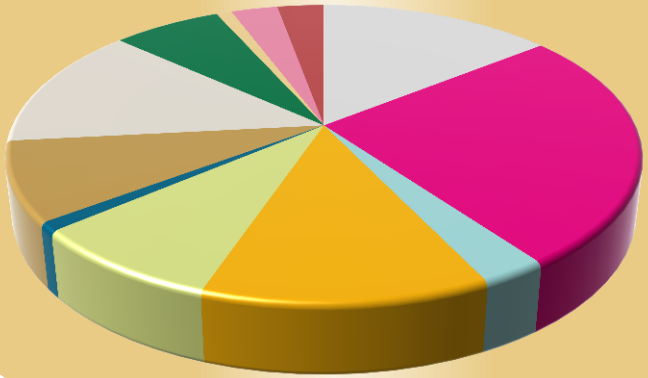
VALIDATION (60 CHEMICALS)



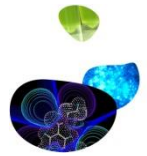
EXTENDED SET (45 CHEMICALS)



FUNCTIONAL GROUPS 105 CHEMICALS



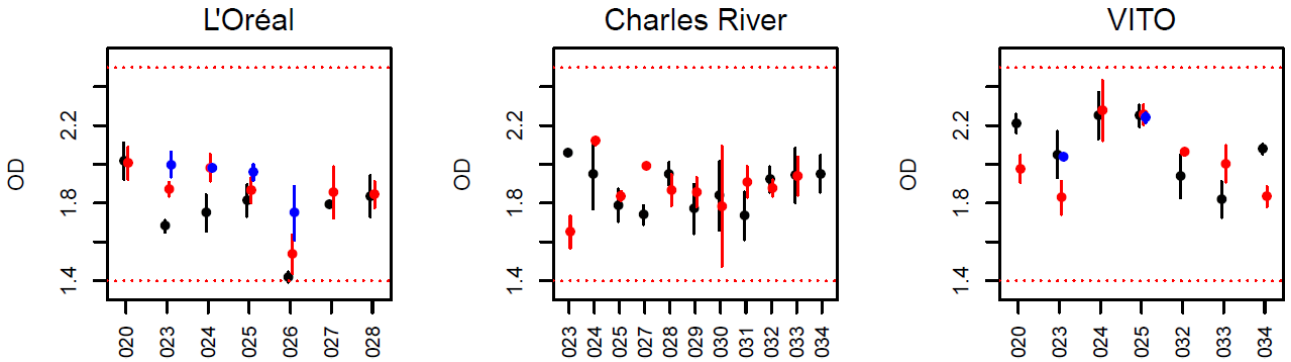
- Carboxylic acids & derivates (ester, amide…)
- Alcohols (allyl alcohols, cyclic alcohols…)
- Alkanes
- Amine, ammonium salt
- Aromatic
- Phosphorates derivates
- Electrophile (Acrylate, aldehyde, ketone...)
- Ether & PolyEther
- Halogenated
- Nitrile
- Silicium derivated
- Thiol, di-sulfure & sulfure oxyde



EITL
CONTROLS REPRODUCIBILITY

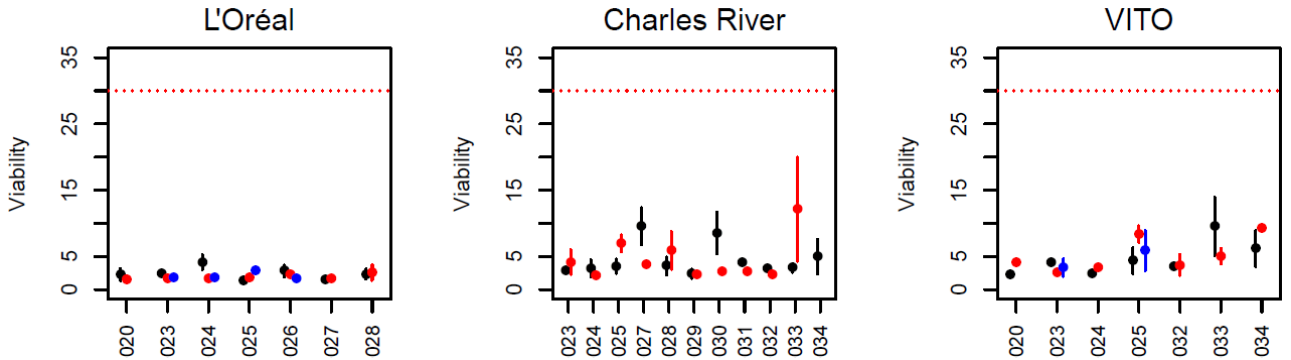
Negative control
PBS-

Acceptance criterion:
$1.4 \leq OD_{NgC} \leq 2.5$



Positive control
methyl acetate

Acceptance criterion:
PCmean viability ≤ 30%



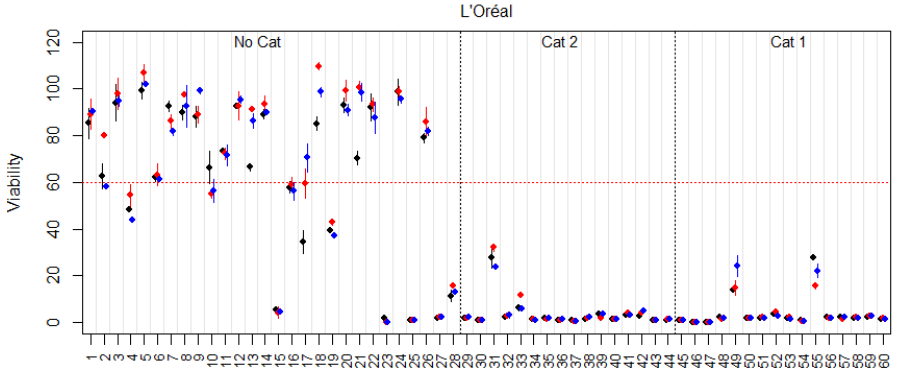
Acceptance criterion:
%Difference between replicates ≤ 20%

Dots represent the mean of two tissues, bars correspond to single values. Up to 3 series were tested (serie 1, serie 2, serie 3)

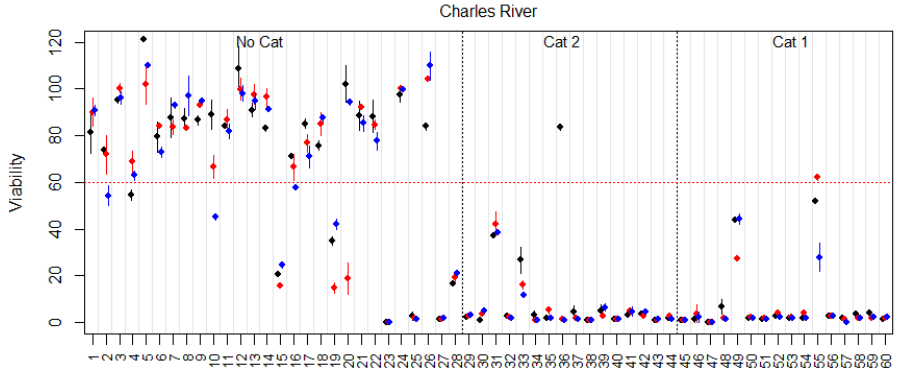
EITL

WITHIN LABORATORY REPRODUCIBILITY

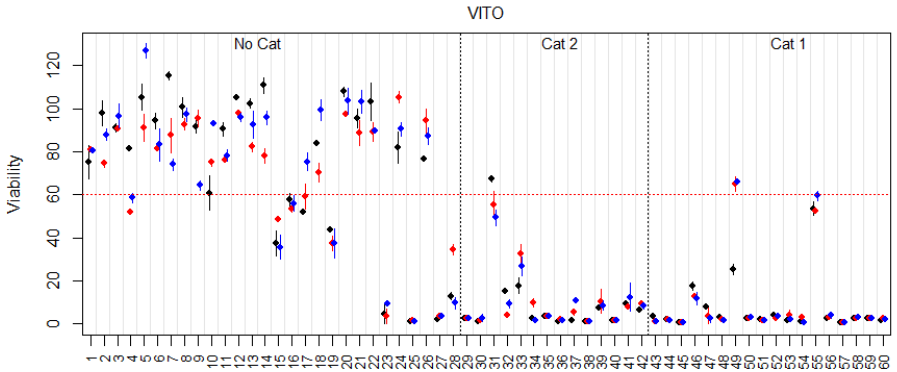
95% (95% CI: 86.3%; 98.3%)



