# The European Commission's science and knowledge service

Joint Research Centre

## A Future Framework for Application of In Vitro Metabolism and QIVIVE Models to Inform Risk Assessment

## Strategies to overcome the "human metabolism" bottleneck in regulatory risk assessment of the 21st century


Sandra Coecke,

Camilla Bernasconi, Alfonso Lostia, Alicia Paini, Giovanna Baron, Joanna Bartnicka, David Asturiol, Andrew Worth, Olavi Pelkonen, Tommy B. Andersson, Minne Heringa, Jochem Louisse, Ans Punt, Betty Hackert EFSA comparative metabolism work group *et al.*

# Overview

1. Introduction
2. Two decades of metabolism **methods** for regulatory purposes
3. Framework and activities to characterise *in vitro* metabolism **methods** (including species differences)
4. An example of standardisation of *in vitro* metabolism **methods**: CYP induction validation study
5. Current regulatory needs for *in vitro* metabolism **methods**

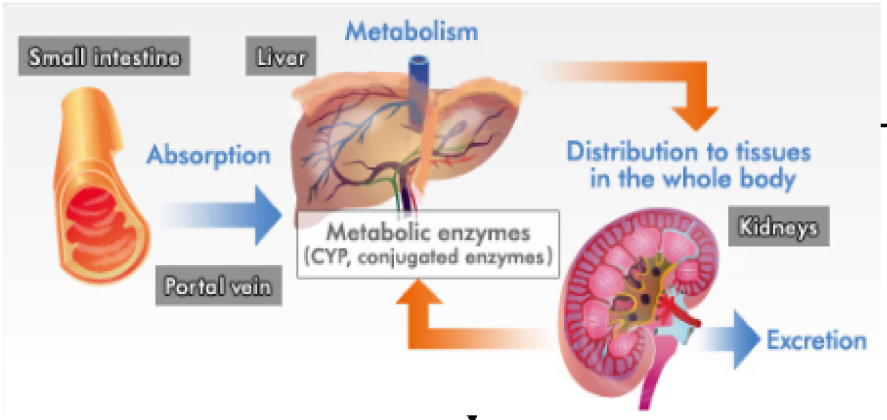
# 1. Introduction

## Strategies to overcome the "human **metabolism**" bottleneck in regulatory risk assessment of the 21st century

- **Early consideration of the multiplicity of factors that govern the biological fate of foreign compounds in living systems is a necessary prerequisite for the quantitative in vitro-in vivo extrapolation (QIVIVE) of toxicity data.**
- **Substantial technological advances in in vitro methodologies have facilitated the study of in vitro metabolism and the further use of such data for in vivo prediction.**
- **However, extrapolation to in vivo with a comfortable degree of confidence, requires continuous progress in the field to address challenges such as e.g., in vitro evaluation of chemical-chemical interactions, accounting for individual variability but also analytical challenges for ensuring sensitive measurement technologies.**
- **Discusses the current status of in vitro metabolism studies for QIVIVE extrapolation, serving today's hazard and risk assessment needs.**

