The European Commission's Science and Knowledge Service

Joint Research Centre

Good *In Vitro* Method Practices (GIVIMP) and its implementation

Sandra Coecke,

Gerard Bowe, Patience Browne

The National Academies of Sciences, Engineering, and Medicine

Strategies and Tools for Conducting Systematic Reviews of Mechanistic Data to Support Chemical Assessments

Lecture Room, NAS Building, Washington, DC, USA, 10-11 December 2018

European Commission

In vitro method development safeguarding scientific integrity and quality

Trusted by decision makers
Used by industry

Need approaches and tools to respond to a more general "reproducibility crisis" or "replication crisis" of scientific results
++ stimulate a "credibility revolution"

https://plato.stanford.edu/entries/scientific-reproducibility/
First published Mon Dec 3, 2018

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GIVIMP

OECD Guidance Document on Good *In Vitro* Method Practices

FOR THE DEVELOPMENT AND IMPLEMENTATION OF IN VITRO METHODS FOR REGULATORY USE IN HUMAN SAFETY ASSESSMENT

GIVIMP is method centred but includes aspects of good practices in reporting of mechanistic data, critical to their credibility

If GIVIMP is properly implemented, this tool will increase confidence in the reliability and integrity of the generated data

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GIVIMP OECD Guidance Document on Good *In Vitro* Method Practices

***It strives to* implement good practices early in the *in vitro* method development process**

Applying the principles described in GIVIMP during the development of in vitro methods will help improve the quality and reliability of methods that are needed to support safety decisions.

Tool to improve the efficiency of the whole process from development to final use.

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GIVIMP OECD Guidance Document on Good *In Vitro* Method Practices

August 2018…Support method developers and end-users working in academic, industry or government laboratories across all 36 OECD member countries and beyond in ***harmonisation efforts*** of *in vitro* methods.

OECD BETTER POLICIES FOR BETTER LIVES

36 **Member Countries** + EC
Accession countries: Russia, Columbia, and Costa Rica
Key Partners: Brazil, India, China, South Africa and Indonesia

The development of GIVIMP was coordinated by the European Commission Joint Research Centre's EU Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) within the context of a project of the OECD Test Guidelines Programme

EURL ECVAM European Union Reference Laboratory for Alternatives to Animal Testing

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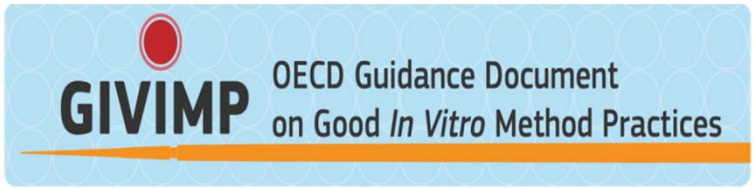


The GIVIMP GD is divided into 10 sections covering:

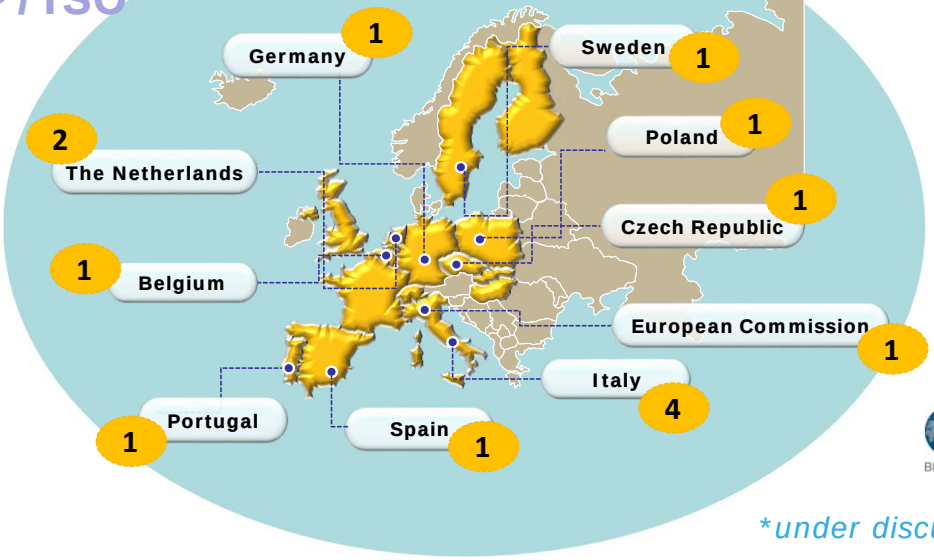
1. Roles and responsibilities
2. Quality considerations
3. Facilities
4. Apparatus, material and reagents
5. Test systems
6. Test and reference/control items
7. Standard operating procedures (SOPs)
8. Performance of the method
9. Reporting of results
10. Storage and retention of records and materials

EURL ECVAM is coordinating a large scale validation study of a set of mechanistically informative alternative methods to detect chemicals that disrupt normal thyroid hormone function, in collaboration with 14 of the 37 European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL) and the method developers for delivering at OECD level.

EU Directive 2010/63/EU on the protection of animals used for scientific purposes

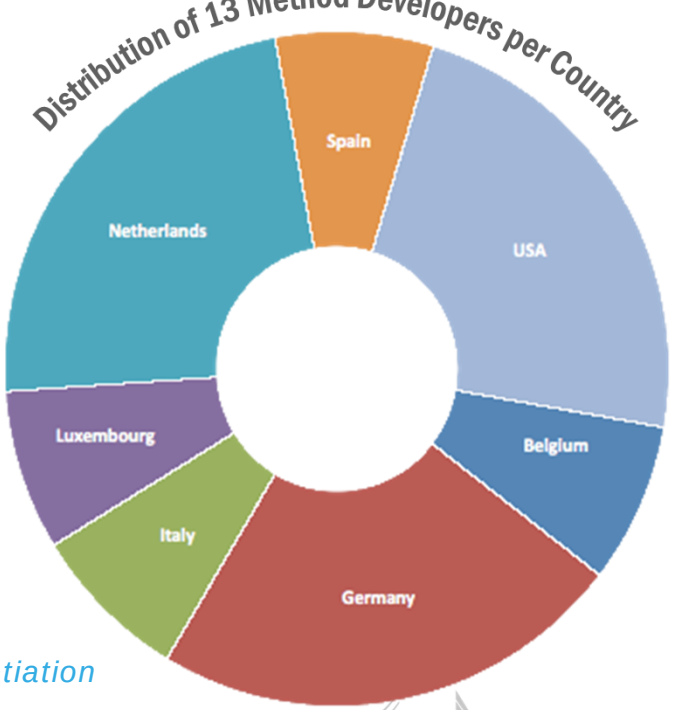
GLP / ISO

14 EU-NETVAL Thyroid Validation Study Participants



EURL ECVAM
European Union Reference Laboratory for Alternatives to Animal Testing

OECD
BETTER POLICIES FOR BETTER LIVES



*under discussion & negotiation

EU-NETVAL test facilities interact with the *in vitro* method developers to define the methods and establishing SOPs that can run in a GLP/routine environment and assess within laboratory reproducibility followed by relevance assessment of the methods using a set of reference compounds.

