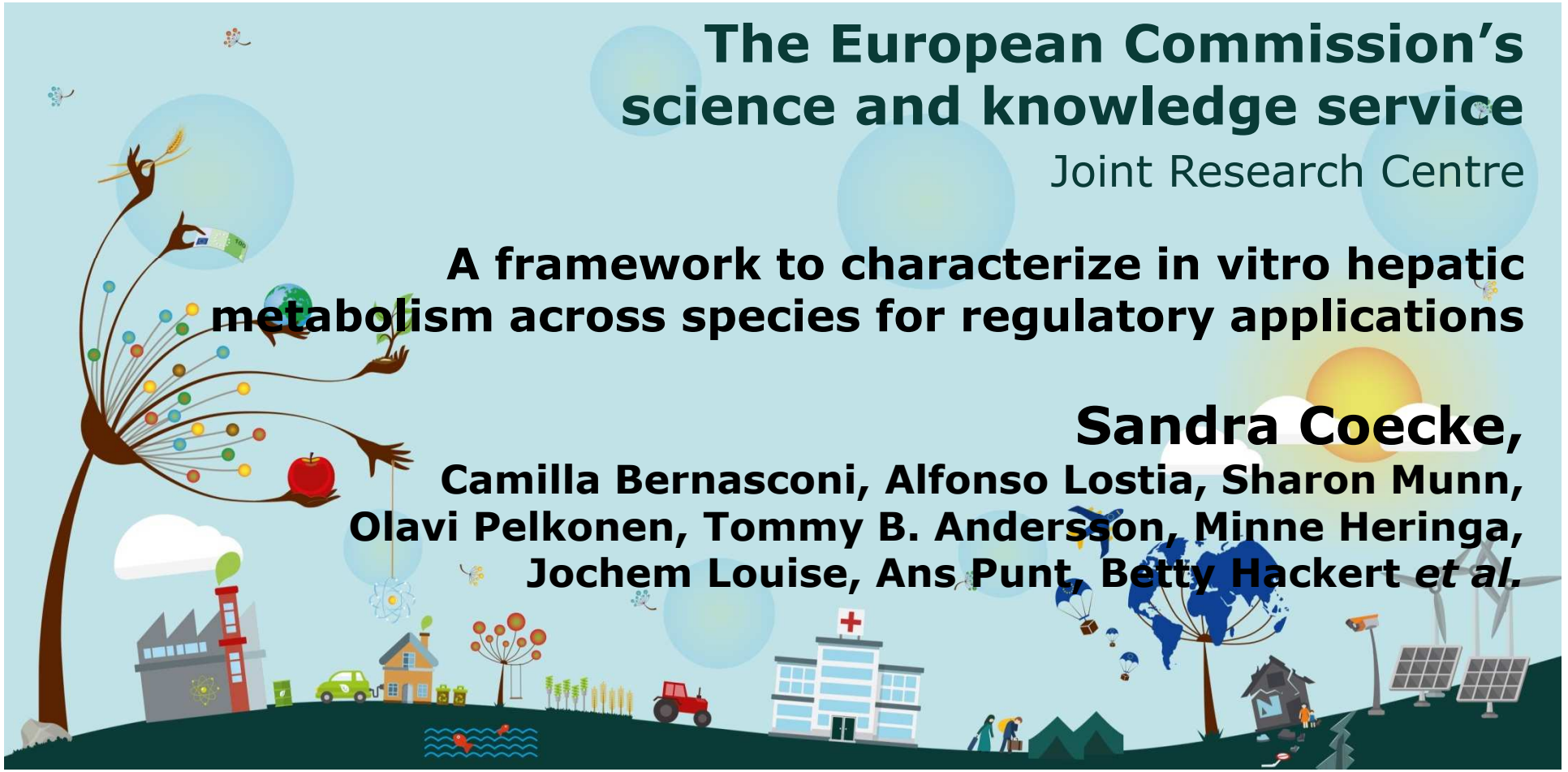


Workshop on "In vitro comparative metabolism studies in regulatory pesticide risk assessment"
EFSA, 15-16 November 2018, Parma, Italy

European Commission

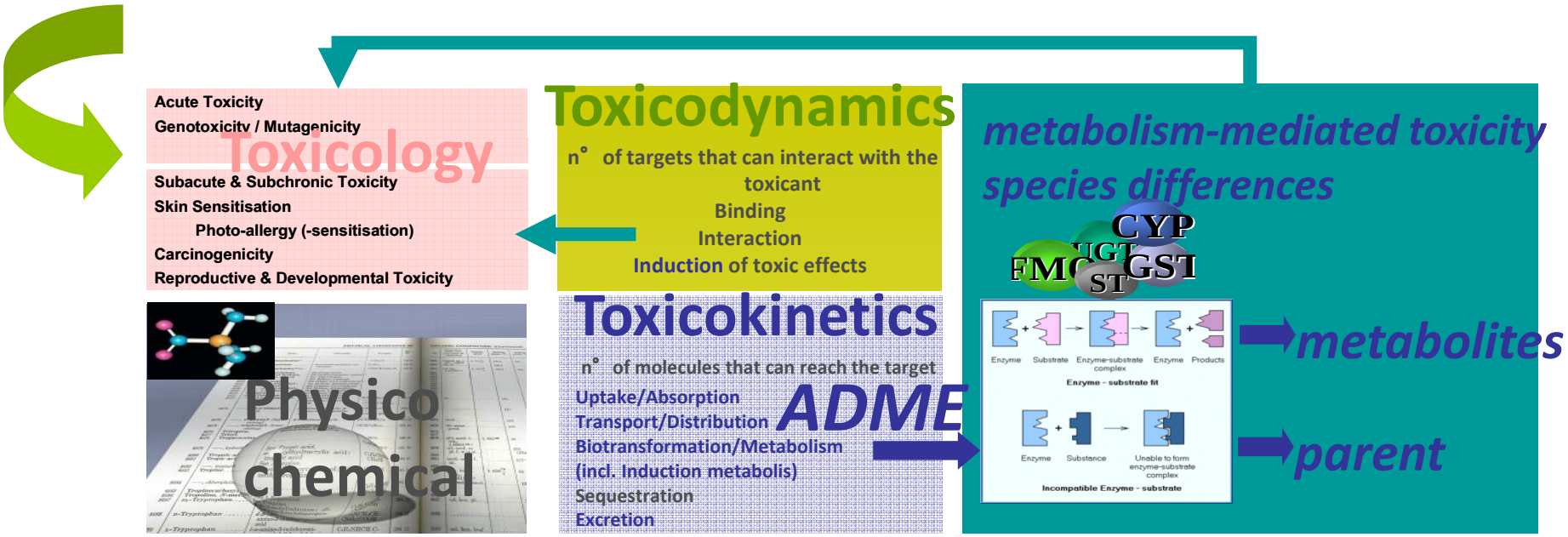
Overview

- Introduction: Metabolism/AD**M**E as essential parts of IATA
- Two decades of *in vitro* methods for test development and validation for regulatory purposes: ADME *in vitro* methods
- Framework and activities to characterise *in vitro* metabolism methods (including species differences)
- An example of standardisation of *in vitro* metabolism methods: CYP induction
- Current regulatory needs for *in vitro* metabolism methods

Introduction

Metabolism/ADME as essential parts of IATA

ADME systems as essential parts of IATA's for systemic toxicity



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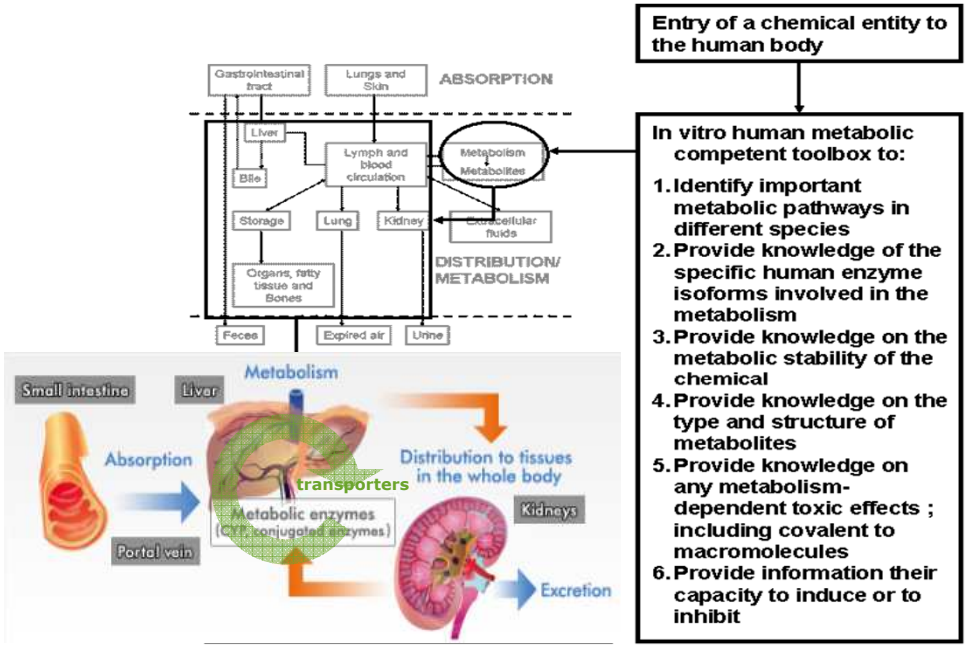
ADME / Metabolism as key components of IATA