## **Metabolism methods** are key components in any framework for the application of new approach methods

*Assessing systemic toxicity and toxicokinetics*




**Entry of a chemical entity to the human body**

**In vitro human metabolic competent toolbox to:**

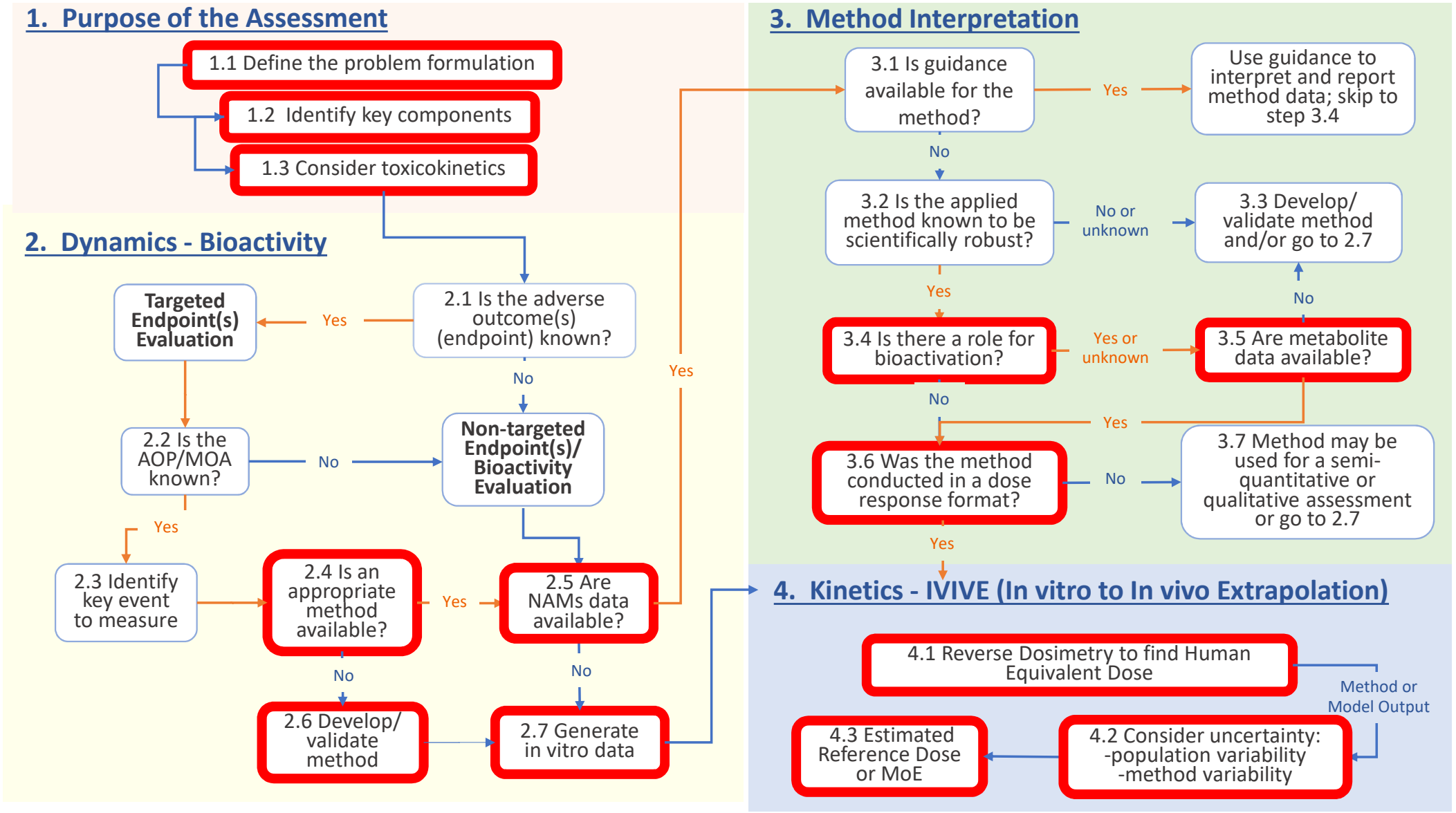
1. **Identify important metabolic pathways in different species**
2. **Provide knowledge of the specific human enzyme isoforms involved in the metabolism**
3. **Provide knowledge on the metabolic stability of the chemical** → **Human test system**; → **CYP, UGT; SULT**
4. **Provide knowledge on the type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

**reproducible** **transferable** **mechanistic or predictive relevance**

**Identification of TARGET ORGAN and/or TARGET SYSTEM toxicity:**

- **Sensitisation**
- **Systemic toxicity**
- **Mutagenicity**
- **Carcinogenicity**
- **Reproductive toxicity**
- **Endocrine disrupting function**