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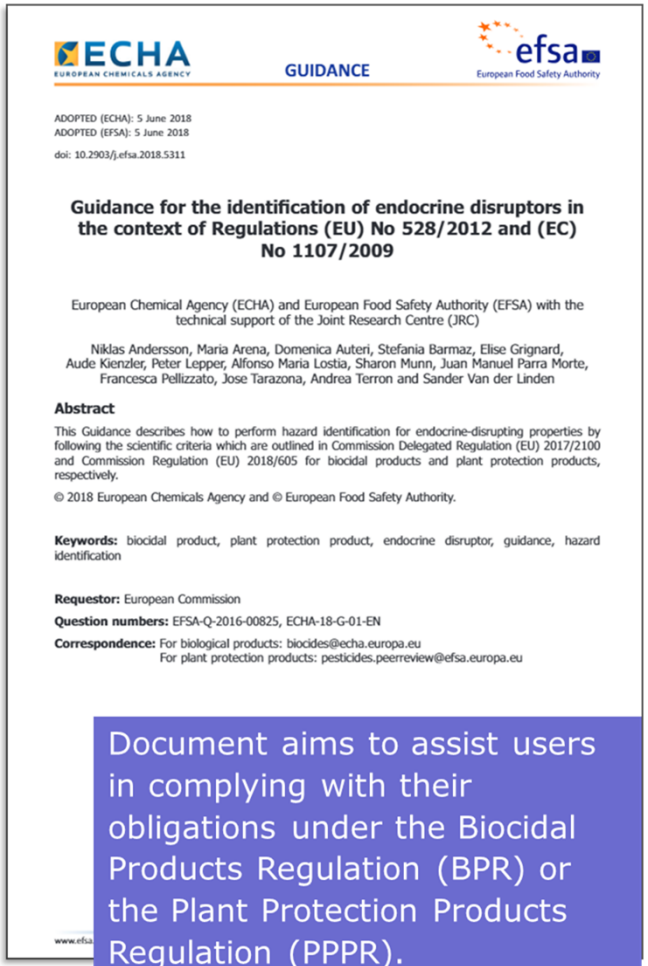

ECHA EUROPEAN CHEMICALS AGENCY

GUIDANCE

efsa European Food Safety Authority

ADOPTED (ECHA): 5 June 2018
ADOPTED (EFSA): 5 June 2018

doi: 10.2903/j.efsa.2018.5311

Guidance for the identification of endocrine disruptors in the context of Regulations (EU) No 528/2012 and (EC) No 1107/2009

European Chemical Agency (ECHA) and European Food Safety Authority (EFSA) with the technical support of the Joint Research Centre (JRC)

Niklas Andersson, Maria Arena, Domenica Auteri, Stefania Barmaz, Elise Grignard, Aude Kienzler, Peter Lepper, Alfonso Maria Lostia, Sharon Munn, Juan Manuel Parra Morte, Francesca Pellizzato, Jose Tarazona, Andrea Terron and Sander Van der Linden

Abstract

This Guidance describes how to perform hazard identification for endocrine-disrupting properties by following the scientific criteria which are outlined in Commission Delegated Regulation (EU) 2017/2100 and Commission Regulation (EU) 2018/605 for biocidal products and plant protection products, respectively.

Keywords: biocidal product, plant protection product, endocrine disruptor, guidance, hazard identification

Requestor: European Commission

Question numbers: EFSA-Q-2016-00825, ECHA-18-G-01-EN

Correspondence: For biological products: biocides@echa.europa.eu
For plant protection products: pesticides.peerreview@efsa.europa.eu

Document aims to assist users in complying with their obligations under the Biocidal Products Regulation (BPR) or the Plant Protection Products Regulation (PPPR).

Appendix A – Additional considerations on how to assess the potential for thyroid disruption for human health

2. Comparative studies of enzyme activity induced by the test substance in liver *in vitro* systems should be measured in both the relevant test species (e.g. rat, mouse and dog) and humans. The metabolism of the specific substance (ADME properties) in both test species and humans, and the activity of possible metabolites must be considered when this comparison is conducted.

3. The presence of other possible thyroid-disrupting modes of action such as interference with TH synthesis should also be excluded, e.g. by evaluating *in vitro* the potential for inhibition of the sodium-iodide symporter (NIS) (Cianchetta et al., 2010; Hallinger et al., 2010; Kogai et al., 2012) and thyroid peroxidase (TPO) (Kambe et al., 1997; Paul et al., 2014; Paul Friedman et al., 2016; Wu et al., 2016). It must however be acknowledged that substances may interfere with the thyroid hormone system through many different mechanisms of action, and that currently validated/standardized *in vitro* assays do not exist to investigate all these different pathways and a reasonable effort is anticipated, based on available tools and current understanding of thyroid physiology.

An example of a postulated mode of action is reported below:

The assessment of qualitative/quantitative differences in hepatic induction can therefore be part of the WoE and used to provide evidence of non-human relevance.

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A total of 17 methods have been identified as candidates by EURL ECVAM taking primarily into account the information reported in an OECD scoping document on *in vitro* and *ex vivo* methods for the identification of modulators of thyroid hormone signalling (OECD No. 207), but also an OECD Detailed Review Paper (OECD No. 178), and feedback received at various Expert Group meetings.

1. Central regulation

1a. CHO-K1 cells thyrotropin-releasing hormone (TRH) receptor activation (beta-galactosidase) measuring agonist and antagonist activities

1b. CHO-K1 cells thyrotropin-stimulating hormone (TSH) receptor activation based on cAMP measurement

2. Thyroid Hormone (TH) synthesis

2a. Thyroperoxidase (TPO) inhibition based on oxidation of Amplex UltraRed

2b. Thyroperoxidase (TPO) inhibition based on oxidation of Luminol

2c. Tyrosine iodination using liquid chromatography

2d. Activation of the sodium iodide symporter (NIS) based on Sandell-Kolthoff reaction

3. Secretion and transport in serum

3a. Thyroxine-binding prealbumin (TTR) / thyroxine-binding globulin (TBG) using fluorescence displacement (ANSA)

3b. Thyroxine-binding prealbumin (TTR) using fluorescence displacement (T4-FITC)

4. Metabolism and excretion

4a. Deiodinase 1 activity based on Sandell-Kolthoff reaction

4b. Glucuronidation of thyroid hormones (THs) using liquid chromatography/mass spectrometry (LC/MS)

4c. Inhibition of thyroid hormones (THs) sulfation using liquid chromatography

5. Local cellular concentrations

5a. Inhibition of monocarboxylate transporter 8 (MCT8) based on Sandell-Kolthoff reaction

6. Cellular responses

6a. Human thyroid hormone receptor alpha (TRα) and Human thyroid hormone receptor beta (TRβ) reporter gene transactivation measuring agonist and antagonist activities

6b. CALUX human thyroid hormone receptor beta (TRβ) reporter gene transactivation measuring agonist and antagonist activities

7. Relevant short term methods integrating multiple MoA

7a. Measurement of intrafollicular thyroxine (T4) using zebrafish eleutheroembryos

8. Integrative cellular *in vitro* methods

8a. Measurement of proliferation of rat pituitary-derived cell line GH3

8b. Proliferation, migration and oligodendrocyte differentiation of human neural progenitor cells

PART 1: Definition of the *in vitro* method

- ✓ produce the related set of SOPs
- ✓ Further development of the method if so needed
- ✓ assessing robustness and reliability
- ✓ Few chemicals: Reference and controls
- ✓ Experimental study of 5 valid runs …**data to assess within laboratory reproducibility**