In vitro ADME platform for assessing metabolism and toxicity



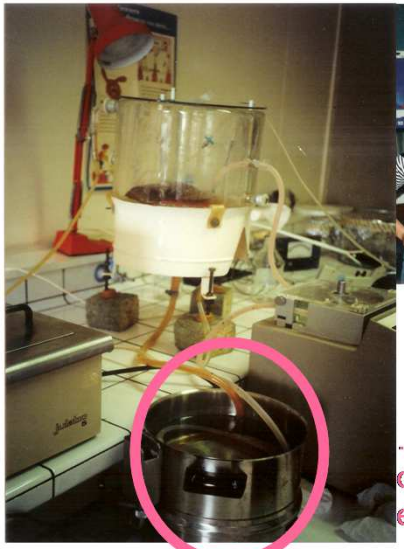
Metabolism/Biotransformation: need for reliable, relevant, easily accessible human metabolic competent test systems

PBTK is currently regarded as the most adequate approach to simulate the fate of compounds in the human body (1R)

Two decades of *in vitro* methods for test development and validation for regulatory purposes:

ADME *in vitro* methods

…….and the age of liver perfusions and metabolism



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

Human liver perfusion, Marseille, April 1992

Metabolism

dynamics

kinetics

ATLA 22, 231-241, 1994 231

The Practical Applicability of Hepatocyte Cultures in Routine Testing

The Report and Recommendations of ECVAM Workshop 1 [1,2]

Bas J. Blaauboer[3], Alan R. Boobis[4], Jose V. Castell[5], Sandra Coecke[6], Geny M.M. Groothuis[7], Andre Guillouzo[8], Tony J. Hall[9], Gabrielle M. Hawksworth[10], Giocondo Lorenzon[11], Herbert G. Miltenburger[12], Vera Rogiers[13], Paul Skett[14], Pia Villa[15] and Friedrich J. Wiebel[16]

ATLA 27, 579–638, 1999 579

The Use of Long-term Hepatocyte Cultures for Detecting Induction of Drug Metabolising Enzymes: The Current Status

ECVAM Hepatocytes and Metabolically Competent Systems Task Force Report 1

Sandra Coecke,[1] Vera Rogiers,[2] Martin Bayliss,[3] José Castell,[4] Johannes Doehmer,[5] Gérard Fabre,[6] Jeffrey Fry,[7] Armin Kern[8] and Carl Westmoreland[9]

ATLA 34, 49–84, 2006

Metabolism: A Bottleneck in *In Vitro* Toxicological Test Development

The Report and Recommendations of ECVAM Workshop 54[1]

Sandra Coecke,[1] Hans Ahr,[2] Bas J. Blaauboer,[3] Susanne Bremer,[1] Silvia Casati,[1] José Castell,[4] Robert Combes,[5] Raffaella Corvi,[1] Charles L. Crespi,[6] Michael L. Cunningham,[7] Greetje Elaut,[8] Brighitta Eletti,[1] Andreas Freidig,[9] Alessandra Gennari,[1] Jean-François Ghersi-Egea,[10] Andre Guillouzo,[11] Thomas Hartung,[1] Peter Hoet,[12] Magnus Ingelman-Sundberg,[13] Sharon Munn,[14] Walter Janssens,[15] Bernhard Ladstetter,[16] David Leahy,[17] Anthony Long,[18] Annarita Meneguz,[19] Mario Monshouwer,[20] Siegfried Morath,[21] Fred Nagelkerke,[22] Olavi Pelkonen,[23] Jessica Ponti,[1] Pilar Prieto,[1] Lysianne Richart,[24] Enrico Sabbioni,[1] Beatrice Schaack,[25] Winfried Steiling,[26] Emanuela Testai,[27] Joan-Albert Vericat[28] and Andrew Worth[14]

1992 **1994** **1997** **1999..** **2002** **2005** **2006** **2007**

ATLA 25, 17–31, 1997 17

Pharmacokinetics in Early Drug Research

The Report and Recommendations of ECVAM Workshop 22[1,2]

David E. Leahy,[3] Ruth Duncan,[4] Hans J. Ahr,[5] Martin K. Bayliss,[6] A. (Bert) G. de Boer,[7] Ferenc Darvas,[8] Julia H. Fentem,[9] Jeffrey R. Fry,[10] Robert Hopkins,[11] J. Brian Houston,[12] Johan Karlsson,[13] Gregory L. Kedderis,[14] Margaret K. Pratten,[15] Pilar Prieto,[3] Dennis A. Smith[16] and Donald W. Straughan[17]

ATLA – Altern. Lab. Animal. 33, 147-175, 2005.

Toxicokinetics and metabolism.

A report prepared in the context of the 7th Amendment of the Cosmetics Directive for establishing a timetable for phasing out animal testing

Coecke S, Blaauboer BJ, Elaut G, Freeman S, Freidig A, Gennaandel N, Hoet P, Kapoulas VM, Ladstetter B, Langley G, Leahy D, Mannens G, Meneguz A, Monshouwer M, Nemery B, Pelkonen O, Pfaller W, Prieto P, Proctor N, Rogiers V, Rostami-Hodjegan A, Sabbioni E, Steiling W, van de Sandt JJ.).

ATLA 35, 603–671, 2007 601

Physiologically-based Kinetic Modelling (PBK Modelling): Meeting the 3Rs Agenda

The Report and Recommendations of ECVAM Workshop 63[a]

Michel Bouvier d'Yvoire,[1] Pilar Prieto,[1] Bas J. Blaauboer,[2] Frederic Y. Bois,[3] Alan Boobis,[4] Céline Brochot,[3] Sandra Coecke,[1] Andreas Freidig,[5] Ursula Gundert-Remy,[6] Thomas Hartung,[1] Miriam N. Jacobs,[1] Thierry Lavé,[7] David E. Leahy,[8] Hans Lennernäs,[9] George D. Loizou,[10] Bette Meek,[11] Camilla Pease,[12] Malcolm Rowland,[13] Martin Spendiff,[10] Jiansong Yang[14] and Marco Zeilmaker[15]

European Commission

Metabolism

dynamics

kinetics

new EU Cosmetics Regulation (EC 1223/2009)

2010 ECVAM DG Sanco

Arch Toxicol (2011) 85:367–485

Alternative (non-animal) methods for cosmetics testing: current status and future prospects—2010

Sarah Adler · David Basketter · Stuart Creton · Olavi Pelkonen · Jan van Benthem · Valérie Zuang · Klaus Ejner Andersen · Alexandre Angers-Loustau · Aynur Aptula · Anna Bal-Price · Emilio Benfenati · Ulrike Bernauer · Jos Bessems · Frederic Y. Bois · Alan Boobis · Esther Brandon · Susanne Bremer · Thomas Broschard · Silvia Casati · Sandra Coecke · Raffaella Corvi · Mark Cronin · George Daston · Wolfgang Dekant · Susan Felter · Elise Grignard · Ursula Gundert-Remy · Tuula Heinonen · Ian Kimber · Jos Kleinjans · Hannu Komulainen · Reinhard Kreiling · Joachim Kreysa · Sofia Batista Leite · George Loizou · Gavin Maxwell · Paolo Mazzatorta · Sharon Munn · Stefan Pfuhler · Pascal Phrakonkham · Aldert Piersma · Albrecht Poth · Pilar Prieto · Guillermo Repetto · Vera Rogiers · Greet Schoeters · Michael Schwarz · Rositsa Serafimova · Hanna Tähti · Emanuela Testai · Joost van Delft · Henk van Loveren · Mathieu Vinken · Andrew Worth · José-Manuel Zaldivar

Volume 130, Issue 1
November 2012

Three-Dimensional HepaRG Model As An Attractive Tool for Toxicity Testing

Sofia B. Leite, Iwona Wilk-Zasadna, Jose M. Zaldivar, Elodie Airola, Marcos A. Reis-Fernandes, Milena Mennecozzi, Christiane Guguen-Guillouzo, Christopher Chesne, Claude Guillou, Paula M. Alves ... Show more

Toxicological Sciences, Volume 130, Issue 1, 1 November 2012, Pages 106–116, https://doi.org/10.1093/toxsci/kfs232

Published: 27 July 2012 **Article history**

Protocols in In Vitro Hepatocyte Research pp 143-159 | Cite as 2015

Differentiation-Promoting Medium Additives for Hepatocyte Cultivation and Cryopreservation

Authors Authors and affiliations

Varvara Gouliarmou, Olavi Pelkonen, Sandra Coecke

2007…2009 2011 2012 2013 2014 2015 2018 2019??