# Framework for the Application of New Approach Methods: Metabolism considerations

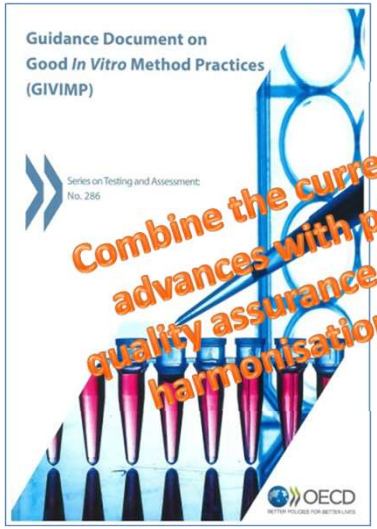


# 2. Two decades of metabolism methods for regulatory purposes

**…….and the age of liver perfusions and metabolism**


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

*Human liver perfusion, Marseille, April 1992*




Combine the current scientific advances with principles of quality assurance and strives for harmonisation at all levels

# 2. Two decades of metabolism methods for regulatory purposes (cont.)

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] Josè 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 Richert,[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, Gensmantel 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, 661–671, 2007 661

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

**The Report and Recommendations of ECVAM Workshop 63**[a]

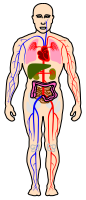
Michel Bouvier d'Yvoire,[1] Pilar Prieto,[1] Bas J. Blaauboer,[2] Frederic Y. Bois,[3] Alan Boobis,[4] Céline Brochot,[3] Sandra Coecke,[1] Andreas Freidig,[5] Ursula Gundert-Remy,[6] Thomas Hartung,[1] Miriam N. Jacobs,[1] Thierry Lavé,[7] David E. Leahy,[8] Hans Lennernäs,[9] George D. Loizou,[10]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

# 2. Two decades of metabolism methods for regulatory purposes (cont.)

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

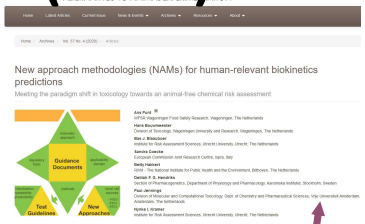
Volume 130, Issue 1
November 2012

**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**



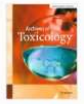

Protocols in In Vitro Hepatocyte Research pp 143-159 | Cite as **2015**

**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 2020**



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], Juho Hokkanen[2], Jouko Uusitalo[2], Michel Bouvier d'Yvoire[1] and Sandra Coecke[3]


**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]


Review

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

K. Schroeder[a,a], 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[h], R. Note[c], A. Poth[m], M. Radtke[s], V. Rogiers[t], J. Scheel[u], T. Schulz[v], H. Steinkellner[w], M. Toerock[x], M. Whelan[h], P. Winkler[y], W. Diembeck[z]

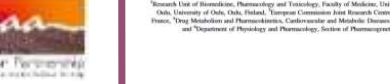

Workshop Report

**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[e], Eva-Maria Collnot[g], Walter Diembeck[h], Lucian Romeo Farcal[e], Liesbeth Geraets[a], Ursula Gundert-Remy[i], Nynke Kramer[j], Gabriele Küsters[k], Sofia B. Leite[a,l], Olavi R. Pelkonen[m], Klaus Schröder[n], Emanuela Testai[o], Iwona Wilk-Zasadna[e], José-Manuel Zaldivar-Comenges[a,l]


**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,*]




**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[2], Sandra Coecke[2], Lysiane Richert[3], Tommy B. Andersson[3,4] and Olavi Pelkonen[1,2]


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

Varvara Gouliarmou[a,1], Alfonso Maria Lautia[a,1], Sandra Coecke[a,c,*], 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[g], Olavi Pelkonen[f], Siegfried Morath[a], Barbara A. Wetmore[f], Andrew Worth[a], Ilaio Zanelli[a], Maria Chiara Zorzoli[a], Maurice Whelan[a]