PART 2: Relevance

- ✓ Will start only when the Definition has proven to be successful
- ✓ Overall relevance, based on the underlying mechanisms, of the selected *in vitro* methods: a set of chemicals (max. 30) for all methods
- ✓ Experimental study of 3 valid runs per test items

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Thyroid Validation Study

1.1 *In vitro* method developers

- Sign declaration; inform on IPR
- Declare GM elements
- Provide input to first draft of the outline protocol
- Provide input for choice of reference and control items

1.2 Test system providers

- Sign Material Transfer Agreement, IPR
- Declare GM elements

1.3. Validation bodies (EC JRC EURL ECVAM)

- Overall coordination (incl. all legal agreements…35)
- Provision test systems (characterisation and Qc), compounds, outline protocols

1.5 Suppliers of equipment, materials and reagents

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Thyroid Validation Study

2. Quality considerations

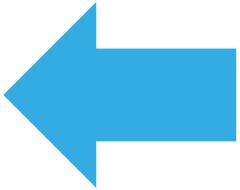
- Discusses quality assurance versus quality control
- Examines quality risk-based assessment and quality control requirements for development and implementation of *in vitro* methods
- Provides quality considerations regarding the integrity of the data

2.4 Quality control of test systems

2.5 Quality control of consumables and reagents

Applicability of integrity checks on cell cultures

Attributes	Original Source	Early Stocks	Cell Banks	Routine Cultures
Morphology	✓	✓	✓	✓
Viability	✓	✓	✓	✓[a]
Identity	✓	✓	✓	
Doubling time[b]	✓	✓	✓	✓
Mycoplasma	✓	✓	✓	✓
Viruses	✓ (donor only)		✓ (master bank only)	
Bacteria and Fungi			✓	✓[c]
Function/phenotype		✓	✓	✓[d]
Genetic stability			✓	✓[e]
Absence of reprogramming vectors (iPSC[f] lines)		✓	✓	



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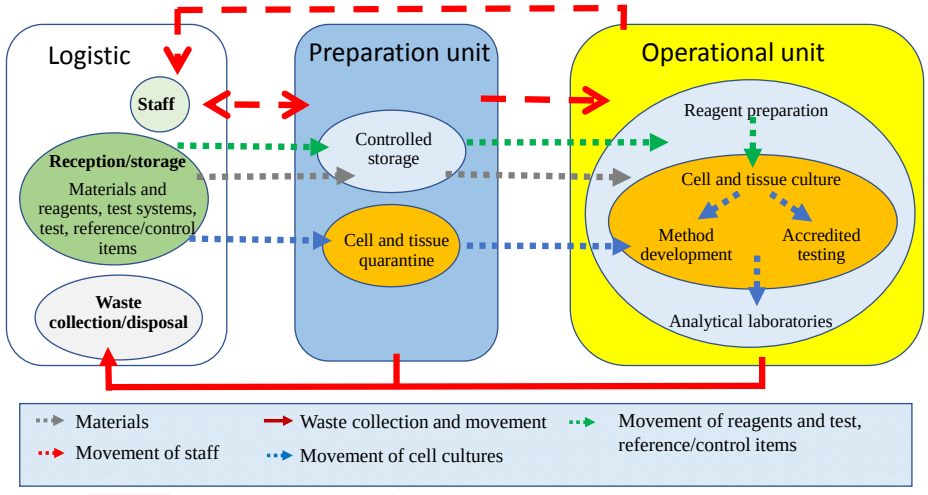
Thyroid Validation Study

3. Facilities

- Recommends fit for purpose facilities and a detailed understanding of the work flow
- Indicates that facility design and safety risk assessment and management should be adequate
- Prescribes strategies to avoid cross-contamination



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Thyroid Validation Study

4. Apparatus, materials and reagents

- Highlights the importance of regular maintenance, calibration, and validation
- Instructs on sourcing of materials and reagents (e.g. from well-established suppliers) to ensure the integrity and reliability
- Discusses the use of media in cell culture, including alternatives to animal-sourced serum

Antibiotics

…may arrest or disrupt fundamental aspects of cell biology, and, while they are effective against prokaryotic cells (i.e. bacteria), they can causing toxic effects in mammalian cells.

Foetal calf serum

Consensus Report ALTEX. 2018;35(1):99-118

Fetal Bovine Serum (FBS):
Past – Present – Future

Jan van der Valk[1], Karen Bieback[2], Christiane Buta[3], Brett Cochrane[4], Wilhelm G. Dirks[5], Jianan Fu[6], James J. Hickman[7], Christiane Hohensee[8], Roman Kolar[9], Manfred Liebsch[10], Francesca Pistollato[11], Markus Schulz[12], Daniel Thieme[13], Tilo Weber[9], Joachim Wiest[14], Stefan Winkler[15] and Gerhard Gstraunthaler[16]

- The use of serum has been discouraged: the undefined nature of the medium
- batch variability
- potential limitation
- availability of supply.

https://fcs-free.org/

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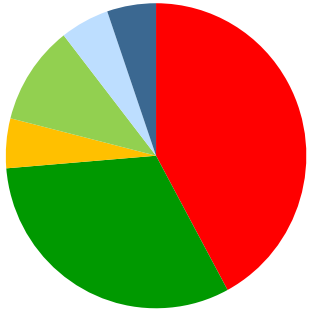
Thyroid Validation Study

5. Test systems

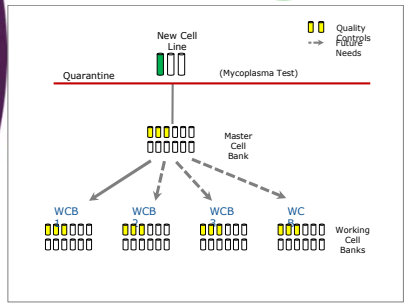
- Elaborates the importance of Good Cell Culture Practice (GCCP)
- Advises the setting of acceptance criteria already at the development stage
- Describes identification and characterisation, sourcing, cell-banking and cryopreservation
- Suggests good practices for contaminants screening, including sterility, mycoplasma, virus testing

19 Test systems



- 8 animal cell lines (5 with human inserts)
- 6 human cell lines
- 1 human primary cells
- 2 proteins
- 1 cellular fraction
- 1 whole organism

*under discussion & negotiation



Methodologies testing
Test system acceptance criteria

Annex 1

ATLA 33, 261–287, 2005 REFERENCE 02 from EURL ECVAM

Guidance on Good Cell Culture Practice

A Report of the Second ECVAM Task Force on Good Cell Culture Practice

Sandra Coecke,[1] Michael Balls,[2] Gerard Bowe,[1] John Davis,[3] Gerhard Gstraunthaler,[4] Thomas Hartung,[1] Robert Hay,[5] Otto-Wilhelm Merten,[6] Anna Price,[1] Leonard Schechtman,[7] Glyn Stacey[8] and William Stokes[9]