Archives of Toxicology
March 2012, Volume 86, Issue 3, pp 393–40 | Cite as

Screening of chemicals for human bioaccumulative potential with a physiologically based toxicokinetic model

Authors Authors and affiliations

Arnaud Tonnelier, Sandra Coecke, José-Manuel Zaldivar

ALTEX 2009

Comparison of Metabolic Stability and Metabolite Identification of 55 ECVAM/ ICCVAM Validation Compounds between Human and Rat Liver Homogenates and Microsomes – a preliminary Analysis*

Olavi Pelkonen[1], Ari Tolonen[2], Timo Rousu[2], Larissa Tursas[1], Mia Turpeinen[1], Juha Hokkanen[2], Jouko Uusitalo[2], Michel Bouvier d'Yvoire[3] and Sandra Coecke[3]

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Toxicokinetics as a key to the integrated toxicity risk assessment based primarily on non-animal approaches

Sandra Coecke [a], Olavi Pelkonen [b,*], Sofia Batista Leite [a,c], Ulrike Bernauer [d], Jos GM Bessems [e], Frederic Y. Bois [f], Ursula Gundert-Remy [g], George Loizou [h], Emanuela Testai [i], José-Manuel Zaldivar [j]

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

Review

Report from the EPAA workshop: *In vitro* ADME in safety testing used by EPAA industry sectors

K. Schroeder [a,d], K.D. Bremm [b,*], N. Alépée [c], J.G.M. Bessems [d], B. Blaauboer [e], S.N. Boehn [f], C. Burek [g], S. Coecke [h], L. Gombau [i], N.J. Hewitt [j], J. Heylings [k], J. Huwyler [l], M. Jaeger [m], M. Jagelavicius [n], N. Jarrett [o], H. Ketelslegers [p], I. Kocina [q], J. Koester [r], J. Kreysa [b], R. Note [s], A. Poth [m], M. Radtke [t], V. Rogiers [u], J. Scheel [v], T. Schulz [w], H. Steinkellner [w], M. Toeroek [x], M. Whelan [h], P. Winkler [y], W. Diembeck [z]

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Workshop Report

PBTK modelling platforms and parameter estimation tools to enable animal-free risk assessment
Recommendations from a joint EPAA – EURL ECVAM ADME workshop

Jos G. Bessems [a,*], George Loizou [b], Kannan Krishnan [c], Harvey J. Clewell III [d], Camilla Bernasconi [e], Frederic Bois [f], Sandra Coecke [g], Eva-Maria Collnot [h], Walter Diembeck [h], Lucian Romeo Farcal [i], Liesbeth Geraets [a], Ursula Gundert-Remy [j], Nynke Kramer [a], Gabriele Klüsters [k], Sofia B. Leite [g,l], Olavi R. Pelkonen [m], Klaus Schröder [n], Emanuela Testai [o], Iwona Wilk-Zasadna [g], José-Manuel Zaldivar-Comenges [g,p]

Toxicology in Vitro

journal homepage: www.elsevier.com/locate/toxinvit

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 [k], Ugo Zanelli [l], Maria Chiara Zorzoli [a], Maurice Whelan [a]

MiniReview

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

Toxicology

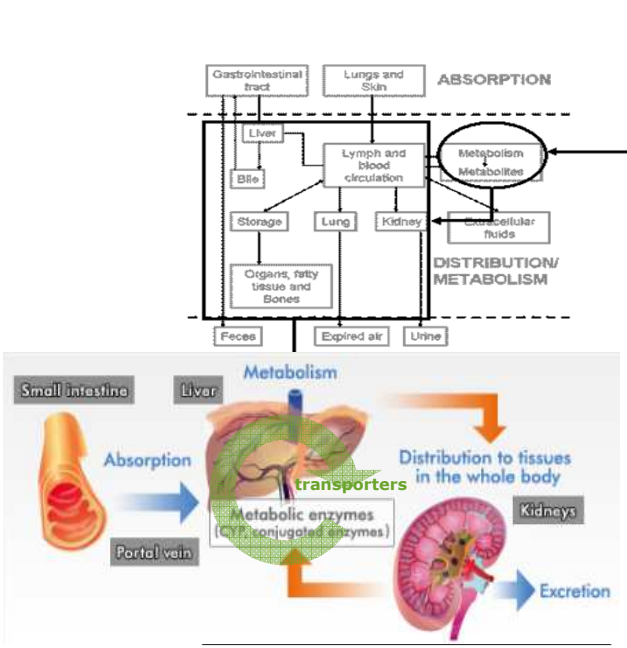
journal homepage: www.elsevier.com/locate/toxicol

Biotransformation *in vitro*: An essential consideration in the quantitative *in vitro*-to-*in vivo* extrapolation (QIVIVE) of toxicity data

Iwona Wilk-Zasadna [a], Camilla Bernasconi [a], Olavi Pelkonen [b], Sandra Coecke [a,*]

TEN YEARS LATER

Framework and activities to characterise *in vitro* metabolism methods (including species differences)



Entry of a chemical entity to the human body

In vitro human metabolic competent toolbox to:

1. **Identify important metabolic pathways in different species**
2. **Provide knowledge of the specific human enzyme isoforms involved in the metabolism**
3. **Provide knowledge on the metabolic stability of the chemical**
4. **Provide knowledge on the type and structure of metabolites**
5. **Provide knowledge on any metabolism-dependent toxic effects ; including covalent to macromolecules**
6. **Provide information their capacity to induce or to inhibit**

→ **Clearance**

2009: Identify the main metabolites and the HUMAN clearance rates of the parent compound and/or its metabolites

Comparison of Metabolic Stability and Metabolite Identification of 55 ECVAM/ ICCVAM Validation Compounds between Human and Rat Liver Homogenates and Microsomes – a preliminary Analysis[1]

Olavi Pelkonen[1], Ari Tolonen[2], Timo Rousu[2], Larissa Tursas[1], Miia Turpeinen[1], Juho Hokkanen[2], Jouko Uusitalo[2], Michel Bouvier d'Yvoire[3] and Sandra Coecke[3]

[1]University of Oulu Department of Pharmacology and Toxicology, Oulu, Finland; [2]Novamass Ltd, Oulu, Finland; [3]EU Joint Research Centre, European Centre for the Validation of Alternative Methods (ECVAM), Ispra, Italy

Received 1st April 2009; received in revised form and accepted for publication 19th June 2009

[1] Project of In-Vitro Toxicology Unit/ECVAM Contract No CCR.IHCP.C432889.X

ALTEX 26, 3/09

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TEST SYSTEM??

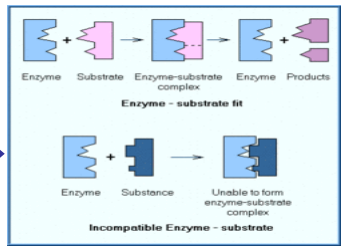
microsomes

homogenates

Hepatocytes

HepaRG

Qualitative and quantitative species differences



metabolites

parent

13

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Comprehensive categorical survey of the results

		Apparent disappearance[1]		Metabolites formed[2]	
Compound	Preparation	Human	Rat	Human	Rat
Carbamazepine	Homogenate	very slow	slow	1	1
	Microsome	very slow	very slow	1	1
Amitriptyline	Homogenate	very slow	very fast	4 (1)	9 (4)
	Microsome	very slow	very fast	4 (1)	9 (5)
Digoxin	Homogenate	very slow	very slow	none	none
	Microsome	very slow	very slow	none	none
Orphenadrine	Homogenate	slow	fast	3	4 (1)
	Microsome	slow	moderate	3	4 (1)
Propranolol	Homogenate	moderate	fast	5 (1)	7 (3)
	Microsome	slower	slow	5 (1)	7 (3)
Compound	Preparation	Human	Rat	Human	Rat
Methadone	Homogenate	slow	moderate	1 (1)	1 (1)
	Microsome	slow	moderate	1 (1)	1 (1)
Thioridazine	Homogenate	moderate	moderate	11 (1)	17 (3)
	Microsome	moderate	moderate	11 (1)	17 (3)
Maprotiline	Homogenate	slow	moderate	1	4 (1)
	Microsome	slow	slow	4	9 (3)
Diphenhydramine	Homogenate	slow	moderate	3	6 (1)
	Microsome	slow	moderate	3	6 (2)
Haloperidol	Homogenate	slow	slow	6 (2)	6 (2)
	Microsome	slow	slow	6 (2)	7 (3)
Atropine	Homogenate	very slow	fast	3	5 (1)
	Microsome	very slow	slow	2	5 (1)
Disopyramide	Homogenate	slow	slow	1	3
	Microsome	very slow	slow	1	3
Diphenylhydantoin	Homogenate	very slow	slow	1	1
	Microsome	very slow	slow	1	1
Warfarin	Homogenate	slow	slow	3	4
	Microsome	slow	slow	3	3
Chloramphenicol	Homogenate	?	moderate	none	1
	Microsome	?	very slow	none	1
Rotenone	Homogenate	moderate	fast	7	7
	Microsome	moderate	moderate	8	8
Diethylphalate	Homogenate	very fast	very fast	1	1
	Microsome	very fast	very fast	1	1
Dibutylphtalate	Homogenate	very fast	very fast	2 (1)	2 (1)
	Microsome	very fast	very fast	2 (1)	3 (1)
Ibuprofen	Homogenate	fast	moderate	1	1
	Microsome	moderate	moderate	1	1
Gibberellic acid	Homogenate	no met	very slow	none	none
	Microsome	no met	no met	none	none
Propylparaben	Homogenate	very fast	very fast	2 (1)	2 (1)
	Microsome	very fast	very fast	3 (1)	3 (1)
Nicotine	Homogenate	moderate	moderate	3 (1)	1
	Microsome	slow	slow	3 (1)	none
Quinidine	Homogenate	moderate	moderate	5 (1)	6 (1)
	Microsome	slow	slow	5 (1)	5 (1)
Verapamil	Homogenate	fast	fast	8 (2)	9 (2)
	Microsome	moderate	moderate	7 (2)	8 (2)
Diazepam	Homogenate	slow	moderate	3 (2)	7 (2)
	Microsome	slow	moderate	3 (2)	6 (3)
Malathion	Homogenate	rapid	rapid	4	4
	Microsome	fast	fast	4	3
Phenobarbital	Homogenate	none	slow	none	none
	Microsome	none	slow	none	none
Pentobarbital	Homogenate	very slow	slow	none	2
	Microsome	very slow	slow	none	1
Fenpropathrin	Homogenate	slow	moderate	3	5
	Microsome	moderate	moderate	4	5
Chlorpyrifos	Homogenate	moderate	moderate	6 (3)	3
	Microsome	moderate	moderate	2 (1)	2 (1)
Carbaryl	Homogenate	moderate	moderate	3 (1)	2
	Microsome	slow	slow	4 (2)	4

Comparison of Metabolic Stability and Metabolite Identification of 55 ECVAM/ ICCVAM Validation Compounds between Human and Rat Liver Homogenates and Microsomes – a preliminary Analysis[1]

Olavi Pelkonen[1], Ari Tolonen[2], Timo Rousu[2], Larissa Tursas[1], Miia Turpeinen[1], Juho Hokkanen[2], Jouko Uusitalo[2], Michel Bouvier d'Yvoire[3] and Sandra Coecke[3]

[1]University of Oulu Department of Pharmacology and Toxicology, Oulu, Finland; [2]Novamass Ltd, Oulu, Finland; [3]EU Joint Research Centre, European Centre for the Validation of Alternative Methods (ECVAM), Ispra, Italy

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ALTEX 26, 3/09

[1]Categories for substrate depletion: very slow (<5%), slow (5-19%), moderate (20-50%), fast (50-80%), very fast (>80%) in the first 15-min incubation. Consistency of substrate loss curve was generally assessed on the basis of the whole substrate depletion curve over 60 minutes.