**Validation of *in vitro* methods for human cytochrome P450 enzyme induction: Outcome of a multi-laboratory study**

Camilla Bernasconi[a], Olavi Pelkonen[b,*], Tommy B. Andersson[c,d], Judy Strickland[a], Iwona Wilk-Zasadna[a], David Asturiol[a], Thomas Cole[a], Roman Liska[a], Andrew Worth[a], Ursula Müller-Vieira[a], Lysiane Richert[c], Christophe Chesne[c], Sandra Coecke[a,*]

TEN YEARS LATER

European Commission

JRC SCIENCE AND POLICY REPORT

# EURL ECVAM strategy for achieving 3Rs impact in the assessment of toxicokinetics and systemic toxicity

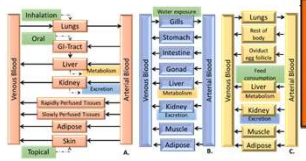
Jos Bessems
Sandra Coecke
Varvara Gouliarmou
Maurice Whelan
Andrew Worth

2015

- ✓EU frameworks REACH, Cosmetics, Plant protection products and Biocides require and/or recommend ADME information
- ✓ADME information is critical to reduce and replace animal testing
- ✓Perform safety assessment based on internal concentrations
- ✓Tissue/ internal concentrations are considered better dose descriptors (animal toxicity studies as well as human exposure)
  - ❖ Improved scientific basis for route-to-route extrapolation
  - ❖ Facilitating use of in vitro points of departure (BMC10, EC50) for human risk assessment purposes

## Framework for the Application of New Approach Methods: **Metabolism considerations**

| ***Strategic aim 1:*** **ADME methods** | ***Strategic aim 2:*** **Kinetic modelling** | ***Strategic aim 3:*** **Data collection** | ***Strategic aim 4:*** **Regulatory anchoring** |
|---|---|---|---|
| Development of **standards** (1.1) | One-stop kinetic **modelling portals** (2.1) | **Collection** human *in vitro* ADME and *in vivo* TK information (3.1) | |
| Human **distribution** and protein binding (1.3) | | **Databases** (3.2) | |
| Human **metabolic stability**-clearance (1.4) | | Sampling strategies, **analytical** determination (3.3) | |
| Human route-specific **absorption** (1.2) | Modelling expert group and **good kinetic modelling practice** (2.2) | | **Guidance** on use of human ADME/TK data in IATA (4.1) |
| Human xenobiotic metabolic pathway **profiling** (1.5) | | | **Legislative** anchoring **human ADME** and **TK** data (4.2) |
| Human route-specific **excretion** (1.6) | | | |



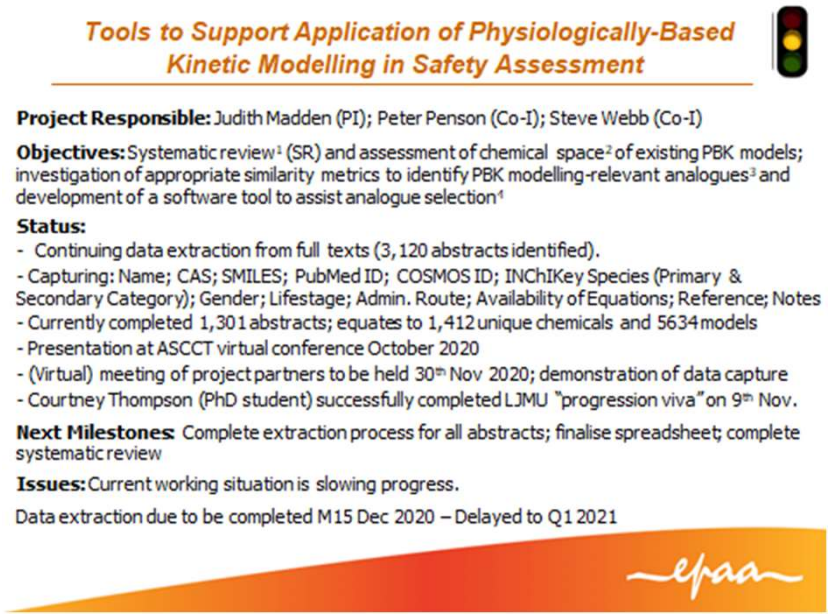
OECD Guidance on the Characterisation, Validation, and Reporting of Physiologically Based Kinetic (PBK) Models (2021)