Annex 2

ALTEX 34(1), 2017

t4 transatlantic think tank for toxicology

t4 workshop report*

Good Cell Culture Practice for Stem Cells and Stem-Cell-Derived Models

David Pamies[1], Anna Bal-Price[2], Anton Simeonov[3], Danilo Tagle[3], Dave Allen[4], David Gerhold[3], Dechong Yin[5], Francesca Pistollato[2], Takashi Inutsuka[6], Kristie Sullivan[7], Glyn Stacey[8], Harry Salem[9], Marcel Leist[10], Mardas Daneshian[10], Mohan C. Vemuri[11], Richard McFarland[12], Sandra Coecke[2], Suzanne C. Fitzpatrick[12], Uma Lakshmipathy[11], Amanda Mack[13], Wen Bo Wang[13], Daiju Yamazaki[14], Yuko Sekino[14], Yasunari Kanda[14], Lena Smirnova[1] and Thomas Hartung[1,10]

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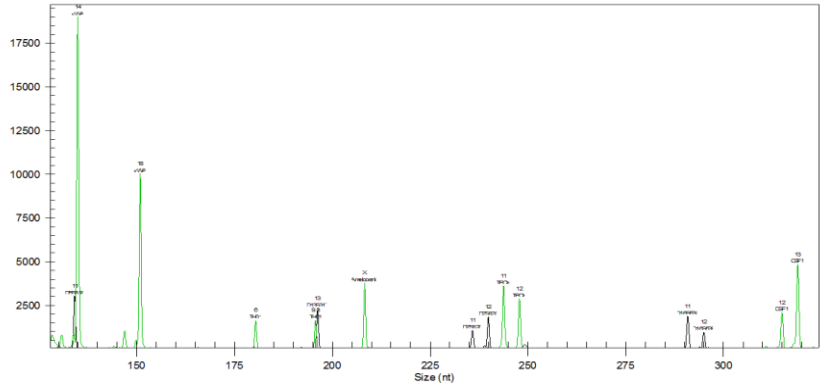
Cell line identity & purity

Authentication of human cell lines with DNA profiling using 8 different and highly polymorphic short tandem repeat (STR) loci.

Human samples also tested for presence of mitochondrial DNA sequences from rodent cells as mouse, rat, Chinese and Syrian hamster. At a detection limit of 1:10^5 mitochondrial sequences from mouse, rat or Chinese and Syrian hamster cells were not detected in the samples.

Identification of animal species with DNA Barcoding of Cytochrome Oxidase subunit 1.



5`- TTTANAGTACACNTATAGTCTCACCCACCCACCAAGAATATCGGTACTTTATACTTACTA
TTGGGAGCATGAGCCGGTATAGTAGGCACTGCCTTGAGCCTCCTCATCCGAGCCGAACTA
GGTCAGCCCGGTACTTTACTAGGTGACGATCAAATTTATAATGTCATCGTAACCGCCCAT
GCTTTCGTAATAATCTTCTTCATAGTCATGCCCATCATAATTGGGGGCTTTGGAAACTGA
CTAGTGCCGTTAATAATTGGTGCTCCGGACATGGCATTCCCCCGAATAAATAACATGAGC
TTCTGACTCCTTCCTCCATCCTTTCTTCTACTATTAGCATCTTCTATGGTAGAAGCAGGT
GCAGGAACGGGATGAACCGTATACCCCCCACTGGCTGGCAATCTGGCCCATGCAGGAGCA
TCCGTTGACCTTACAATTTTCTCCTTACACTTAGCCGGAGTCTCTTCTATTTTAGGGGCA
ATTAATTTCATCACTACTATTATCAACATAAAACCCCCTGCAATATCCCAGTATCAAACT
CCCCTGTTTGTATGATCAGTACTAATTACAGCAGTTCTACTCTTACTATCCCTGCCTGTA
CTGGCTGCTGGAATTACAATACTTTTAACAGACCGGAATCTTAATACAACATTTTTTGAT
CCCGCTGGAGGAGGAGACCCAATCCTATATCAACACCTATTCTGATTCTTCGGCCAGCCA
AGAGTA-3`

Example of results; STR profile of a human cell line (left) and DNA sequence of an animal cell line (right)

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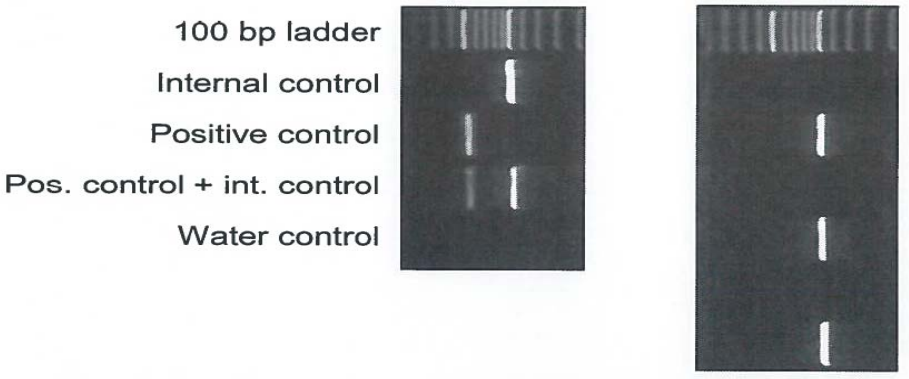
Sterility

- Culturing without antibiotics + microscopic detection of bacteria, fungi, yeast
- Mycoplasma test (PCR and broth agar)
- Presence of human pathogenic viruses (PCR)



The following viruses are checked in rodent cell lines

- Retroviruses (with RT-PCR and ELISA)

The following viruses are checked in human cell lines

- Human Immunodeficiency Virus types 1 and 2
- Hepatitis B and C Viruses
- Human Papilloma Virus
- Xenotropic murine leukemia virus

Example of results; Mycoplasma PCR (left) and microscope image (right)

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Thyroid Validation Study

	sample	parental / reference line	comment / match
1b	CHO-R, JP09	Puck et al, 1958	COI Barcoding analysis revealed Cricetulus barabensis species, species-specific
5a	MDCK1-MCT8	Gaush et al., 1966	COI Barcoding analysis revealed Canis lupus species, species-specific
5a	MDCK1-pcDNA	Gaush et al., 1966	COI Barcoding analysis revealed Canis lupus species, species-specific
6b	TRβ-CALUX	U-2-OS (DSMZ ACC 785)	full-matching STR profile of cell line U-2-OS in the reference database, authentic

Thyroid Validation Study

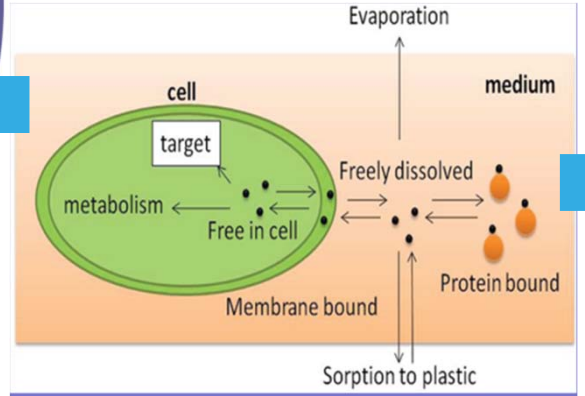
6. Test and reference/control items

- Illustrates the preparation and characterisation of test, reference and control items
- Defines the applicability and limitations of the method
- Recommends to identify potential sources of interference with the test system and/or method endpoint