[2]The first figure is the number of all identified metabolites; the figure in parentheses means the number of major metabolites.

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Comparison of Metabolic Stability and Metabolite Identification of 55 ECVAM/ ICCVAM Validation Compounds between Human and Rat Liver Homogenates and Microsomes – a preliminary Analysis[1]

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ALTEX 26, 3/09

Tab. 4: Similarities and differences in the presence/absence and major/minor metabolite(s) between human and rat liver homogenates and microsomes

	Human homogenate vs microsomes	Rat homogenate vs microsomes	Homogenate human vs rat	Microsomes human vs rat
No metabolites detectable	10	10	8	10
metabolite(s) in one, but not in the other	5	3	6	6
only one metabolite	8	9	7	6
major metabolite(s) same	21	18	14	14
major metabolite(s) different	10	15	20	17
minor metabolite(s) same	11	7	2	2
minor metabolite(s) different	18	22	28	28

Some conclusions:

Comparison of Metabolic Stability and Metabolite Identification of 55 ECVAM/ ICCVAM Validation Compounds between Human and Rat Liver Homogenates and Microsomes – a preliminary Analysis[1]

Olavi Pelkonen[1], Ari Tolonen[2], Timo Rousu[2], Larissa Tursas[1], Miia Turpeinen[1], Juho Hokkanen[2], Jouko Uusitalo[2], Michel Bouvier d'Yvoire[3] and Sandra Coecke[3]

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ALTEX 26, 3/09

- LC-MS–based analytical methods OK for disappearance and formation and tentative identification of metabolites.
- microsomes and homogenates: differences were not large for most of the substances…microsomes as an enzyme source would not produce most phase II metabolites (e.g. paracetamol-sulphate).
- For most compounds, microsomes are still suitable for stability and metabolism screening, but it is difficult to anticipate the extent and significance of wrong conclusions, consequent to the selection of microsomes over homogenates as an enzyme source.

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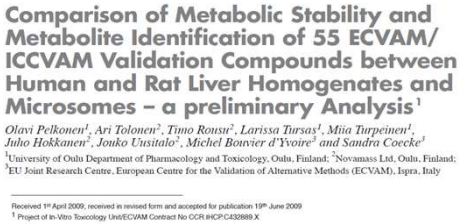
Some conclusions (cont.):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ALTEX 26, 3/09

- A tentative categorical analysis indicated that differences between human and rat preparations were rather modest for most of the substances. There were a number of exceptions, e.g. amitriptyline and aflatoxin B1 regarding substrate loss.

- Qualitative differences in metabolite profiles were relatively common, about a third of compounds displayed a difference in major metabolite(s) and in about a half of the compounds some minor metabolites were different.


ASPET | DRUG METABOLISM AND DISPOSITION

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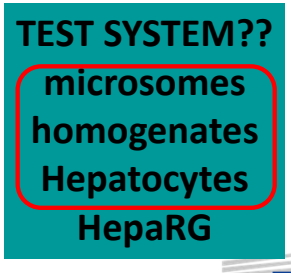
A Decade in the MIST: Learnings from Investigations of Drug Metabolites in Drug Development under the "Metabolites in Safety Testing" Regulatory Guidance

Simone Schadt, Bojan Bister, Swapan K. Chowdhury, Christoph Funk, Cornelis E. C. A. Hop, W. Griffith Humphreys, Fumihiko Igarashi, Alexander D. James, Mark Kagan, S. Cyrus Khojasteh, Angus N. R. Nedderman, Chandra Prakash, Frank Runge, Holger Scheible, Douglas K. Spracklin, Piet Swart, Susanna Tse, Josh Yuan, and R. Scott Obach

Drug Metabolism and Disposition June 2018, 46 (6) 865-878; DOI: https://doi.org/10.1124/dmd.117.079848



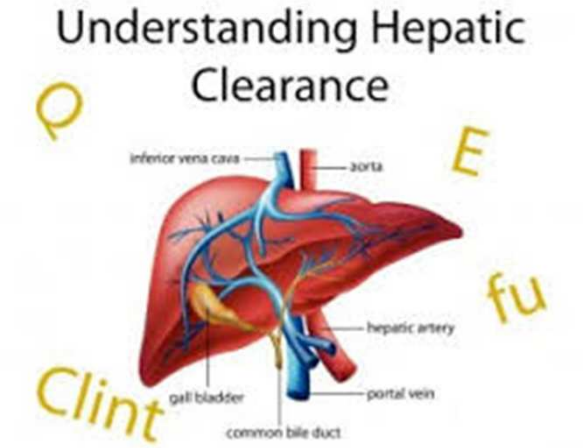
Case Examples of MIST differences in major metabolites in different test systems

European Commission

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]



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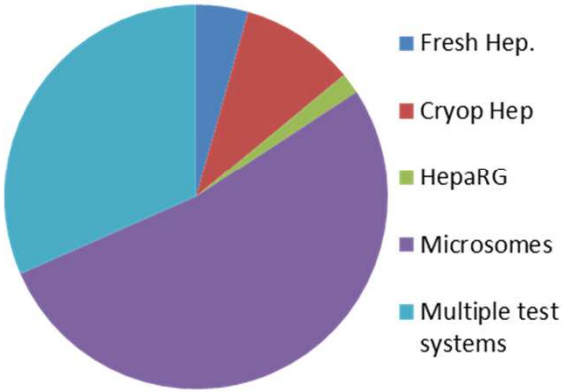
2015 - Literature search and call for clearance methods

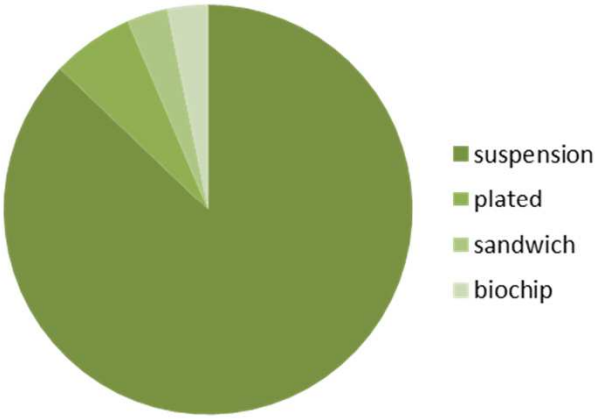
Searching criteria: human based clearance methods and published 1998-2014

Inclusion of **115** published studies

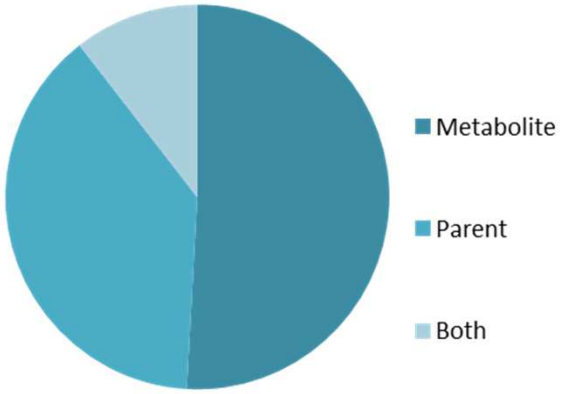
Test system



Test system configuration



Measured parameter



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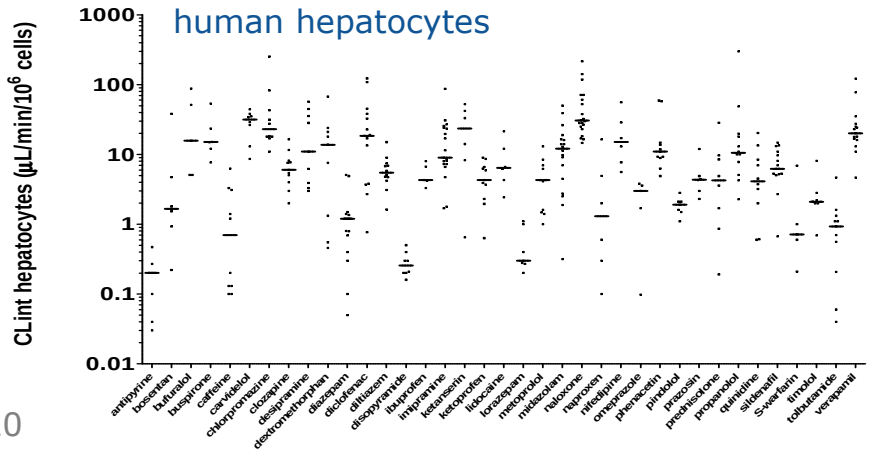
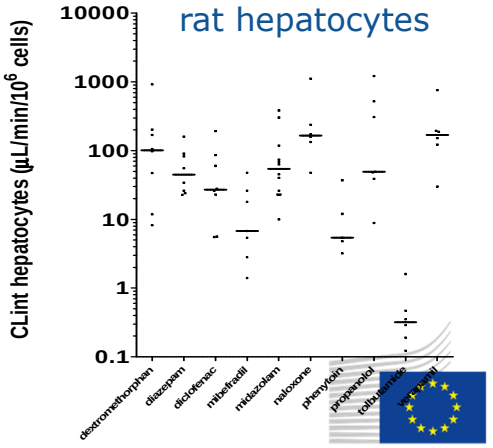
2018 Lead NL: OECD 4.132 Feasibility study TG development