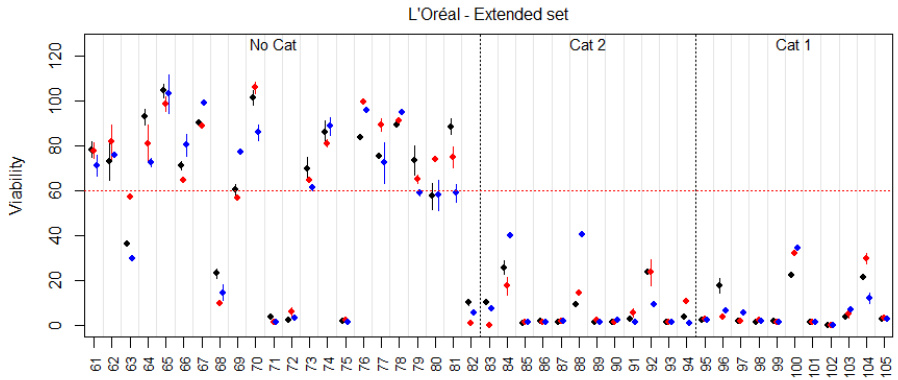
88.3% (95% CI: 77.8%; 94.2%)



93.3% (95% CI: 84.1%; 97.4%)



91.1% (95% CI: 79.3%; 96.5%)

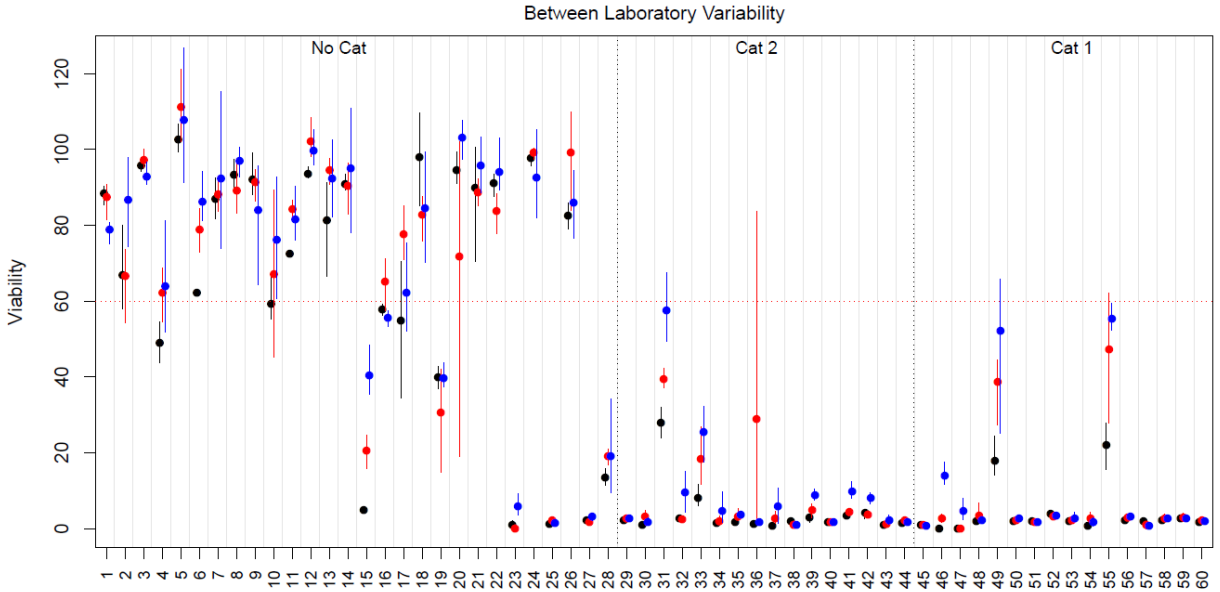


Viability for the chemicals for the three independent experiments (Experiment 1, Experiment 2, Experiment 3). Dots represent the mean of two tissues

EITL

BETWEEN LABORATORIES REPRODUCIBILITY

93.3% (95% CI: 84.1% - 97.4%)



BLR for the pair-wise comparisons

L'Oréal - CRL	L'Oréal - VITO	CRL - VITO
93.3% (56/60)	95.0% (57/60 chemicals)	98.3% (59/60)

Viability for the chemicals for the 3 laboratories. (L'Oréal, CRL,VITO). Dots represent the mean of three independent experiments.

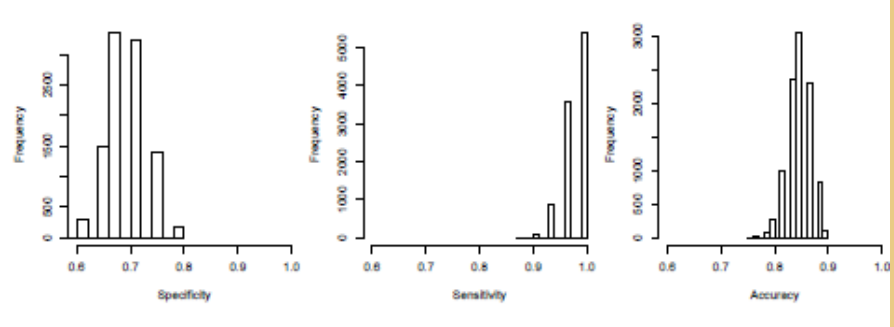
EITL
PREDICTIVE CAPACITY

EITL – 60 chemicals

In vivo	Cumulative		L'Oréal		CRL		VITO	
	C	NC	C	NC	C	NC	C	NC
Classified [A]	**283**	5	**96**	0	**94**	2	**93**	3
No Category	77	**175**	29	**55**	23	**61**	25	**59**
Total	540		180		180		180	
Sensitivity (%)	98.3		100		97.9		96.9	
False Negatives (%)	1.7		0		2.1		3.1	
Specificity (%)	69.4		65.5		72.6		70.2	
False Positives (%)	30.6		34.5		27.4		29.8	
Accuracy (%)	84.8		83.9		86.1		84.4	

[A] UN GHS Cat 1 and Cat 2

Bootstrap resampling

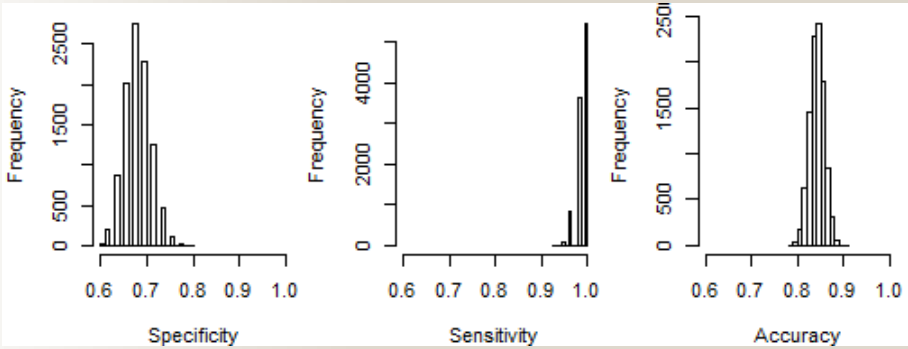


Overall - 105 chemicals

In vivo UN GHS	Cumulative		L'Oréal [a]		Charles River laboratories [b]		VITO [b]	
	I	NI	I	NI	I	NI	I	NI
Classified (n)	**352**	5	**165**	0	**94**	2	**93**	3
No Category (n)	100	**218**	52	**98**	23	**61**	25	**59**
Total (n)	675		315		180		180	
Sensitivity (%)	98.6		100		97.9		96.9	
Specificity (%)	68.6		65.3		72.6		70.2	
Accuracy (%)	84.4		83.5		86.1		84.4	