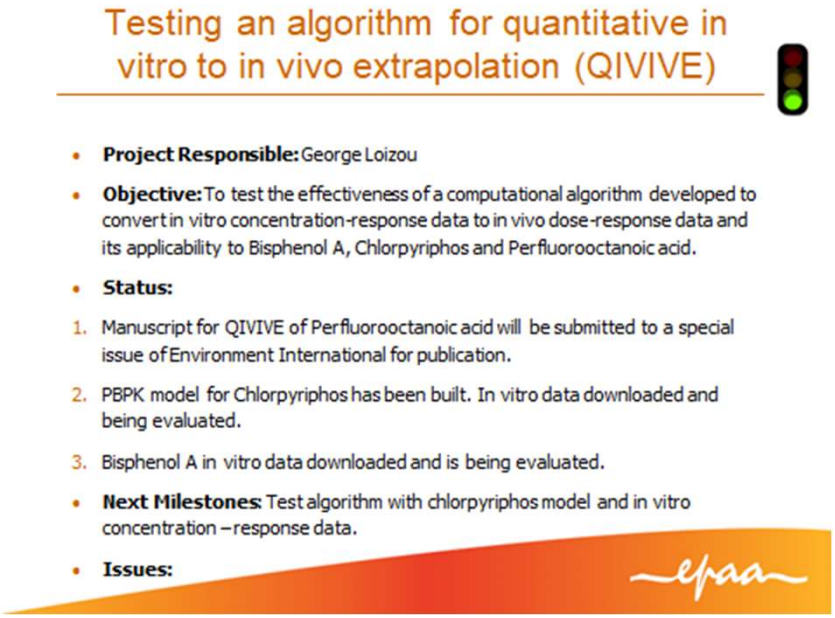
The aim of this document is to provide guidance on the characterisation, validation and reporting of Physiologically Based Kinetic (PBK) models intended for use in the regulatory assessment of chemicals in cases where no in vivo kinetic data are available for model validation.