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)





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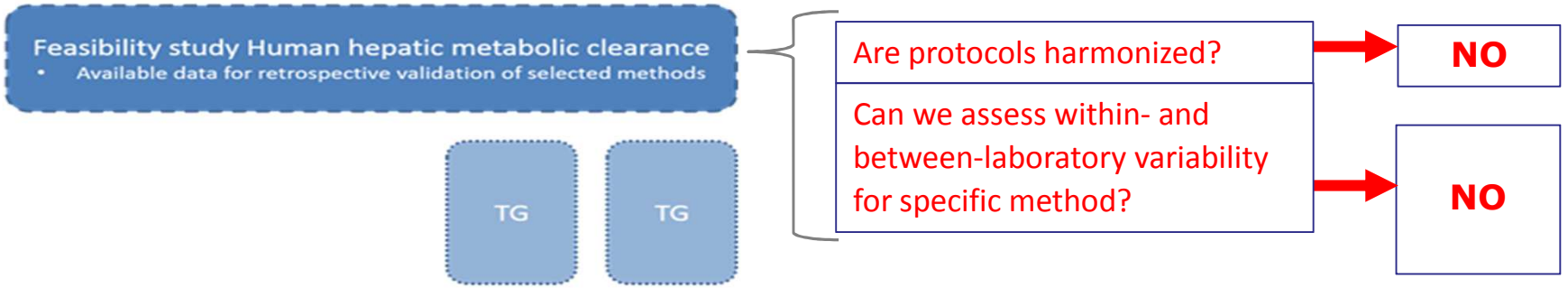
OECD 4.132 Feasibility study TG development

Conclusions:

No harmonised protocol used in literature

Large between-laboratory variability



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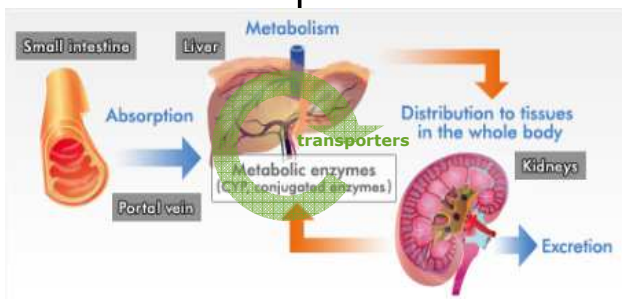
An example of standardisation of *in vitro* metabolism methods: CYP induction

CYP induction



Entry of a chemical entity to the human body

In vitro human metabolic competent toolbox to:

1. Identify important metabolic pathways in different species
2. Provide knowledge of the specific human enzyme isoforms involved in the metabolism
3. Provide knowledge on the metabolic stability of the chemical
4. Provide knowledge on the type and structure of metabolites
5. Provide knowledge on any metabolism-dependent toxic effects ; including covalent to macromolecules
6. Provide information their capacity to induce or to inhibit

→ Human test system (PHH; HepaRG)

→ CYP, UGT; SULT

→ Clearance

→ Thyroid

→ CYP induction validation

UGT; SULT

BCPT Basic & Clinical Pharmacology & Toxicology

Basic & Clinical Pharmacology & Toxicology, 2018, **123**, 42–50 Doi: 10.1111/bcpt.13004

MiniReview

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

(Received 23 January 2018; Accepted 1 March 2018)

European Commission

thyroid

Science of the Total Environment 645 (2018) 97–108

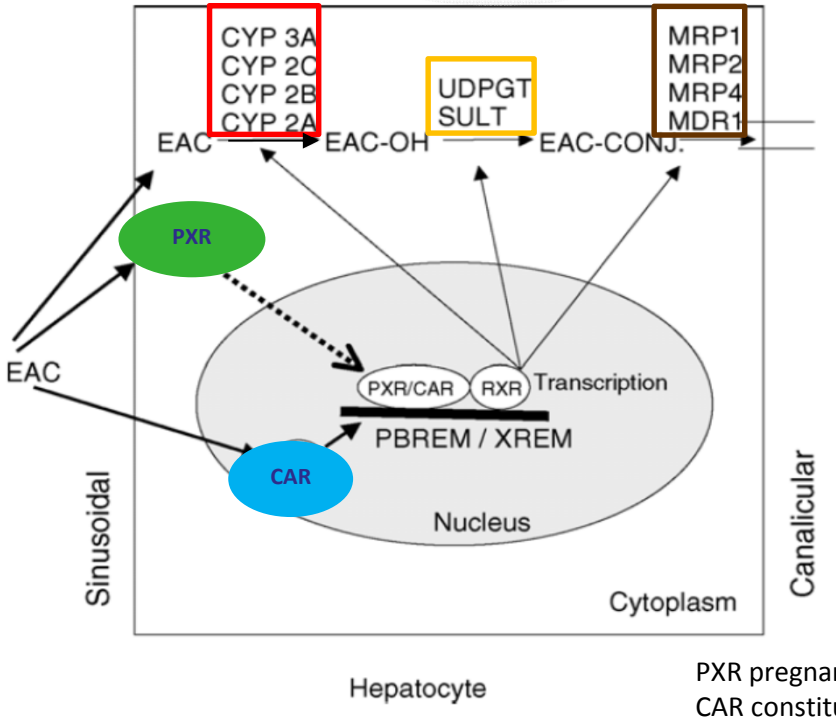
Capturing the applicability of *in vitro-in silico* membrane transporter data in chemical risk assessment and biomedical research

Laure-Alix Clerbaux [a,*], Sandra Coecke [a], Annie Lumen [b], Tomas Kliment [c], Andrew P. Worth [a], Alicia Paini [a]

Human test systems (PHH, HepaRG)

CYP isoforms

CYP induction



PXR pregnane X receptor
CAR constitutive androstane receptor

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PXR and CAR activation by EDCs

steroids

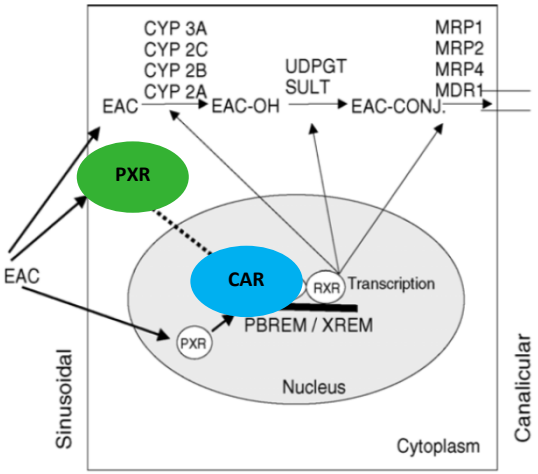
pesticides

phthalates

alkylphenol

PCBs

Type of EDC	EDC	Affected hormone system
Other	Benzophenone (metabolic products)	Estrogen agonist; antiandrogens
	Bisphenol-A (BPA)	Estrogen agonist
	Triclosan	Potential weak androgen
	Cyproterone acetate	Antiandrogen
	Spironolactone	Antiandrogen; aldosterone antagonist; progestational activity
	Alachlor	Disruption of thyroid hormone levels; antiestrogen; antiandrogen
	Aldrin	Potential EDC
	Chlordane	Increases estrogen agonist effects of other EDC (synergism); mimicry of male sex steroids; antiandrogen
	Chlordecone (kepone)	Estrogen agonist and antiestrogen
	Chlorpyrifos	Potential EDC; estrogen agonist
	Cypermethrin	Disruption of reproductive function
	DDE (1,1-dichloro-2,2-bis(*p*-chlorophenyl) ethylene)	Antiandrogen
	DDT	Estrogen agonist; antiandrogen
	Dieldrin	Potential EDC; estrogen agonist; antiandrogen
	Endosulfan	Estrogen agonist; antiandrogen
	Endrin	Potential EDC
	Fenvalerate	Potential EDC
	Lindane (γ-BHC)	Interference with or without estrogen-mediated events (mechanism unknown); disruption of reproductive cycle
	Methoxychlor (metabolic products	ERα agonist, ERβ antagonist, AR antagonist
	Mono-OH-methoxychlor	Estrogen agonist
	Bis-OH-methoxychlor	Estrogen agonist
	Trans-nonachlor	Progesterone and estrogen agonist
	Trifluralin	Reproductive and metabolic effects
	Vinclozolin	Antiandrogen
	Phthalic acid	Antiandrogen
	Mono(2-ethylhexyl) phthalate (MEHP)	Breakdown product of DEHP. Potential antiandrogen
	Di(2-ethylhexyl) phthalate (DEHP)	Antiandrogen
	Di-*n*-butyl phthalate (DBP)	Weakly estrogenic
	Nonylphenol	Estrogen agonist
ls	PCBs (highly chlorinated)	Estrogen agonist; inhibit estrogen catabolism; antiestrogen