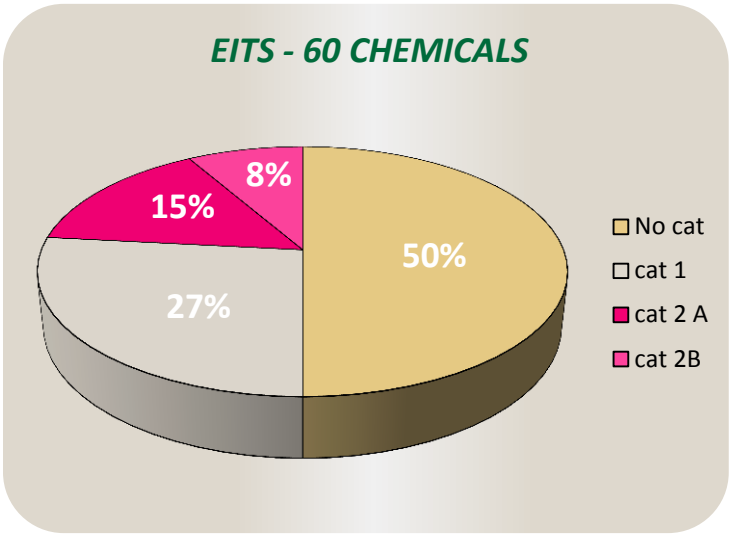
[a] Predictions based on all chemicals (60 from the multicentre study and 45 additional chemicals)

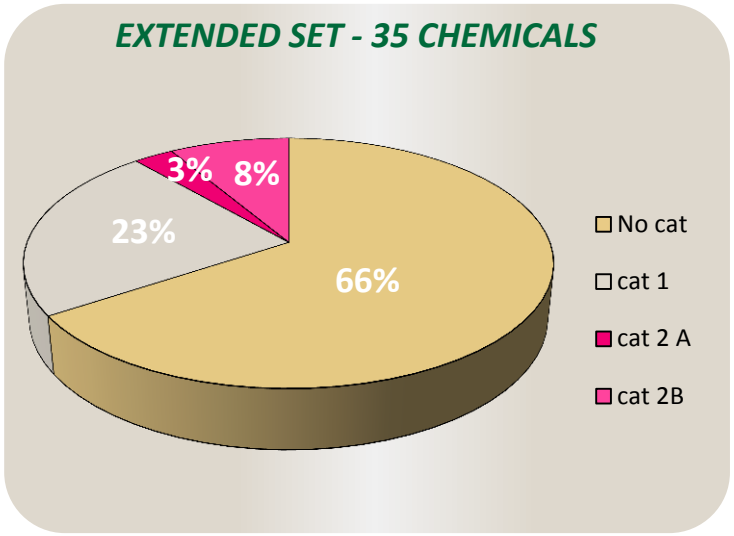
[b] Predictions based on the 60 chemicals from the multicentre study

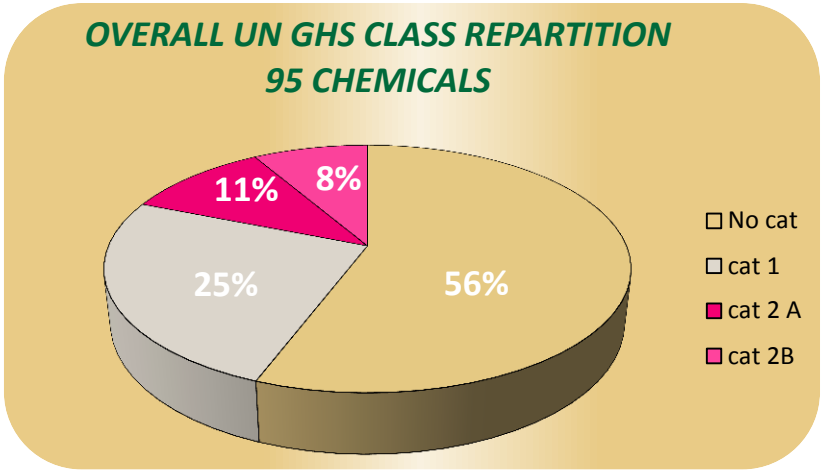


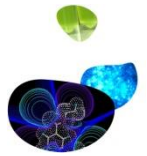
Bootstrap sample consist of 10.000 resamplings of size = 1 per chemical for the extended data set of 105 chemicals.