Comparison between solubility determination methods

Method	Limitations	Specificity	Cut off	Rapidity
Nephelometry (Light scatter)	• Sticky precipitates • Impurities	Low	No	High
UV/VIS 1 (Absorbance)	• Compound must have chromophore • Sticky precipitates • Impurities	Low	<500 nm	High
UV/VIS 1* (Filtration + Absorbance)	• Compound must have chromophore • Sticky precipitates • Impurities • Loss due to filter absorption	Medium	<250 nm	Medium
HPLC-UV*^	• Sticky precipitates	High	No	Low
LC-MS*^	• Sticky precipitates	High	No	Low

** Requires Calibration* *^ High Cost*



Representation of some processes that can cause the final target concentration to be different than the nominal concentration in an in vitro test (Kramer et al., 2012)

6. Test and reference/control items

- 6.1 Reference and control items
- 6.3 Test item preparation
- 6.4 Concentration range
- 6.5 Solubility
- 6.6 Stability
- 6.7 Solvents

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7. Standard operating procedures

- Provides guidance on the development and preparation of *in vitro* method standard operating procedures (SOPs) to ensure consistency and reproducibility of data acquired
- Describes the evolution of a SOP from initial method description to method optimisation and validation

Thyroid Validation Study

Evolution of a Standard Operating Procedure (SOP)

No SOP

Limited reproducibility (operator dependant)

Method optimisation

Describe the in vitro method procedure

Method optimised

Historical data of reference items are generated in a controlled way

SOP Version 01

Advanced in vitro method description
Acceptance criteria for valid/invalid experiments
Lists of needed equipment, reagents, consumables and reference items
Calculation of results

SOP Version xx

Further optimised procedure
Acceptance criteria for valid/invalid experiments
Calculation of results for test items
Data recording Forms, Data Calculation forms
SOP is robust

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Thyroid Validation Study

8. Performance of the method

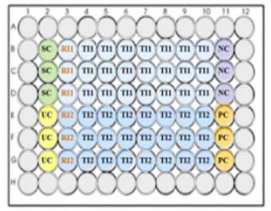
- Analyses development of acceptance criteria for components (e.g..positive and negative controls)
- Reviews the elements of experimental design such as plate layout, the number of replicates, outlier detectin, data analysis
- Examines how to determine the performance of the method, including the assessment of linearity, range, accuracy

Performance of the method

8.1 Acceptance criteria

8.2 Experimental design

- 8.2.1 Plate layout
- 8.2.2 Data analysis
- 8.2.3 Outlier detection and removal
- 8.2.4 Non-monotonic dose and U-shaped curves

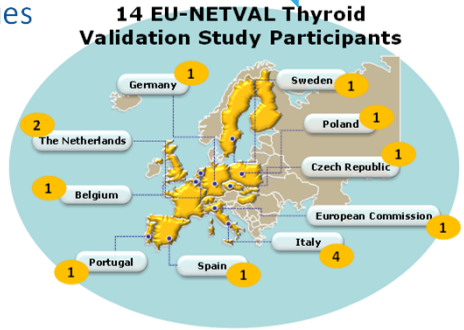


8.3 In-house validation of the measurement endpoint(s)

- 8.3.1 Detection Limits and Cut-off values
- 8.3.2 Linearity and dynamic range
- 8.3.3 Accuracy and precision
- 8.3.4 Sensitivity and specificity
- 8.3.5 Repeatability

8.4 Proficiency chemicals

8.5 Data-intensive *in vitro* methods



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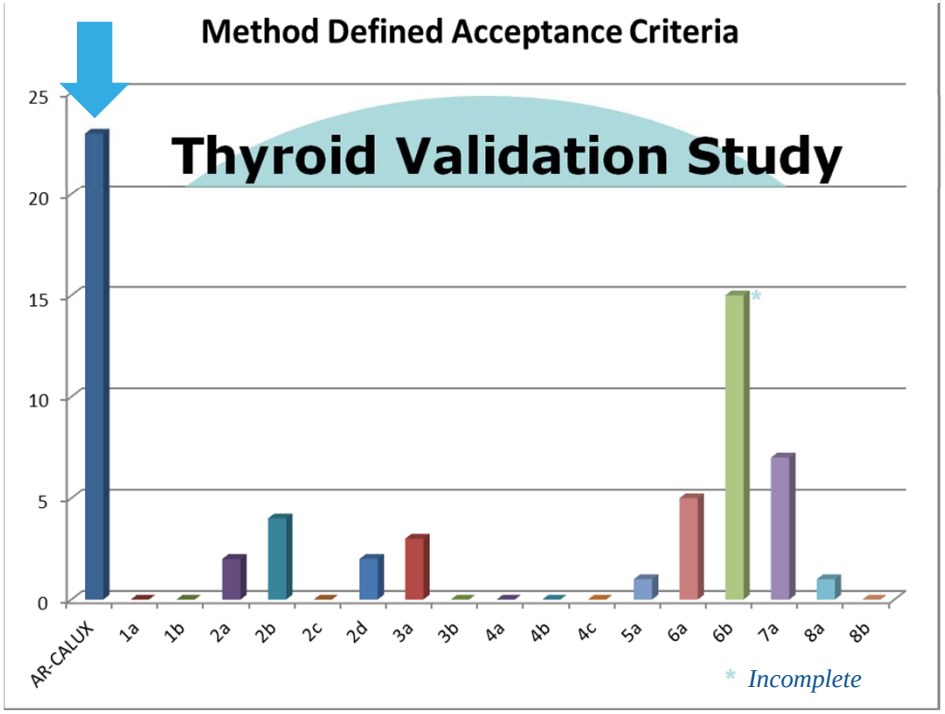
1st EU-NETVAL validation study AR-CALUX Method: QC parameters

Towards a Test Guideline for Androgen Receptor Transactivation Assays (ARTA)

Target: WNT review June 2019 / adoption April 2020



- Thawing -> 75% confluence after 72 +/- 4 hours of incubation
- Passage number > 2 and < 30
- Freedom of contamination
- Incubation time: 16 to 32 hours
- 50% and 90% confluent in all the wells
- Test Items must be soluble by visual inspection
- EC50 range reference item DHT (1×E-10 –1×E-9 M)
- CV of estimated log (EC50) reference item < 1.5%
- Relative induction (%) PC 17α-Methyltestosterone > 30%
- Relative induction (%) NC Corticosterone < 10%
- Minimum fold induction of the highest RI conc. > 20
- Z-factor for DHT data > 0.5
- Maximum 2 concentrations may be excluded from RI curve
- Reference curve 6/8 concentrations acceptable (conditions)
- Max 2 concentrations may be excluded from test item series
- Lowest test item concentration should be at solvent control level
- IC50 range reference item 1×10-7 – 1×10-6 M
- CV of estimated log(IC50) reference item Flutamide < 3%
- Relative induction (%) PC < 60 %
- Relative induction (%) NC > 85 %
- Minimum fold inhibition of the highest RI concentration (C8) > 10
- Z-factor for Flutamide data > 0.5
- Minimum six valid triplicate samples