Adapted from X.C. Kretschmer, 2005
Luthe et al., 2009

European Commission

PXR and CAR: species differences

Agonists	hPXR	Rodent PXR	hCAR	Roden CAR
Alachlor		ag		
Androstanol	ag	ag	ant	ant
Androstenol				ant
Benzophenone		ag		
Bisphenol-A	ag			
Chlordane	ag	ag		
Chlordecone	ag			
Chlorpyrifos	ag			
Corticosterone	ag	ag		
Cypermethrin	ag			
Cyproterone acetate		ag		
Daidzein	ag			
DBP	ag	ag		ag
DDE		ag		ag
DDT	ag			
o,p-DDT	ag			ag
DEHP	ag	ag		
DHT		ag		
Dexamethasone	ag	ag		
Dexamethasone-21-acetate		ag		
Dexamethasone-*t*-butyl acetate	ag	ag		
Dieldrin	ag			ag
Diethylstilbestrol	ag			
Endosulfan	ag			
Endrin	ag			
17β-Estradiol	ag	ag		ag
Estrone				ag
17α-Ethynylestradiol	ag			
Fenvalerate	ag			
Genistein	ag			
Lindane	ag	ag		
Lithocholic acid	ag	ag		
MEHP	ag	ag		
Methoxychlor	ag	ag	ag	ag
Mono-OH-methoxychlor			ag	
Bis-OH-methoxychlor			ag	
Trans-nonachlor	ag	ag		ant
Nonylphenol	ag	ag		ag
PCBs (highly chlorinated)	ant	ag		ag
PCN		ag		
5β-Pregnane-3,20-dione	ag	ag	ag	
Pregnenolone		ag		
6,16α-Dimethyl pregnenolone	ag	ag		
17α-OH-pregnenolone	ag	ag		
Progesterone	ag	ag		ant
17α-OH-progesterone	ag	ag		
RU486	ag	ag		
Spironolactone	ag	ag		
Tamoxifen	ag			

Rifampicin is a potent activator of hPXR but not of mPXR
PCN is a weak activator of hPXR and a potent activator of mPX
Hyperforin (Saint John's Wort) is a potent activator of hPXR but not of mPXR
TCPOBOP is an activator of mCAR but not of hCAR

Clotrimazole is an inverse agonist for hCAR yet has no effects on mCAR

Androstanol is an inverse agonist for mCAR but is inactive in hCAR

Several **estrogenic** EACs are only active on mCAR not on hCAR

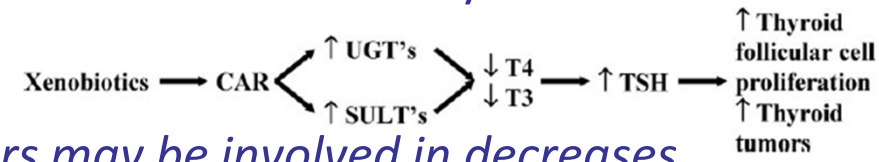
X.C. Kretschmer, 2005
K. Abass et al., 2012
Fujiwara et al., 2018

European Commission

PXR and CAR activation: key mechanistic event in deregulation of homeostasis

Proposed mechanism of thyroid tumor promotion mediated by CAR

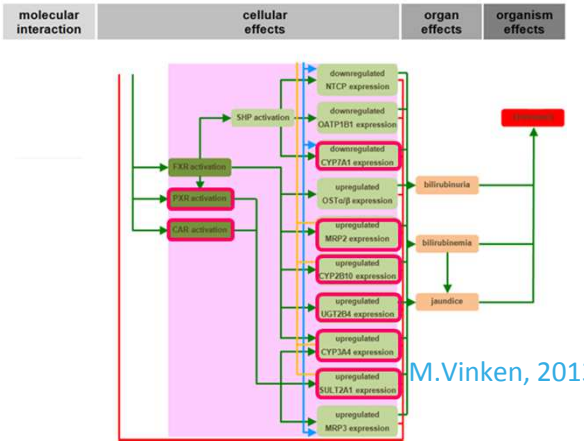
Qatanani M et al., 2005



"*Changes in hepatic UGTs and transporters may be involved in decreases in circulating T4 following BDE 47 exposure* " (in mouse)

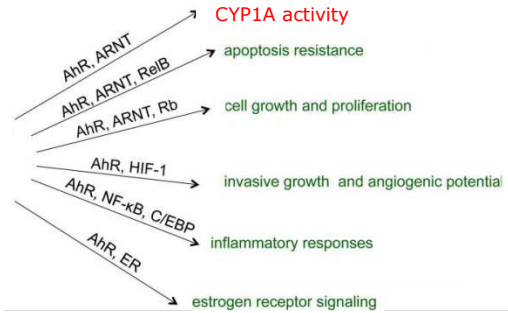
V.M. Richardson et al., 2008

"*BDE-47 induces CYP genes through activation of human CAR in addition to the previously identified pathway through human PXR*" (in PHH) Sueyoshi et al., 2014



M.Vinken, 2013

AhR activation



Vogel CFA et al., 2017

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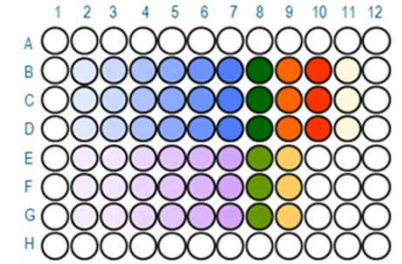
How to measure PXR and CAR activation resulting in phenotypic changes at enzyme activity level? ….Human CYP induction *in vitro* method

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

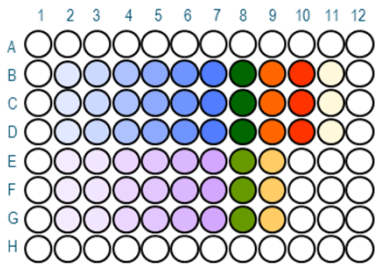
WHY CYP **activity** and not mRNA? CYP induction is a slow process. CYP induction, requiring *de novo* protein synthesis, is a sensitive biomarker for evaluating phenotypic hepatic metabolic competence.

Cell culture

Analytics (LC-MS/MS)

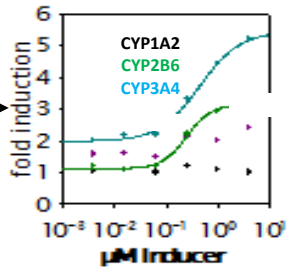


CYP Induction 48-72h Test item exposure

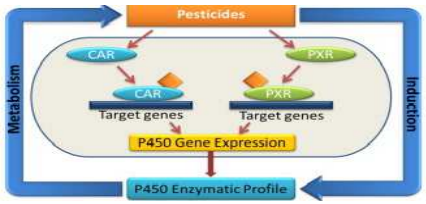


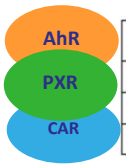
+ enzymatic probe substrates

metabolites measurement



Test items (6 serial dilutions)
Negative control (DMSO)
Medium



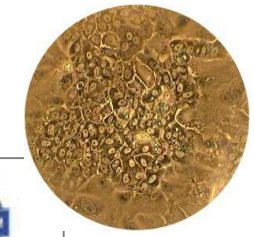


CYP	Reference item for human CYP induction	Enzymatic probe substrate	Metabolite measured
1A2	β-naphthoflavone (BNF) 25 µM	phenacetin	acetaminophen
2B6	Phenobarbital (PB) 500 µM	bupropion	OH-bupropion
3A4	Rifampicin (RIF) 10 µM	midazolam	1-OH-midazolam

Human CYP induction validation study

PHH

HepaRG cells



Pharmacelsus CONTRACT RESEARCH ORGANISATION

janssen PHARMACEUTICAL COMPANIES of Johnson & Johnson

EURL ECVAM THE EUROPEAN UNION REFERENCE LABORATORY FOR ALTERNATIVES TO ANIMAL TESTING

KaLy-Cell

AstraZeneca

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Warren Casey

Michael Paris

NTP National Toxicology Program

NIH NIEHS

NIH National Institutes of Health

NICEATM

BAYER

Momoko Sunouchi

JaCVAM

NIHS

The human CYP induction in vitro method: between and within labs reproducibility

Laboratory	CYP1A2	CYP2B6	CYP3A4	Laboratory	CYP1A2	CYP2B6	CYP3A4
Lab 1	72% (43/60)	75% (45/60)	92% (55/60)	Lab 4	82% (42/51)	60% (30/50)	82% (41/50)
Lab 2	82% (46/60)	75% (45/60)	87% (52/60)	Lab 5	66% (39/59)	78% (46/59)	78% (46/59)
Lab 3	**85%** (51/60)	**78%** (47/60)	**88%** (53/60)	Lab 6	**77%** (54/70)	**60%** (42/70)	**74%** (52/70)

WLR based on based on concordance of predictions between three batches obtained in each laboratory and based on twelve (PHH)/ten (HepaRG cells) test items.

HepaRG cell batch	CYP1A2	CYP2B6	CYP3A4	PHH cell batch	CYP1A2	CYP2B6	CYP3A4
HPR116020	95% (57/60)	82% (49/60)	90% (54/60)	B270808	80% (45/56)	67% (37/55)	71% (39/55)
HPR116035	83% (50/60)	75% (45/60)	95% (57/60)	S240408	58% (35/60)	37% (22/60)	55% (33/60)
HPR116036	68% (41/60)	70% (42/60)	90% (54/60)	S2406A	74% (52/70)	63% (44/70)	61% (43/70)

BLR based on concordance of predictions obtained for one particular batch across the three laboratories and for 12 (PHH)/10 (HepaRG cells) test items.