SKINETHIC™ HCE EITS
UN GHS CLASS REPARTITION



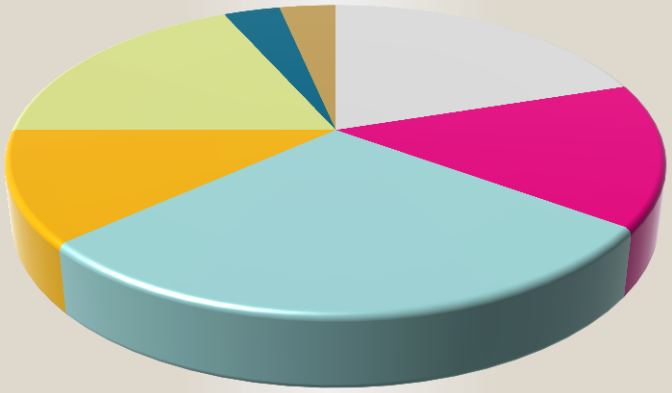




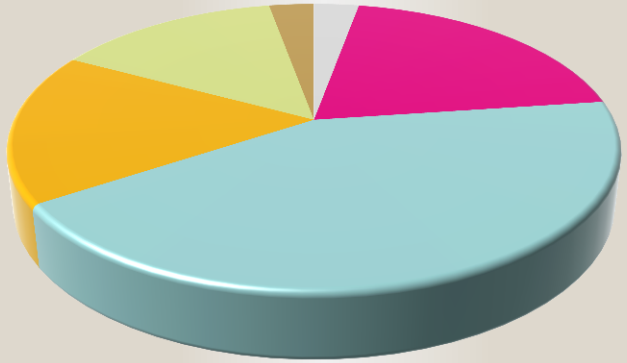


EITS
IN VIVO SOURCES REPARTITION

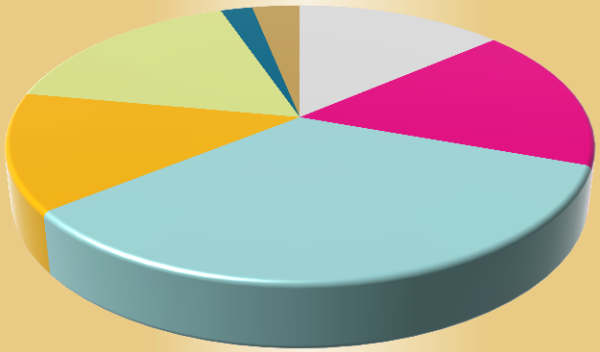
EITS - 60 CHEMICALS



EXTENDED SET - 35 CHEMICALS



OVERALL - 95 CHEMICALS

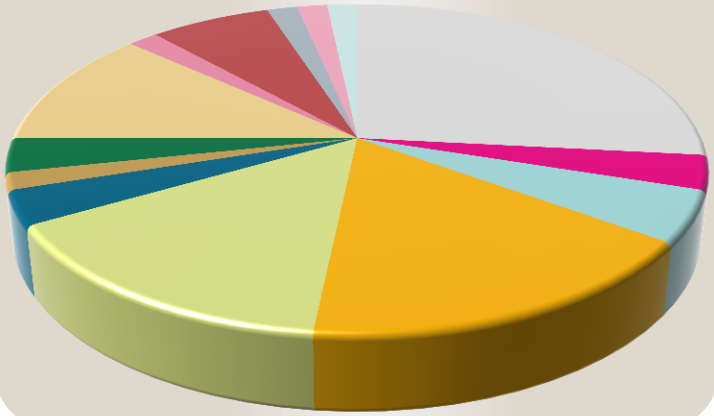


- ECETOC
- NICEATM
- EURL ECVAM
- LNS
- ZEBET
- ECETOC / ZEBET
- ECETOC + LNS

EITS

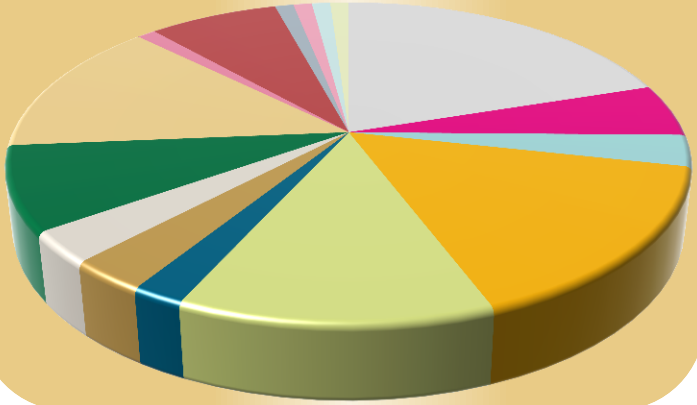
CHEMICAL GROUPS REPARTITION

VALIDATION (60 CHEMICALS)

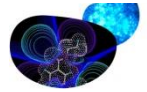


EXTENDED SET (35 CHEMICALS)

FUNCTIONAL GROUPS 95 CHEMICALS



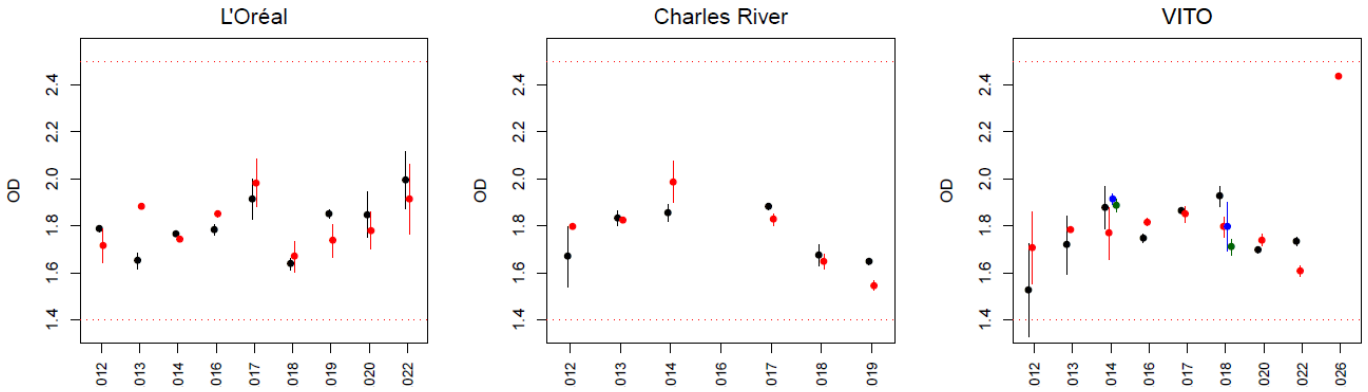
- Carboxylic acids & derivates (ester, amide…)
- Alcohols (allyl alcohols, cyclic alcohols…)
- Alkanes
- Amine, ammonium salt
- Aromatic
- Phosphorates derivates
- Electrophile (Acrylate, aldehyde, ketone…)
- Ether & PolyEther
- Halogenated
- Hétérocycles
- Silicium derivated
- Thiol, di-sulfure & sulfure oxyde
- Inorganic salts
- Nitro derivates
- Urea derivates
- Metal derivates



EITS CONTROLS REPRODUCIBILITY

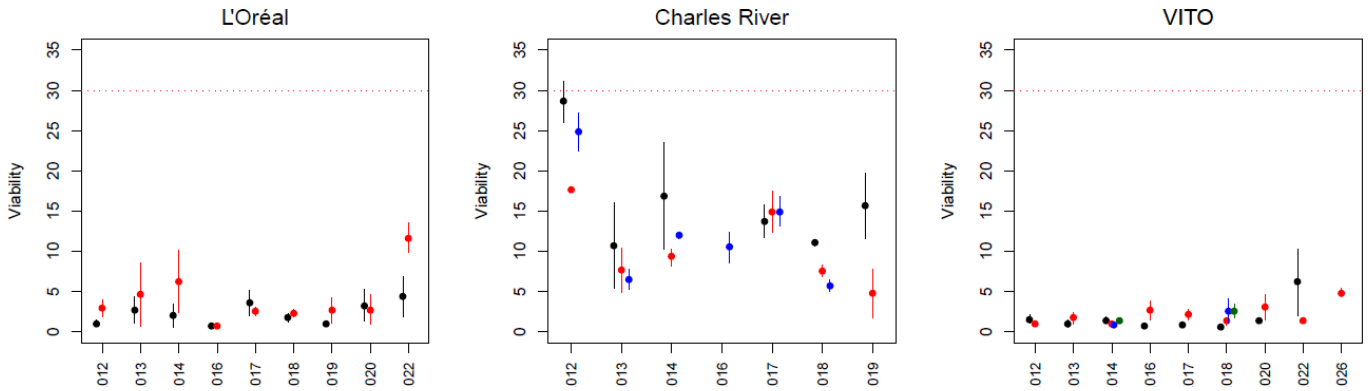
Negative control
PBS-

Acceptance criterion:
1.4 ≤ OD_{NgC} ≤ 2.5



Positive control
methyl acetate

Acceptance criterion:
PCmean viability ≤ 30%



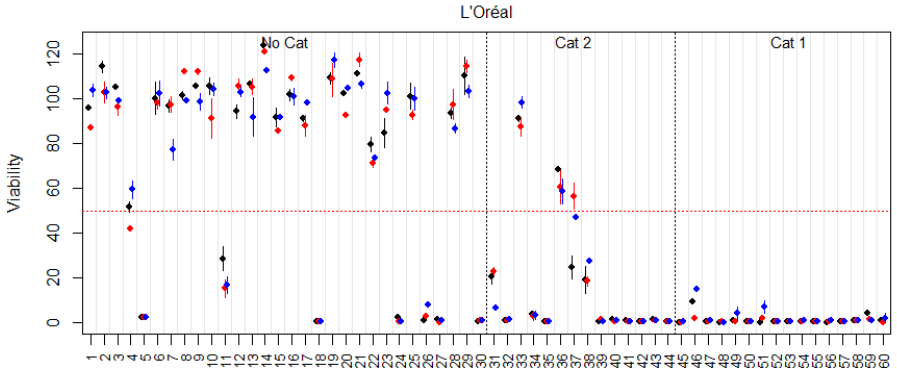
Acceptance criterion:
%Difference between replicates ≤ 20%