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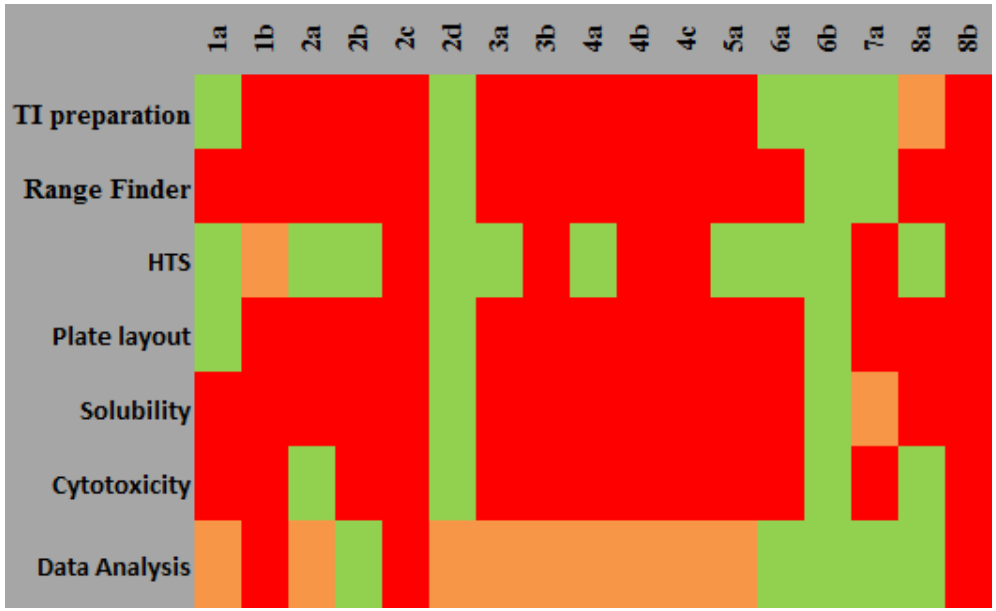
Thyroid Validation Study

Initial assessment of method procedures
(Completeness based on EURL ECVAM's experience)



Once the development and optimisation of the method in the developers' laboratory has been finalised it is recommended to perform an in-house validation of the method prior to routine use.

***In vitro* method development** and **in-house validation** should be considered as continuous and inter-dependent.

Major required elements to subject to in-house validation:

1. Aspects of test system
2. and *in vitro* method details

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Thyroid Validation Study

9. Reporting of results

- Gives guidance on reporting of data for regulatory purposes including the OECD Mutual Acceptance of Data (MAD)
- Recommends publishing of scientific data to promote more transparency and openness
- Reporting of method validation is also discussed

Data sharing in public repositories is encouraged

Publication of method procedures in on-line repositories

Major required elements to be reported:

1. Aspects of test system
2. and *in vitro* method details

Transparency

The Transparency and Openness Promotion (TOP) guidelines https://osf.io/ud578/

The Journal of Negative Results in BioMedicine
https://jnrbm.biomedcentral.com/
Etc.

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GIVIMP OECD Guidance Document on Good *In Vitro* Method Practices

Data sharing

- Public repositories – guarantee data integrity and access
- Electronic data – format critical for future retrieval

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GIVIMP OECD Guidance Document on Good *In Vitro* Method Practices

Major required elements to be reported:

1. Aspects of test system
2. and *in vitro* method details allowing QC

Annex 1

ATLA 33, 261–287, 2005 REFERENCE 02 from EURL ECVAM

Guidance on Good Cell Culture Practice

A Report of the Second ECVAM Task Force on Good Cell Culture Practice

Sandra Coecke,[1] Michael Balls,[2] Gerard Bowe,[1] John Davis,[3] Gerhard Gstraunthaler,[4] Thomas Hartung,[1] Robert Hay,[5] Otto-Wilhelm Merten,[6] Anna Price,[1] Leonard Schechtman,[7] Glyn Stacey[8] and William Stokes[9]

Table 5: Details to be included in papers for publication in journals, using an example of primary/early passage human cell culture

	Details	Supplier details
Type of culture	Primary cell culture	na
Cell/tissue type	Keratinocyte	na
Species	Human	na
Origin	Foreskin	QMC Hospital Trust, Nottingham, UK
Ethical permission	Required	Ethics Committee, QMC Hospital Trust
Supply to other users	Not permitted	
Transport solution	Phosphate-buffered saline	Gibco, Paisley, Scotland
Basic culture medium	Epi-Life® Medium	Cascade Biologics, Mansfield, Notts., UK
Serum	None	na
Antibiotics	100U/ml penicillin, 100µg/ml streptomycin	Gibco
Other additives	HKGS Kit (5-001 5) Calcium chloride	Cascade Biologics In-house
Complete medium	No further comment	na
Frequency of medium change	Every 2 days and at subculture	na
Culture flasks for establishing cultures	24cm^2 tissue culture flasks (163371)	Nunclon, Roskilde, Denmark, or Scientific Laboratory Supplies, Nottingham, UK
Inserts	Not used	na
Surface coating	Not used	na
Subculture	When 50–80% confluent (not when 100% confluent)	na
Subculture split ratio	1:5 or 1:10	na
Detachment solution	0.25% trypsin/EDTA (R-001-100) with trypsin-neutralising solution (R002-100)	Cambrex Bio Science, Wokingham, Berkshire, UK
Usable passage range	1–4	na
Maintenance conditions	37°C, 5% CO_2 in air	na
Storage conditions	Stock cells in liquid nitrogen, in 90% fetal calf serum/10% DMSO	na
Passage number at use	3	na
Culture plates for use	96-well plates (167008)	Nunclon
Use	3T3-NRU phototoxicity test	na
Relevant Standard Operating Procedures/guidelines	OECD TG 427, EU B.29	na
References	14, 15	na
Further comments	None	na

na = not applicable.

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1st EU-NETVAL validation study AR-CALUX Method: QC parameters

Towards a Test Guideline for Androgen Receptor Transactivation Assays (ARTA)

Target: WNT review June 2019 / adoption April 2020

Major required elements to be reported:

1. Aspects of test system
2. and *in vitro* method details

It might beneficial to report some of these criteria….

- Thawing -> 75% confluence after 72 +/- 4 hours of incubation
- Passage number > 2 and < 30
- Freedom of contamination
- Incubation time: 16 to 32 hours
- 50% and 90% confluent in all the wells
- Test Items must be soluble by visual inspection
- EC50 range reference item DHT (1×E-10 –1×E-9 M)
- CV of estimated log (EC50) reference item < 1.5%
- Relative induction (%) PC 17α-Methyltestosterone > 30%
- Relative induction (%) NC Corticosterone < 10%
- Minimum fold induction of the highest RI conc. > 20
- Z-factor for DHT data > 0.5
- Maximum 2 concentrations may be excluded from RI curve
- Reference curve 6/8 concentrations acceptable (conditions)
- Max 2 concentrations may be excluded from test item series
- Lowest test item concentration should be at solvent control level
- IC50 range reference item 1×10-7 – 1×10-6 M
- CV of estimated log(IC50) reference item Flutamide < 3%
- Relative induction (%) PC < 60 %
- Relative induction (%) NC > 85 %
- Minimum fold inhibition of the highest RI concentration (C8) > 10
- Z-factor for Flutamide data > 0.5
- Minimum six valid triplicate samples

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Major required elements to be reported for mechanistic methods and the related study results

1. Aspects of test system ?
2. and *in vitro* method details ?

Thyroid Validation Study

4. Metabolism and excretion

4a. Deiodinase 1 activity based on Sandell-Kolthoff reaction

4b. Glucuronidation of thyroid hormones (THs) using liquid chromatography/mass spectrometry (LC/MS)

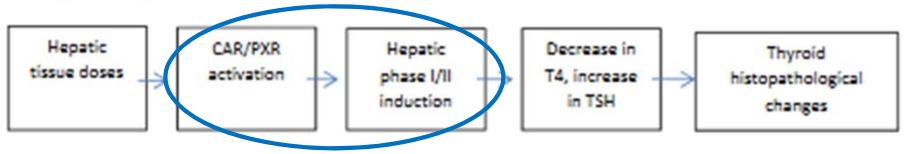
4c. Inhibition of thyroid hormones (THs) sulfation using liquid chromatography

Appendix A – Additional considerations on how to assess the potential for thyroid disruption for human health

2. Comparative studies of enzyme activity induced by the test substance in liver *in vitro* systems should be measured in both the relevant test species (e.g. rat, mouse and dog) and humans. The metabolism of the specific substance (ADME properties) in both test species and humans, and the activity of possible metabolites must be considered when this comparison is conducted.