The human CYP induction in vitro method: predictivity

	HepaRG cells			PHH		
Test item	CYP1A2	CYP2B6	CYP3A4	CYP1A2	CYP2B6	CYP3A4
Omeprazole	N	N	N	N	N	N
Carbamazepine	Y	Y	Y	Y	Y	Y
Phenytoin	Y	Y	Y	Y	Y	Y
Penicillin	N	N	N	N	N	N
Rifabutin	Not tested			N	Y	Y
Sulfinpyrazone	Y	Y	Y	Y	Y	Y
Bosentan	Y	Y	Y	N	Y	Y
Artemisinin	N	Y	N	Y	Y	N
Efavirenz	Not tested			N	Y	Y
Rifampicin	Y	Y	Y	N	Y	Y
Metoprolol	N	N	N	N	N	N
Sotalol	N	N	N	N	N	N

correct *in vitro*-human *in* vivo prediction (i.e. true positive and true negative)

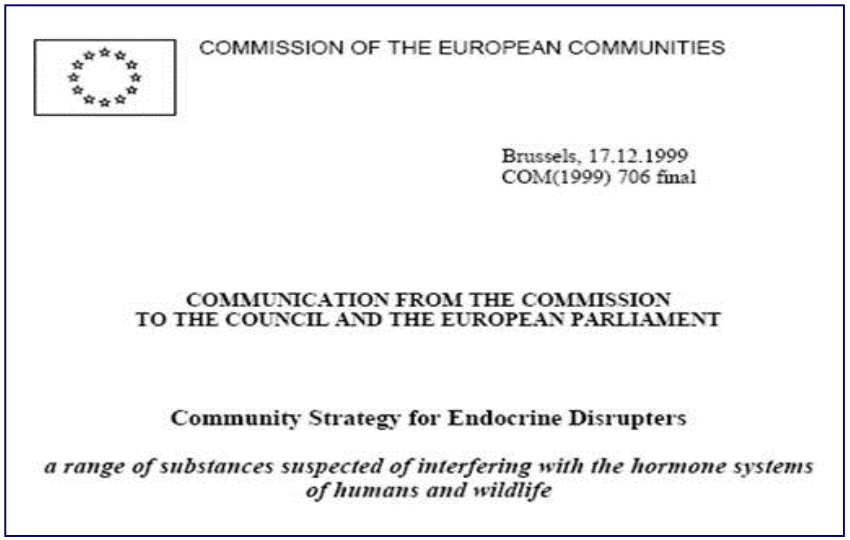
human *in vivo* induction status **unknown** (e.g.no studies) or **conflicting** results (e.g. artemisinin)

incorrect *in vitro*-human *in vivo* prediction.

Current regulatory needs

for *in vitro* metabolism methods

Community Strategy for Endocrine Disrupters - 1999



COMMISSION OF THE EUROPEAN COMMUNITIES

Brussels, 17.12.1999
COM(1999) 706 final

COMMUNICATION FROM THE COMMISSION TO THE COUNCIL AND THE EUROPEAN PARLIAMENT

Community Strategy for Endocrine Disrupters

a range of substances suspected of interfering with the hormone systems of humans and wildlife

- ✓ Coordination framework outlining systematic approach for the identification and assessment of endocrine disruptors that can be applied across the different pieces of legislations.
- ✓ To identify problem of endocrine disruption, its causes and consequences
- ✓ To identify appropriate policy action

http://ec.europa.eu/environment/endocrine/index_en.htm

European Commission

EU Legislation – Criteria for ED identification

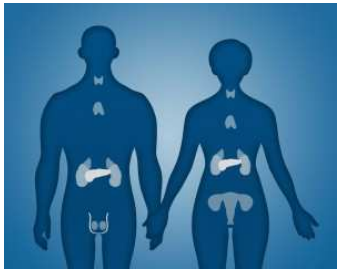
- Publication of scientific criteria for the determination of endocrine disrupting properties for pesticides and biocides

COMMISSION DELEGATED REGULATION (EU) 2017/2100

of 4 September 2017

setting out scientific criteria for the determination of endocrine-disrupting properties pursuant to Regulation (EU) No 528/2012 of the European Parliament and Council

(Text with EEA relevance)



20.4.2018 EN Official Journal of the European Union

COMMISSION REGULATION (EU) 2018/605

of 19 April 2018

amending Annex II to Regulation (EC) No 1107/2009 by setting out scientific criteria for the determination of endocrine disrupting properties

(Text with EEA relevance)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2
concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC
91/414/EEC (1), and in particular Article 78(1)(a) and the second paragraph of point 3.6.5 of Annex II thereto,

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).

Commission

ECHA EUROPEAN CHEMICALS AGENCY

GUIDANCE

efsa European Food Safety Authority

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

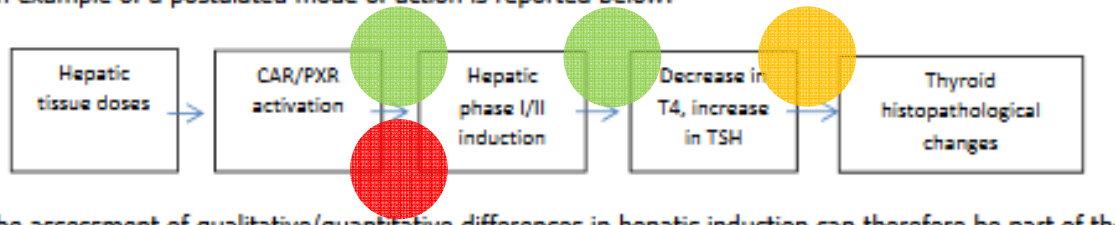
doi: 10.2903/j.efsa.2018.5311

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.

European Commission

BETTER POLICIES FOR BETTER LIVES

OECD encourages the development of no the detection of thyroid disrupters

The OECD Advisory Group on Endocrine Disrupters Testing and Assessment met on 16-17 Oc Guidelines and related documents for the testing and assessment of endocrine disrupters.

One important endocrine system is the thyroid pathway. Thyroid hormones are of great importa Guidelines on laboratory animals are already addressing toxicity to the thyroid hormone systen development.

However, non-animal test methods are also currently needed for more efficient testing. Progres very high priority, in line with the "3-Rs" (Replacement, Reduction, Refinement) principles. A recent review published at OECD aimed at scoping potential tests. OECD countries are strongly encouraged to support the development of non-animal tests for the thyroid pathway that are applicable to the screening and assessment of endocrine disruption in humans and wildlife.

Proposals to develop standardised OECD Test Guidelines for the detection of thyroid disrupters should be made via the National Coordinators of the Test Guidelines Programme.

ENV/JM/MONO(2014)23
Unclassified

Unclassified **ENV/JM/MONO(2014)23**

Organisation de Coopération et de Développement Économiques
Organisation for Economic Co-operation and Development **11-Jul-2014**

English - Or. English

ENVIRONMENT DIRECTORATE
JOINT MEETING OF THE CHEMICALS COMMITTEE AND
THE WORKING PARTY ON CHEMICALS, PESTICIDES AND BIOTECHNOLOGY

NEW SCOPING DOCUMENT ON IN VITRO AND EX VIVO ASSAYS FOR THE IDENTIFICATION OF MODULATORS OF THYROID HORMONE SIGNALLING

Series on Testing and Assessment

No. 207

www.oecd.org/officialdocuments/publicdisplaydocumentpdf/?cote=env/jm/mono(2014)23&doclanguage=en

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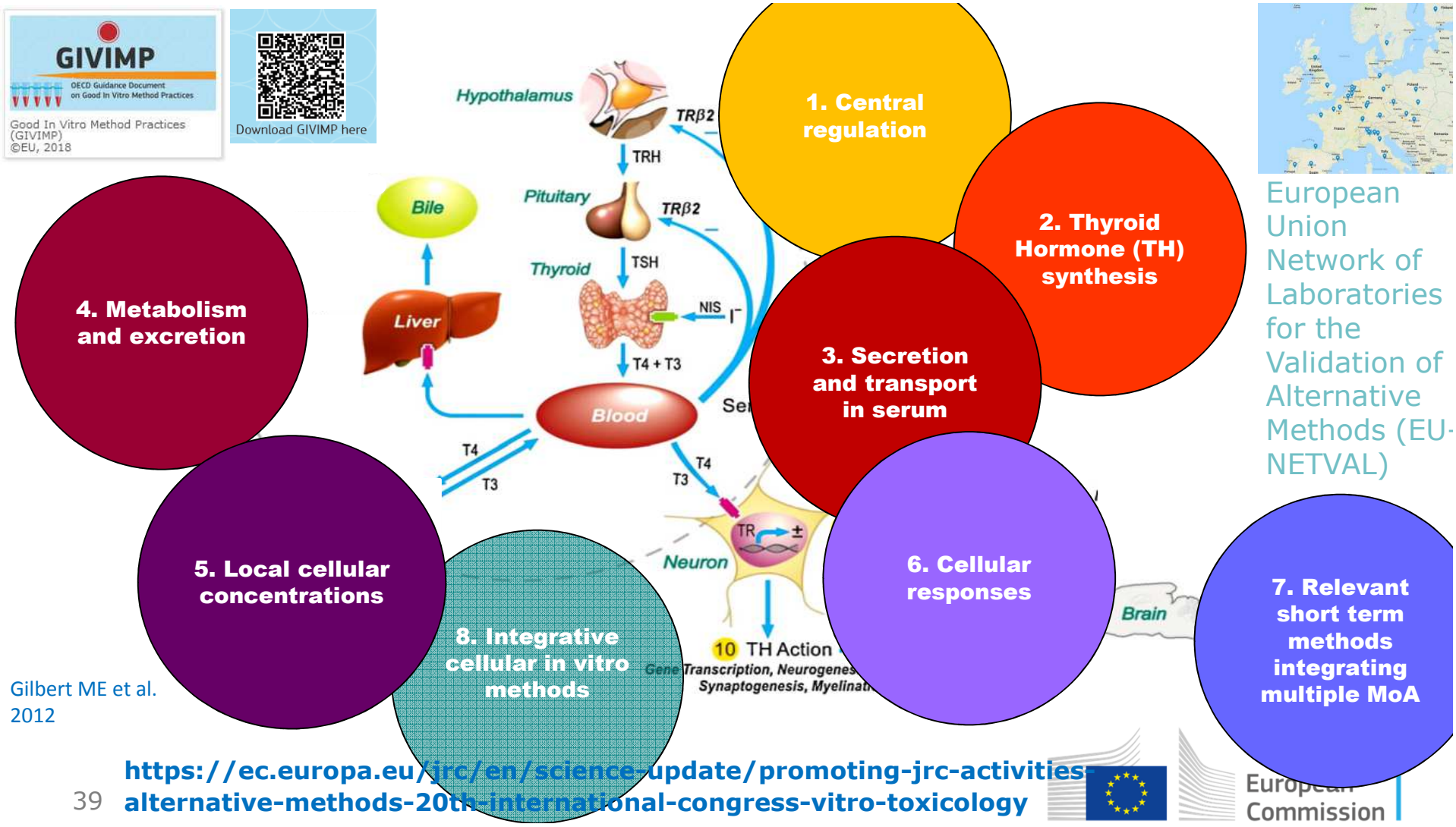