Dots represent the mean of two tissues, bars correspond to single values. Up to 4 series were tested (serie 1, serie 2, serie 3, serie 4)

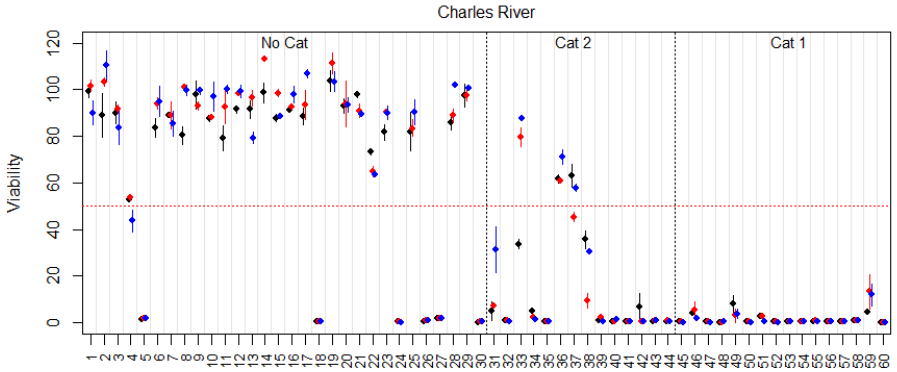
EITS

WITHIN LABORATORY REPRODUCIBILITY

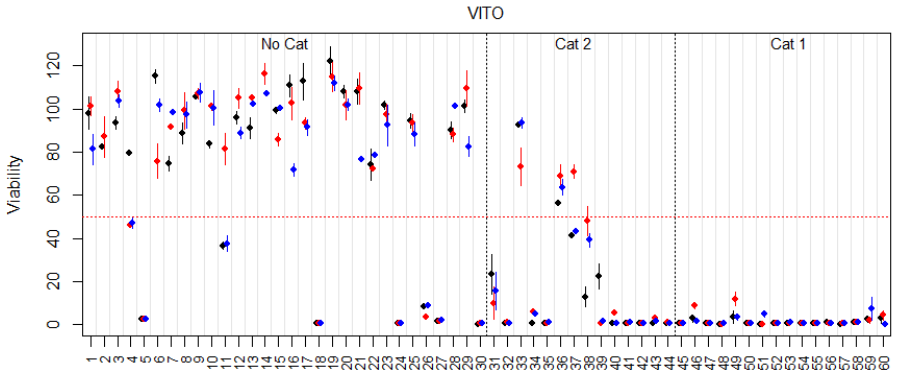
96.7% (95% CI: 88.6%; 99.1%)



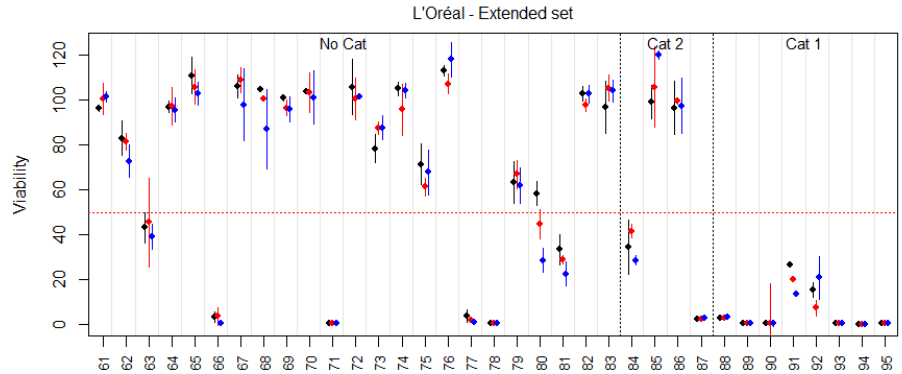
95% (95% CI: 86.3%; 98.3%)



95% (95% CI: 86.3%; 98.43)



97.1% (95% CI: 85.5%; 99.5%)

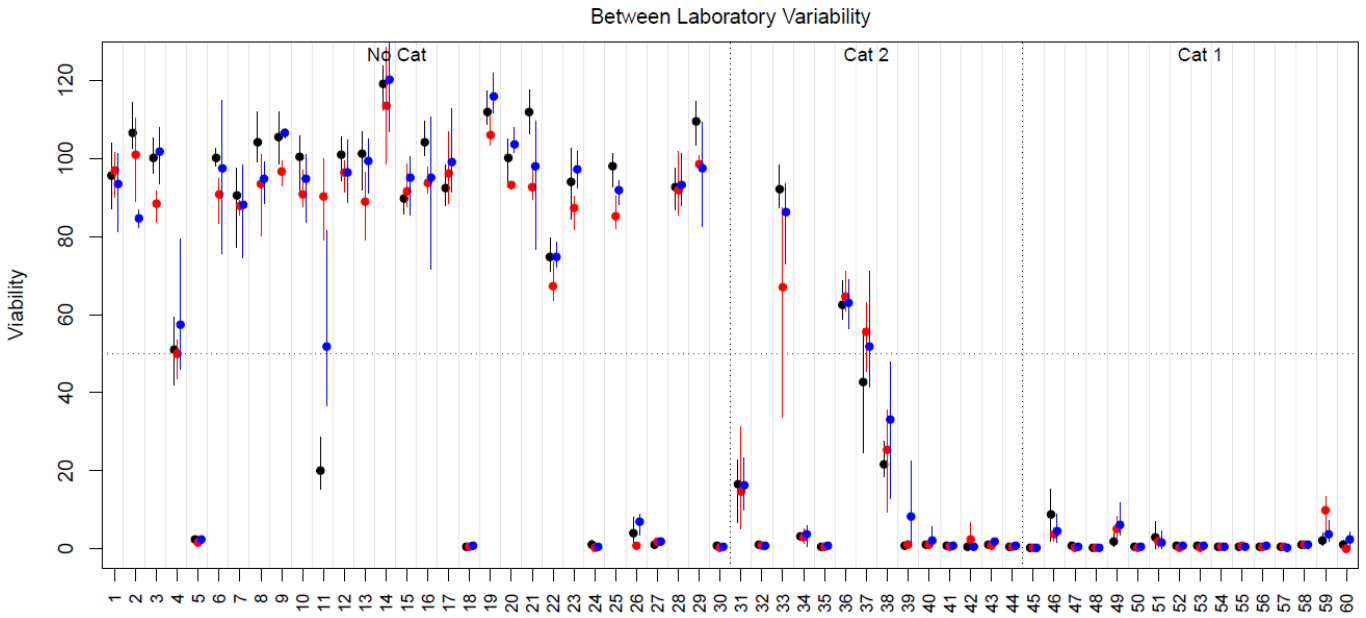


Viability for the chemicals for the three independent experiments (Experiment 1, Experiment 2, Experiment 3). Dots represent the mean of two tissues

EITS

BETWEEN LABORATORIES REPRODUCIBILITY

96.7% (95% CI: 88.6% - 99.1%)



BLR for the pair-wise comparisons

L'Oréal - CRL	L'Oréal - VITO	CRL - VITO
96.7% (58/60)	96.7% (58/60 chemicals)	100% (60/60)

Viability for the chemicals for the 3 laboratories. (L'Oréal, CRL,VITO). Dots represent the mean of three independent experiments.