3. The presence of other possible thyroid-disrupting modes of action such as interference with TH synthesis should also be excluded, e.g. by evaluating *in vitro* the potential for inhibition of the sodium-iodide symporter (NIS) (Cianchetta et al., 2010; Hallinger et al., 2010; Kogai et al., 2012) and thyroid peroxidase (TPO) (Kambe et al., 1997; Paul et al., 2014; Paul Friedman et al., 2016; Wu et al., 2016). It must however be acknowledged that substances may interfere with the thyroid hormone system through many different mechanisms of action, and that currently validated/standardized *in vitro* assays do not exist to investigate all these different pathways and a reasonable effort is anticipated, based on available tools and current understanding of thyroid physiology.

An example of a postulated mode of action is reported below:



The assessment of qualitative/quantitative differences in hepatic induction can therefore be part of the WoE and used to provide evidence of non-human relevance.

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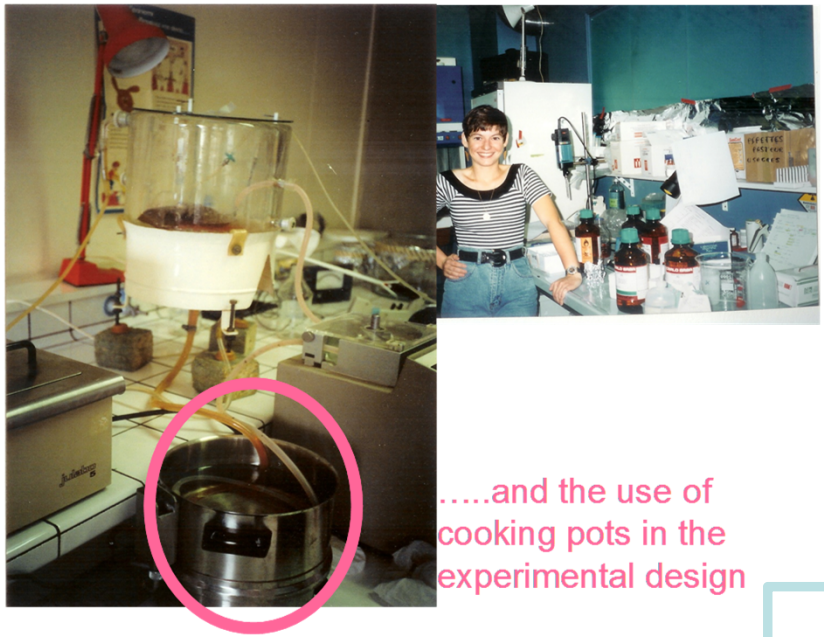
…….and the age of liver perfusions and metabolism



…..and the use of cooking pots in the experimental design

Human liver perfusion, Marseille, April 1992

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EURL ECVAM European Union Reference Laboratory for Alternatives to Animal Testing

In vitro metabolism (CYP) induction method ring trial validation study using cryo pHH (3 labs) and cryoHepaRG (3 labs)

incl. aspects of harmonisation

MiniReview

Basic & Clinical Pharmacology & Toxicology, 2018, 123, 42–50

Cytochrome P450 Induction and Xeno-Sensing Receptors Pregnane X Receptor, Constitutive Androstane Receptor, Aryl Hydrocarbon Receptor and Peroxisome Proliferator-Activated Receptor α at the Crossroads of Toxicokinetics and Toxicodynamics

Jukka Hakkola[1,2], Camilla Bernasconi[3], Sandra Coecke[3], Lysiane Richert[4], Tommy B. Andersson[5,6] and Olavi Pelkonen[1,2]

[1]Research Unit of Biomedicine, Pharmacology and Toxicology, Faculty of Medicine, University of Oulu, Oulu, Finland, [2]Medical Research Center Oulu, University of Oulu, Oulu, Finland, [3]European Commission Joint Research Centre, EURL ECVAM, Ispra, Italy, [4]Kaly-Cell, Plobsheim, France, [5]Drug Metabolism and Pharmacokinetics, Cardiovascular and Metabolic Diseases, IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden and [6]Department of Physiology and Pharmacology, Section of Pharmacogenetics, Karolinska Institutet, Stockholm, Sweden

https://tsar.jrc.ec.europa.eu/search-test-methods-a?search_combined_anonymous=cyp+induction

2018 Reproducibility

Within and between lab reproducibility for both methods

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Toxicology in Vitro
Volume 53, December 2018, Pages 233-244

Establishing a systematic framework to characterise *in vitro* methods for human hepatic metabolic clearance

Varvara Gouliarmou [a, 1], Alfonso Maria Lostia [a, 1], Sandra Coecke [a], Camilla Bernasconi [a], Jos Bessems [a, 2], Jean Lou Dorne [b], Stephen Ferguson [c], Emanuela Testai [d], Ursula Gundert Remy [e], J. Brian Houston [f], Mario Monshouwer [g], Andy Nong [h], Olavi Pelkonen [i], Siegfried Morath [a], Barbara A. Wetmore [j], Andrew Worth [a], Ugo Zanelli [k], Maria Chiara Zorzoli [a], Maurice Whelan [a]

EURL ECVAM European Union Reference Laboratory for Alternatives to Animal Testing

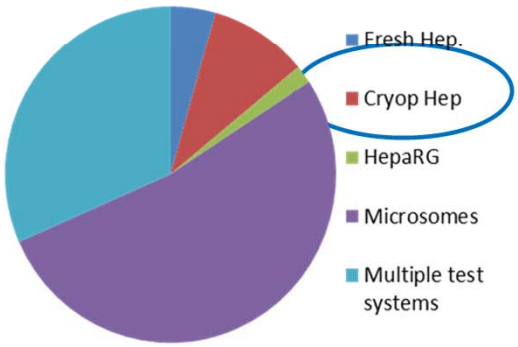
2018 Issues on Within and Between Laboratory Reproducibility

2015 - Literature search and call for clearance methods

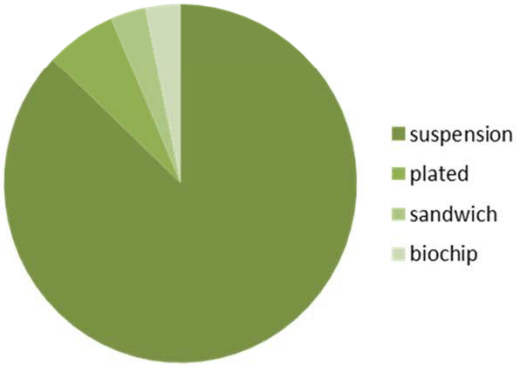
Searching criteria: human based clearance methods and published 1998-2014