Gilbert ME et al. 2012

European Union Network of Laboratories for the Validation of Alternative Methods (EU-NETVAL)

https://ec.europa.eu/jrc/en/science-update/promoting-jrc-activities-alternative-methods-20th-international-congress-vitro-toxicology

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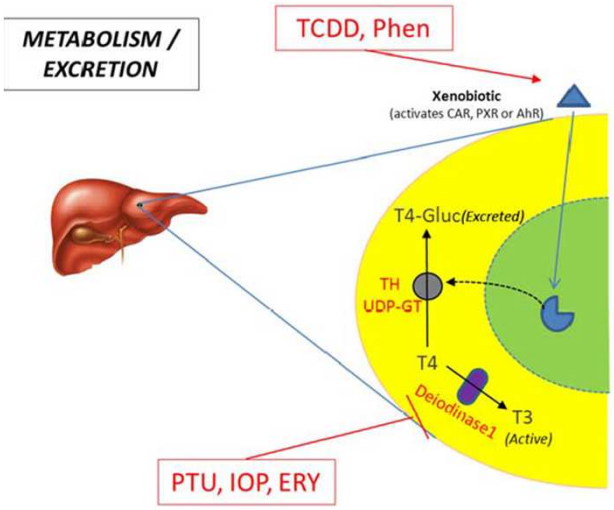
4. Metabolism and excretion



Method	Principle of the test	Test system	Readout
4a. Deiodinase inhibition	redox reaction (Sandell-Kolthoff)	Liver Hepatocytes/ microsomes GMO cells Type I, II, II iodo thyronine deiodinase	spectrophotometry
4b. Glucuronidation	Inhibition/ induction UDPGT	Cryohepatocytes	Chromatography mass spectrometry (LCMS)
4c. TH sulfation	Inhibition/ induction of sulfotransferase	Cryohepatocytes	Chromatography mass spectrometry (LCMS)

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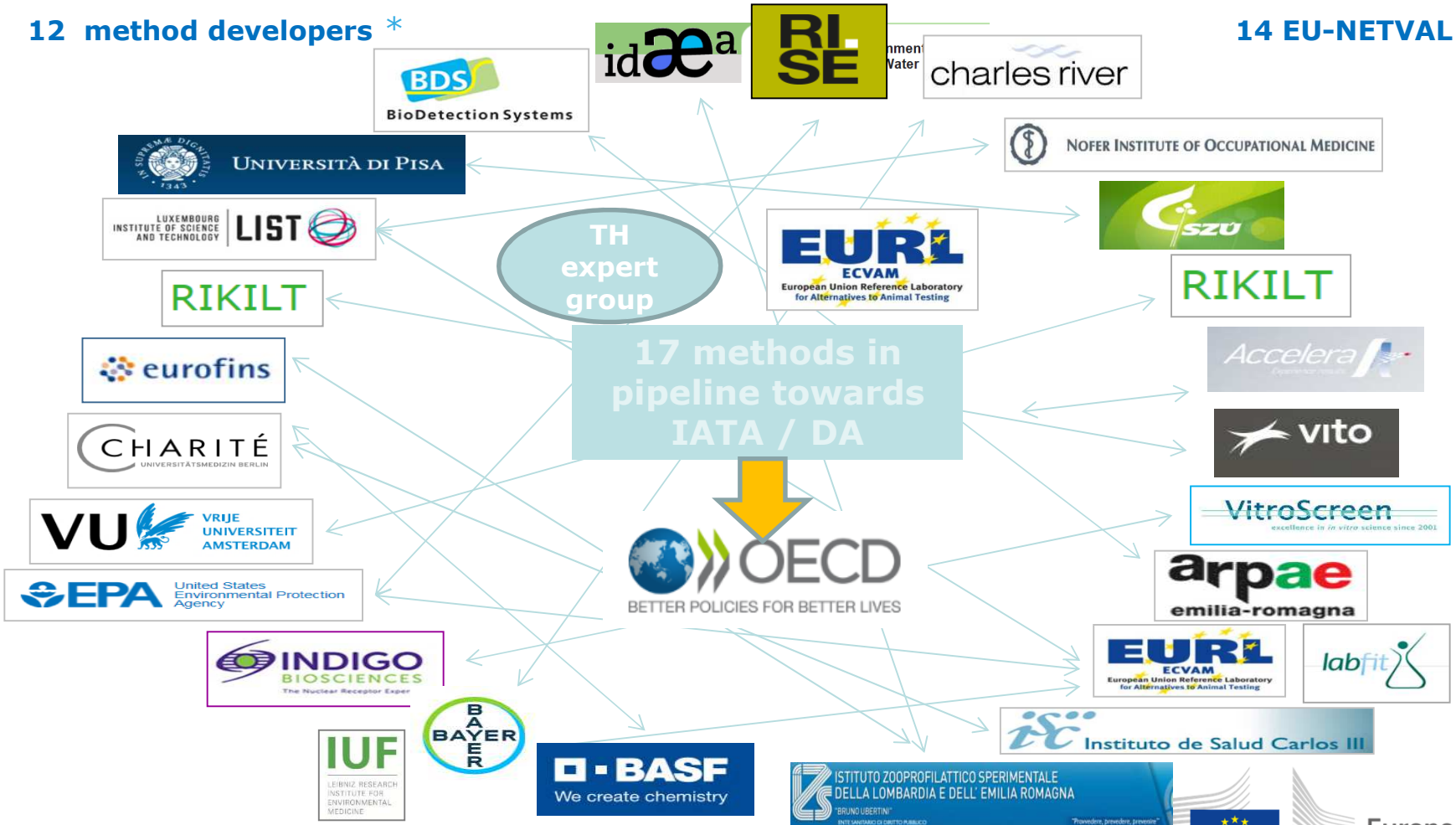
12 method developers *

14 EU-NETVAL labs*



*under negotiation

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Environmental Toxicology

Adverse Outcome Pathway Networks II: Network Analytics

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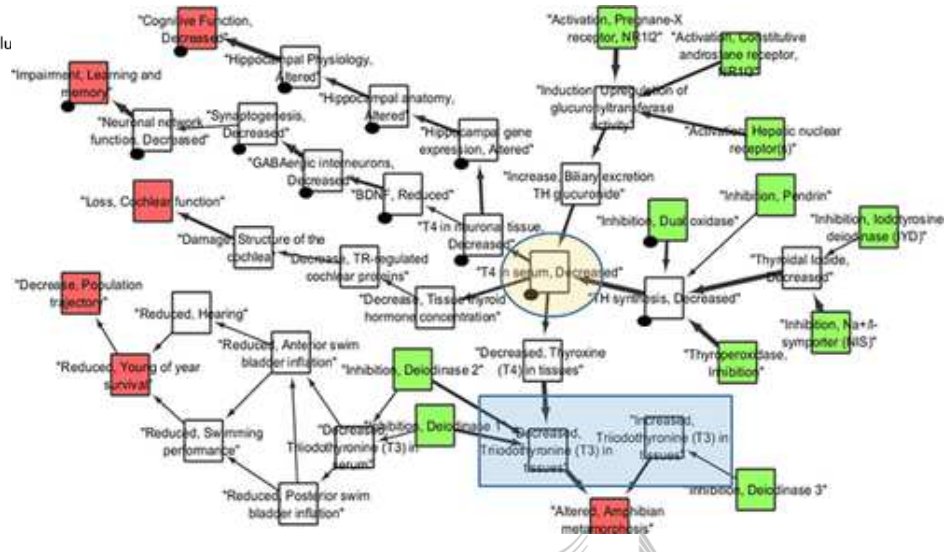
Example adverse outcome pathway (AOP) network 2 (thyroxine [T4]-AOP network). Shown is the network of 14 AOPs related to disruption of thyroid hormone signalling (Society for the Advancement of Adverse Outcome Pathways 2017; AOPs 8, 42, 54, 155, 156, 157, 158, 175, 188, 189, 190, 191, 192, and 193;



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Thanks to the colleagues at EURL ECVAM and all experts that have collaborated to the progress of *in vitro* methods in the metabolism and thyroid field

Collaboration = faster progress

... and many more

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