EITS
PREDICTIVE CAPACITY

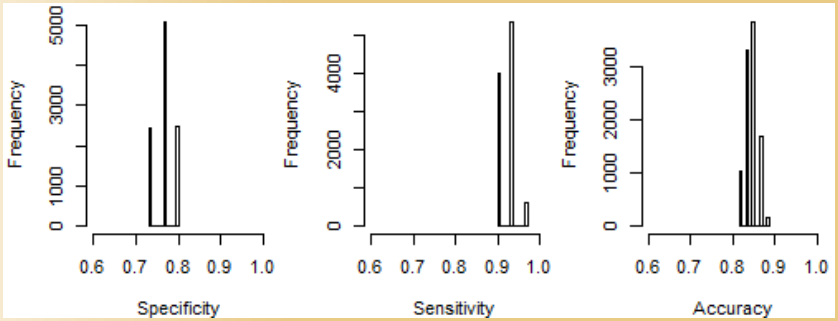
EITS Set (60)

In vivo	Cumulative		L'Oréal		CRL		VITO	
	C	NC	C	NC	C	NC	C	NC
Classified [A]	**249**	21	**83**	7	**83**	7	**83**	7
No Category	63	**206**	22	**68**	19	**71**	22	**67**
Total	539		180		180		179 [B]	
Sensitivity (%)	92.2		92.2		92.2		92.2	
False Negatives (%)	7.8		7.8		7.8		7.8	
Specificity (%)	76.6		75.6		78.9		75.3	
False Positives (%)	23.4		24.4		21.1		24.7	
Accuracy (%)	84.4		83.9		85.6		83.3	

[A] UN GHS Cat 1 and Cat 2

[B] For chemical No. 2 only two valid results were obtained over the 5 tests

Bootstrap resampling



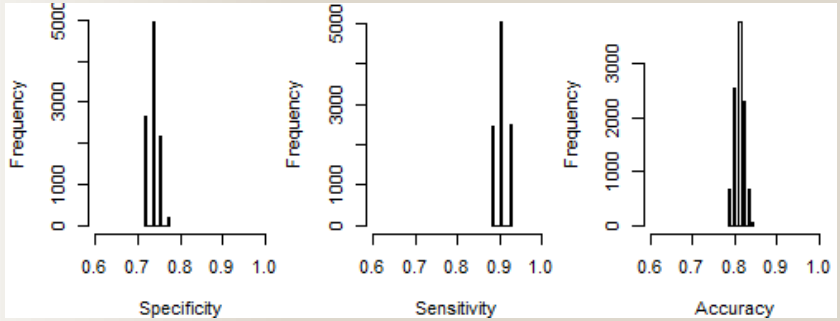
Overall Set (95)

In vivo UN GHS	Cumulative		L'Oréal [a]		CRL [b]		VITO [b]	
	C	NC	C	NC	C	NC	C	NC
Classified (n)	**279**	27	**113**	13	**83**	7	**83**	7
No Category (n)	83	**255**	42	**117**	19	**71**	22	**67**
Total (n)	644		285		180		179 [c]	
Sensitivity (%)	91.2		89.7		92.2		92.2	
Specificity (%)	75.4		73.6		78.9		75.3	
Accuracy (%)	82.9		80.7		85.6		83.3	

[a] Predictions based on all chemicals (60 from the multicentre study and 35 additional chemicals)

[b] Predictions based on the 60 chemicals from the multicentre study

[c] For chemical No. 2 only two valid runs were obtained over the five runs



Bootstrap sample consist of 10.000 resamplings of size = 1 per chemical for the extanded data set of 95 chemicals.

RELIABILITY
RhT TEST METHODS

Liquid chemicals

	WLR				BLR	Prediction		
	Nb chem	Lead Lab	Lab 1	Lab 2	Perf	Sensitivity	Specificity	Accuracy
SkinEthic™ HCE EIT	60	95.0%	88.3%	93.3%	93.3%	98.3%	69.4%	84.8%
EpiOcular EIT	52	96.3%	98.1%	98.1%	94.4%	98.3%	66.7%	81.9%

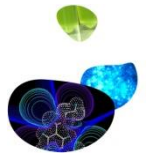
Solid chemicals

	WLR				BLR	Prediction		
	Nb chem	Lead Lab	Lab 1	Lab 2	Perf	Sensitivity	Specificity	Accuracy
SkinEthic™ HCE EIT	60	96.7%	95%	95%	96.7	92.2%	76.6%,	84.4%
EpiOcular EIT	60	96.6 *	-	-	92.0% **	93.5	60.7	78.0

*** Original protocol: 94.0%, 90.2%, 94.1%**
**** from original protocol**

EIVS Acceptance criterion
WLR≥ 85% BLR ≥ 80% Sens ≥ 90% Spe ≥ 60%

From different chemical sets: no strict comparison should be made



ANALYSIS OF MISPREDICTIONS FOR LIQUIDS

4 FNs out of the 55 *in vivo* classified chemicals

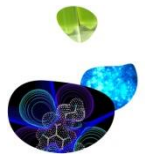
- 4 functional groups (alcohol; ester, ketone; amine-silane; and polyether-acrylate)
- Stability issue for 1 chemical (viability increased // crystal formation reported upon storage)
- 2 Cat 2 based on corneal opacity (98.6% correctly predicted)
- 1 Cat 1 based on iritis (1 out of 9 runs) (95.2% correctly predicted)

22 FPs out of the 50 *in vivo* Not Cat chemicals

- 3 esters misclassified also in the BCOP and EpiOcular™ EIT.
- 9 others representing 8 different functional groups
- Subgroups CO > 0 and CO > 0 ** were often over-predicted (44% and 100% of the runs, respectively).

There is not a dominant functional group for FPs and FNs as the groups with higher frequencies also correspond to the most populated groups.
There is no dominant *in vivo* drivers of classification for FNs

CO > 0: at least one animal for at least one observed time point,
CO > 0 **: at least one animal had a mean of the scores of days 1-3 above the classification cut-off for at least one endpoint but not enough animals to generate a classification



ANALYSIS OF MISPREDICTIONS FOR SOLIDS

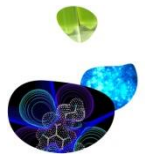
5 FNs out of the 42 *in vivo* classified chemicals

- 4 different functional groups (nitro-compound, two esters, phenol and ether)
- 1 Cat 2A and 2 Cat 2 based on conjunctival effects only (82.8% correctly predicted)
- 2 Cat 2 based on corneal opacity (71.4% correctly predicted)