Inclusion of **115** published studies

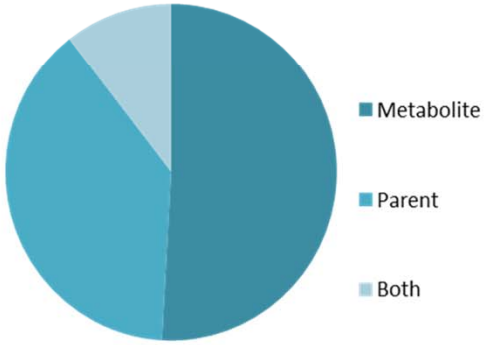
Test system



Test system configuration



Measured parameter



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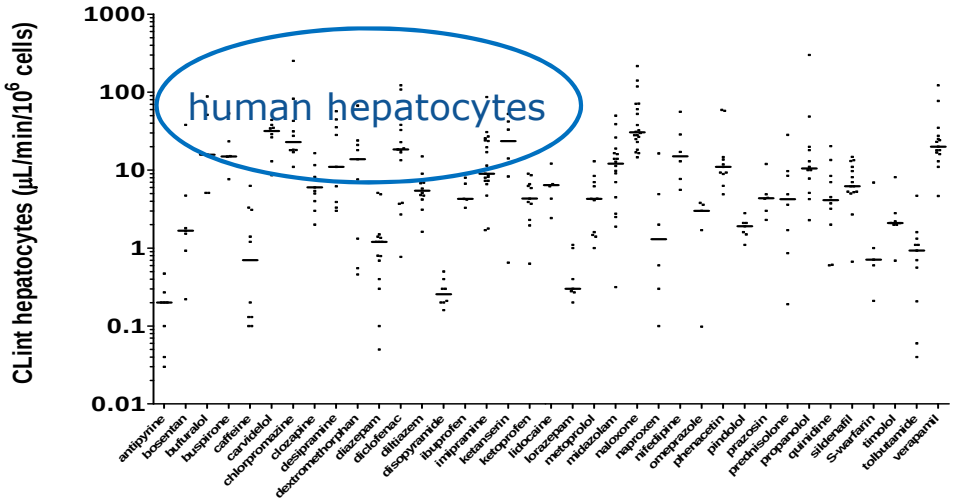
Metabolism 2018 Lead NL: OECD 4.132 Feasibility study TG development

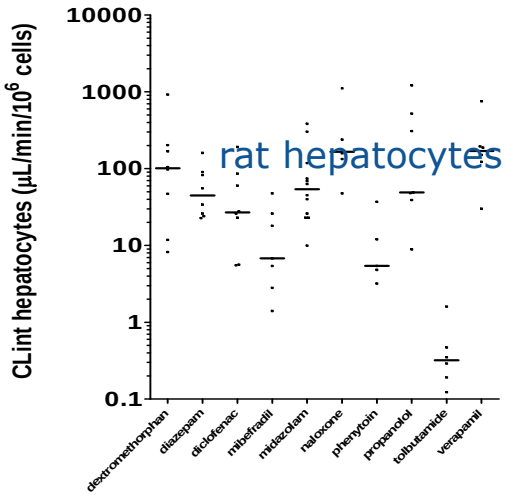
Results 2018 literature analysis:

- *Human data on 37 chemicals from 30 publications*
- *Rat data on 10 chemicals from 15 publications*
- *Large variation in protocols observed*
- *Limited information on within-laboratory variation*
- *Large between-laboratory variation (partly human variability)*

A framework to characterize in vitro hepatic metabolism across species for regulatory applications, Sandra Coecke*, Camilla Bernasconi, Alfonso Lostia, Alicia Paini, Sharon Munn, Olavi Pelkonen, Tommy B. Andersson, ,* Minne Heringa, Jochem Louise, Ans Punt, Betty Hackert *et al.*

Presentation Workshop on "In vitro comparative metabolism studies in regulatory pesticide risk assessment" , EFSA, 15-16 November 2018, Parma, Italy





Reproducibility

Too few data to assess within laboratory reproducibility

Too large variation (%CV) between labs → non-harmonised protocols

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GIVIMP OECD Guidance Document on Good *In Vitro* Method Practices

Major required elements to be reported for mechanistic methods and the related study results????

Reporting aspects of **test system** and ***in vitro* method details (protocols)** and **acceptance criteria** allowing to identify those methodological aspects that are likely to affect the study values obtained -> required step for international harmonisation of *in vitro* mechanistic study data

Metabolism 2018 Lead NL: OECD 4.132 Feasibility study TG development

OECD BETTER POLICIES FOR BETTER LIVES

Examples of some test system and *in vitro* method details		
Parameters	Protocol EC JRC EURL ECVAM	Harmonised
Human CryoHepatocytes	primary	Yes
Source	cryopreserved (pooled 10 donors)	No
Culture condition	in suspension	Yes
Culture material	96-well plate	No
Medium	WME, 15 mM HEPES, 2 mM L-glutamine, Insulin, Transferrin, Selenium (ITS)	Yes/No
Concentration substrate	1 µM	No
Solvent	0.01% DMSO	No
Incubation time	0, 15, 30, 60, 90, 120 min	No
Use of CO2 incubator	yes	No
Medium movement	yes, shaking	Yes
Sampling	medium + cells	Yes
Cell concentration	$0.5 \cdot 10^6$ cells/mL (or $1.0 \cdot 10^6$ cells/mL ?)	No
Incubation volume	0.1 mL	No
Stop reaction	add equal part solvent (ACN) with internal standard (70 µL sample + 70 µL solvent)	No

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Applying GIVIMP during the development of in vitro methods is one of the tools used to improve the quality and reliability of in vitro methods and their resulting data contributing to a credibility revolution of scientific results

The quality and relevance of mechanistic in vitro method study data generated are related to the methodological aspects

Reporting test system aspects and the full in vitro method (incl. acceptance criteria) allows for detailed systematic reviews understanding variability in the mechanistic study data when evaluating their validity

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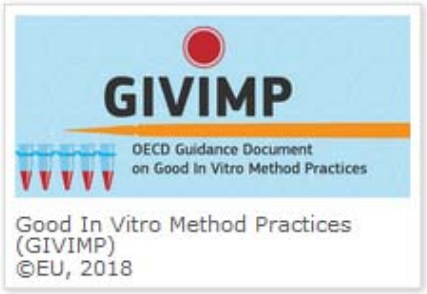


Download GIVIMP here

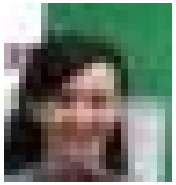
Available on OECD e-Library https://doi.org/10.1787/20777876

Also available on the
OECD Series for Testing and Assessment No. 286

Collaboration = faster progress

Thyroid team

EC JRC Chemicals Safety and Alternative Methods hosting EURL ECVAM



OECD team

EURL ECVAM first GIVIMP writing team

EU-NETVAL meeting participants

DG ENV

OECD GIVIMP expert group

et al.

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