Project Started in October 2017 under OECD working groups WPHA and EAGMST

# EURL ECVAM & The European Partnership for Alternative Approaches to Animal Testing (EPAA) – Modelling projects

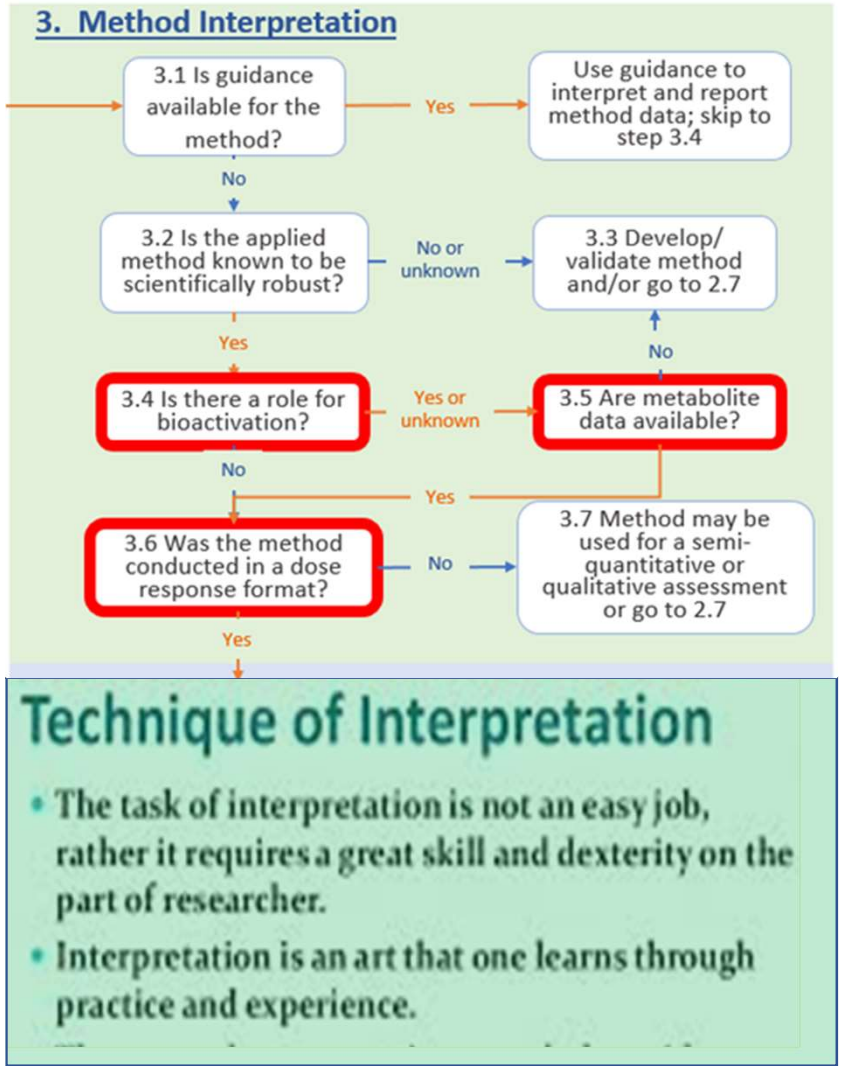
- **EURL ECVAM Strategy Document on Toxicokinetics (2015)–Objectives to enable prediction of systemic toxicity by applying new approach methods**
- **Workshop on physiologically based kinetic modelling(2016)–Establishing model credibility, dealing with uncertainty**

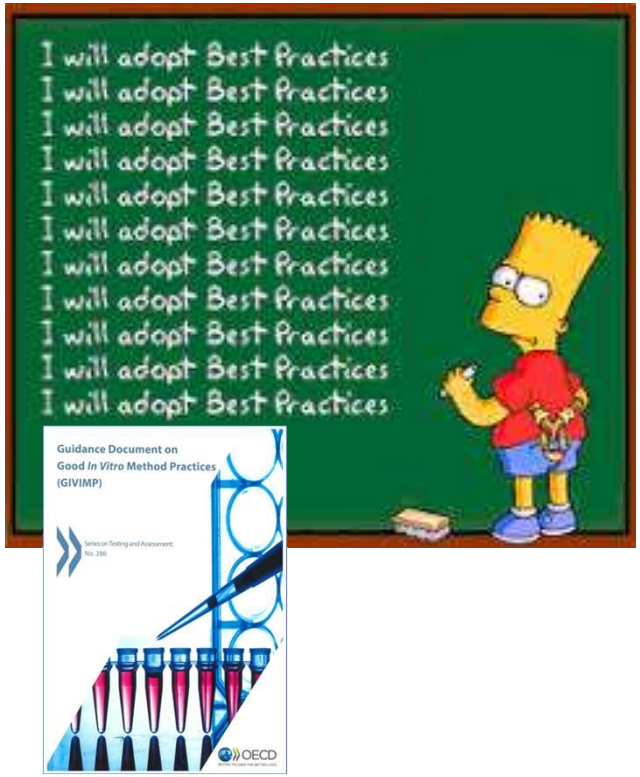



**PROSPERO Protocol:** Systematic review to determine the chemical space of existing physiologically-based kinetic (PBK) models; Courtney Thompson, Judith Madden, Peter Penson. PROSPERO 2020 CRD42020171130 https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42020171130

# 3. Framework and activities to characterise in vitro metabolism methods (including species differences)



# Metabolic stability and metabolite identification

## 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

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 |

ALTEX 2009; 26(3):214-222.