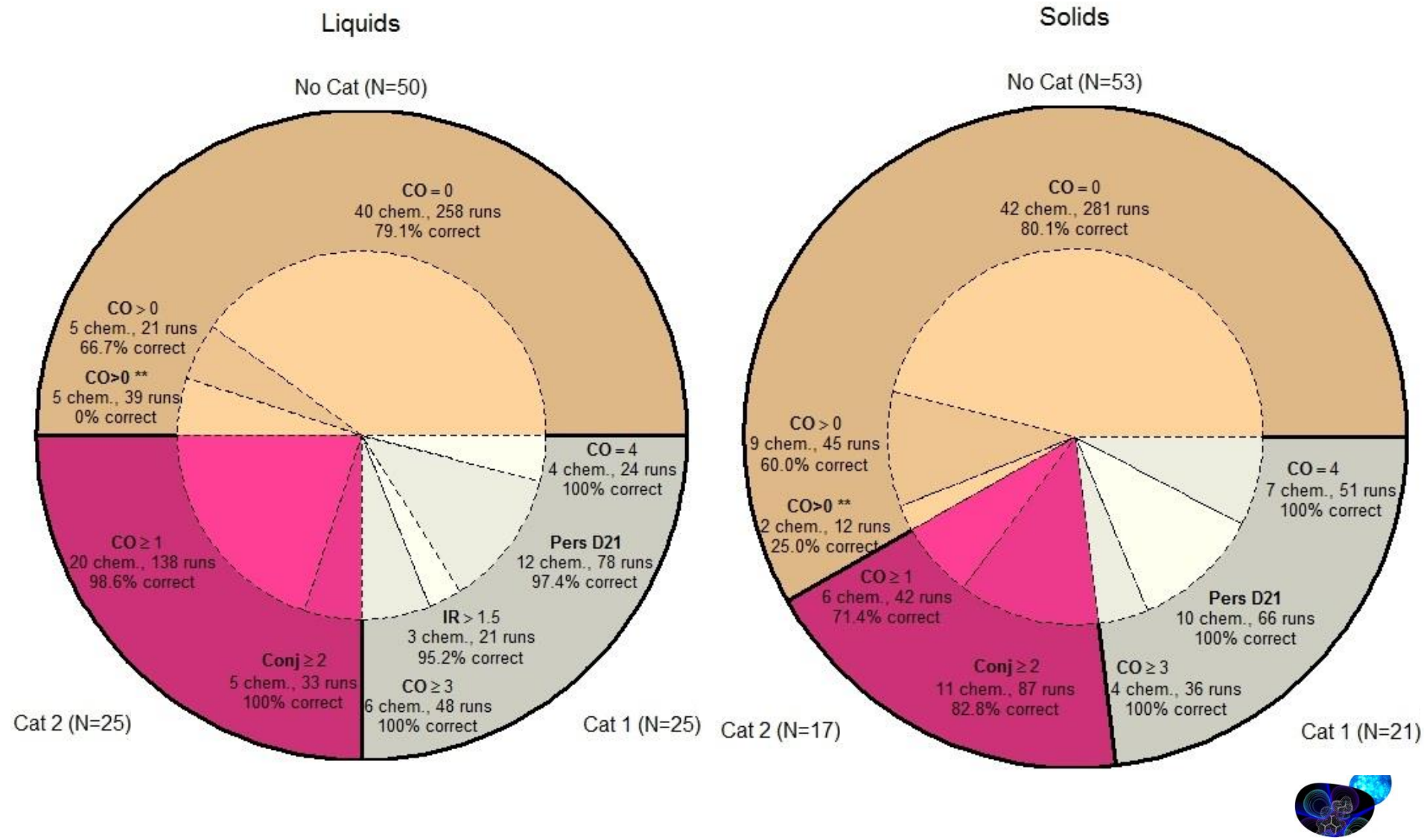
15 FPs of the 53 *in vivo* No Cat chemicals,

- 12 consistently predicted C.
- 12 different functional groups
- Subgroups CO > 0 and CO > 0 ** were often over-predicted (40% and 75% of the runs, resp.).

No relation was observed between the FPs / FNs and the functional group of the chemical
There is no dominant *in vivo* drivers of classification for FNs



SKINETHIC™ HCE EIT PREDICTION
IN VIVO DRIVERS OF CLASSIFICATION*



L'ORÉAL
Research & Innovation

*From subgroups as defined by Barroso et al. Cosmetics Europe compilation of historical serious eye damage/eye irritation in vivo data analysed by drivers of classification to support the selection of chemicals for development and evaluation of alternative methods/strategies: the Draize eye test Reference Database (DRD). Submitted to Arch Toxicol 2015.

SKINETHIC™ HCE EIT
APPLICABILITY DOMAIN

Based on 105 liquids and 95 solids:

- **Different functional groups** (soap / surfactant, neutral organic, organic acid, organic base, inorganic acid, neutral inorganic, inorganic base...);
- **Different functional classes** (carboxylic acid & derivatives (ester, amide), alcohol, amine, electrophile (acrylate, aldehyde, ketone), ether…);
- **Different UN GHS Categories** (51 Cat 1, 29 Cat 2A, 17 Cat 2B and 103 No Cat;
- **Mono-constituent substances** or **multi-constituent substances** (including polymers);
- **Neat** (175 chemicals) or **in dilution** (0.1 to 30%) tested chemicals;
- **MTT reducers** (7 liquids and 12 solids);
- **Colour interfering chemicals** (1 liquid and 7 solids);
- **MTT reducing colorants** (1 liquid and 10 solids).

Results generated in the validation study have demonstrated that SkinEthic™ HCE EIT is applicable those categories

HPLC/UPLC- SPECTROPHOTOMETRY VS ABSORBANCE (OD)

Formazan extraction

IF THE CHEMICAL IS SLIGHTLY COLORANT OR MTT REDUCER OR NO INTERFERENCE

Viability assessment by OD

Read OD at 570 nm

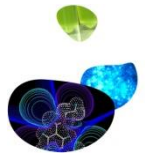
ANY CHEMICAL INCLUDING HIGHLY COLORED AND/OR MTT REDUCER CHEMICAL

Viability assessment by HPLC/UPLC

Analyse by HPLC/UPLC spectrophotometry

HPLC/UPLC- SPECTROPHOTOMETRY

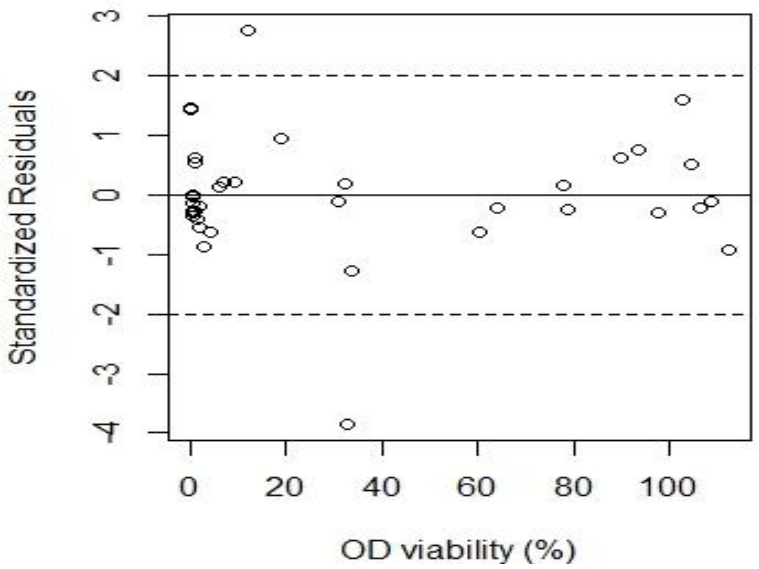
Physical form	Total	Chemical			
		MTT Interference	Coloured Interference	MTT and Coloured Interference	No Interference
Solid	13	2	0	8	3
Liquid	11	2	0	3	6
Total	24	4	0	11	9

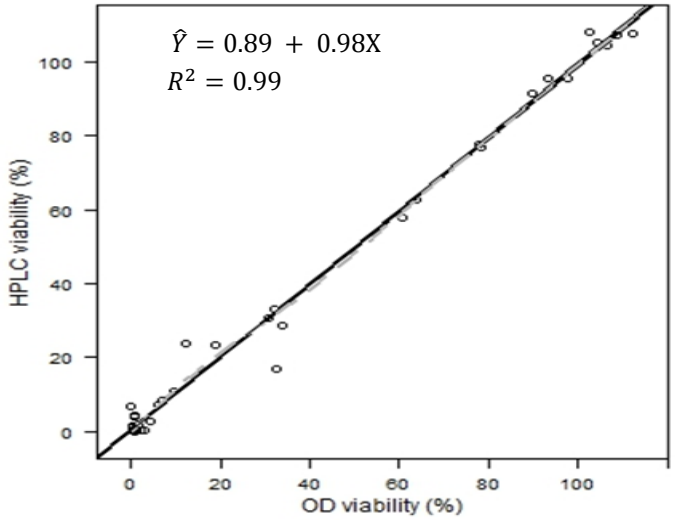


OD – HPLC/UPLC-spectrophotometry correlation

Tissue viability (%) for 19 chemicals when both OD and HPLC/UPLC-spectrophotometry possibly quantified

The assumption of linearity was verified with a scatter plot of the standardized residuals versus the viability and the normality of the residuals was verified with a QQ-plot.





From the residuals plot, it can be observed that the mean absolute difference in viability between both methods was on average ≤ 6.7%.

The high fit (R^2 = 0.99) and the slope of the regression model which is close to 1 (slope = 0.98 with 95% CI: 0.95; 1.02) confirm that, for chemicals that are compatible with use of OD, a high correlation with use of HPLC/UPLC-spectrophotometry.

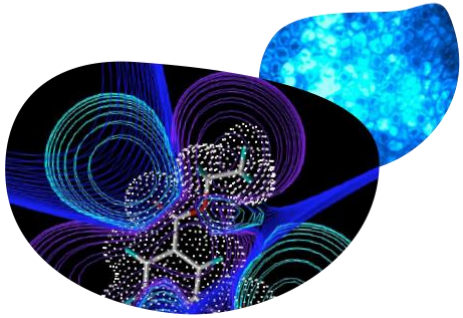
High agreement is observed between measurement of tissue viability by OD and HPLC/UPLC-spectrophotometry

CONCLUSIONS

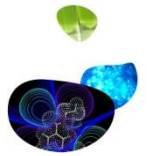
- The SkinEthic™ HCE EIT (EITL and EITS protocols) is reproducible and accurate to identify chemicals not requiring classification for serious eye damage/eye irritation according to UN GHS
- A wide range of chemicals was tested and no clear limitations could be identified. Therefore , the SkinEthic™ HCE EIT as being applicable to the testing of all types of chemicals
- The standard absorbance (OD) measurement used with this and other test methods is appropriate to assess direct MTT-reducers and coloured chemicals.
- For coloured chemicals interfering too strongly with the MTT-reduction assay an alternative endpoint detection system should be used (e.g., HPLC/UPLC-spectrophotometry)

As soon as the Peer-review & EURL ECVAM recommendation are made available, it will be provided (restricted distribution) to the WNT and the OECD eye irritation expert group

THANK YOU FOR YOUR ATTENTION!

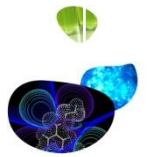


BACK UP SLIDES

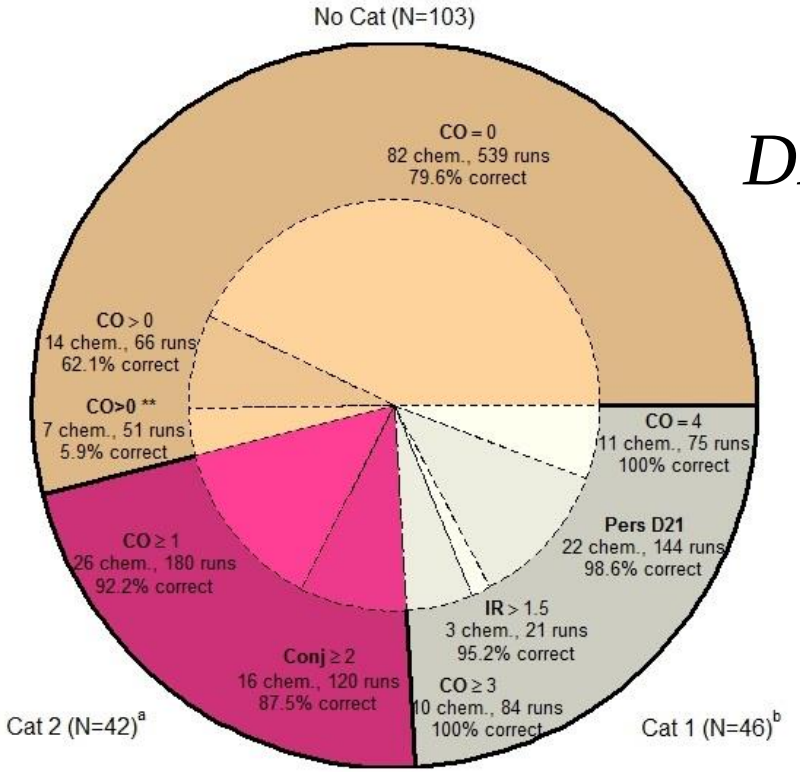


FUNCTIONAL CLASS

Carboxylic acids & derivates (ester, amide…)	Aromatic	Alcohols (allyl alcohols, cyclic alcohols…)	Alkanes	Amine, ammonium salt	Phosphorates derivates	Electrophile (Acrylate, aldehyde, ketone…)	Ether & PolyEther	Halogenated	Nitrile	Silicium derivated	Thiol, di-sulfure & sulfure oxyde	Hétérocycles	Nitro derivates	Urea derivates	Metal derivates
Amide	Aromatic	Alcohol	Alkali	Alkylamonium salt	Organophosphoric	Acid chloride	Ether oxide	Fluoroborate salts	Nitrile	Silane	Disulfure	Heterocyclic ether	Nitro	Urea	Hydroxyde aluminium
Amine	Aromatic alcohol	Alcohol acid	Alkane	Alkyl-pyridinium	Phenyl phosphite	Acrylate	Sulfuric ether	Halogenated		Silicium	Sulfoxide	Heterocyclic aromatic			
Carboxylic acid	Aromatic amide	Alcyne	Alkane cyclic	Amine		Aldehyde	Polyether	Isocyanate		Siloxane	Thiol	Heterocyclic phenol			
Cyclic Ester	Aromatic ether	Allyl alcohol	Alkyl	Aminoacid salt		Amonium		Nitro			Thiolesther	Heterocyclic pyrazole			
Ester	Aromatic Polyether	Cyclic Alcohol	Halide	Amonium		Epoxyde		Organophosphates				Heterocyclic thio-urea			
Ketone	Cycloalcene	Ether Polyols	Hydrocarbon	Amonium salt		Ketone		Pyridine							
Nitrile	Heterocyclic	Polyols	Hydrocarbon-diene	Guanidine halogenated											
Phenol	Hydrocarbon cyclic			Imidazolium											
Polyether	Phenol			Nitrate											
Salt				Nitrile											
Siloxy				Pyridine											
				Thioimine											



SKINETHIC™ HCE EIT
DRIVERS OF CLASSIFICATION*



200 CHEMICALS**

No Cat

CO=0: Corneal Opacity (CO) scores equal to 0 in all animals and all observed time points in the Draize eye test;
CO>0: at least one animal for at least one observed time point;
CO>0**: at least 1 animal had mean scores of days 1-3 above the classif cut-off for ≥ 1 endpoint but not enough animals to generate a classif.

Cat 2

CO≥1: mean CO scores of days 1-3 ≥ 1 in ≥ 60% of the animals;
Conj≥2: mean Conj. Redness (CR) and/or Conj. Chemosis (CC) during the first 3 days ≥ 2 in ≥ 60% of animals in absence of "CO mean ≥ 1;

Cat 1

CO≥3: mean CO scores of days 1-3 ≥ 3 in ≥ 60% of the animals;
IR>1.5: mean Iritis (IR) scores of days 1-3 > 1.5 in ≥ 60% of the animals in absence of CO mean ≥ 3;
Pers D21: persistence of any ocular effect on day 21 in the absence of severity (CO mean ≥ 3 and IR mean > 1.5);
CO=4: at any observation time during the study in the absence of both severity and persistence

*From subgroups as defined by Barroso et al. Cosmetics Europe compilation of historical serious eye damage/eye irritation in vivo data analysed by drivers of classification to support the selection of chemicals for development and evaluation of alternative methods/strategies: the Draize eye test Reference Database (DRD). Submitted to Arch Toxicol 2015.
**The results of 9 chemicals (4 solids & 5 liquids) were not included in the pie charts since the driver could not be identified or because multiple studies were available for the same chemical and the driver differed between the repeat studies.