EVENT REPORT

APPROVED: 08 April 2019

doi:10.2903/sp.efsa.2019.EN-1618

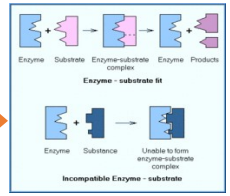
## EFSA Workshop on *in vitro* comparative metabolism studies in regulatory pesticide risk assessment

European Food Safety Authority

201

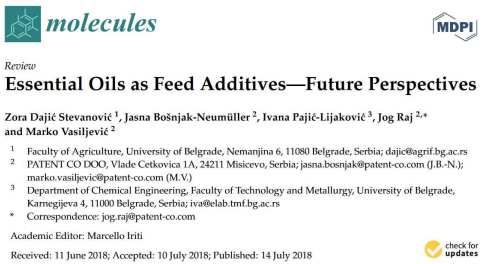
**TEST SYSTEM??**
**microsomes**
**homogenates**
**Hepatocytes**
**HepaRG**

- Unique metabolites
- Disproportionate metabolites


metabolites

parent



molecules

MDPI

*Review*

**Essential Oils as Feed Additives—Future Perspectives**

Zora Dajić Stevanović [1], Jasna Bošnjak-Neumüller [2], Ivana Pajić-Lijaković [3], Jog Raj [2,*] and Marko Vasiljević [2]

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[2] PATENT CO DOO, Vlade Cetkovica 1A, 24211 Misicevo, Serbia; jasna.bosnjak@patent-co.com (J.B.-N.); marko.vasiljevic@patent-co.com (M.V.)
[3] Department of Chemical Engineering, Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11000 Belgrade, Serbia; iva@elab.tmf.bg.ac.rs
* Correspondence: jog.raj@patent-co.com

Academic Editor: Marcello Iriti

Received: 11 June 2018; Accepted: 10 July 2018; Published: 14 July 2018

check for updates

# Essential oils as feed additives and interspecies metabolic difference

1. Phytogenic feed additives' (PFA): **Essential oils**, spices, herbs or plant extracts, combine bioactive ingredients and flavouring substances; categorised as 'sensory additives' according to European legislation. PFAs improve growth rate, nutrient digestibility and gut health in animals. These properties of PFAs project them as a suitable alternative to Antibiotic growth promoters (AGPs) in animal production.
2. The inconsistency of phytogenic feed additives' (PFA) effects on the livestock industry
3. Risk for their use as **a replacement for antibiotic growth promoters**.
4. Information is limited about the PFA mode of action.
5. Complexity of compounds present in essential oils(EOs) and factors that influence biological effects of PFA.
6. Need various controls and optimization parameters that influence the processes for the standardization of these products.
7. The chemical composition of EOs depends on plant genetics, growth conditions, development stage at harvest, and processes of extracting active compounds.
8. Their biological effects are further influenced by the interaction of phytochemicals and their bioavailability in the gastrointestinal tract of animals.
9. PFA effects on animal health and production are also complex due to various EO antibiotic, antioxidant, anti-quorum sensing, anti-inflammatory, and digestive fluids stimulating activities.
10. Focus on reliable methods to identify and control the quality and effects of EOs.

Safety and efficacy of **feed additives** consisting of expressed lemon **oil** and its fractions from *Citrus limon* (L.) Osbeck and of lime **oil** from *Citrus aurantiifolia* (Christm.) Swingle for use in all anin species (FEFANA asbl)

EFSA Panel on **Additives** and Products or Substances used in Animal **Feed** (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Maryline Kouba, Mojca Fašmon Durjava, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Paul Brantom, Andrew Chesson, Johannes Westendorf, Jaume Galobart, Paola Manini, Fabiola Pizzo, Birgit Dusemund

EFSA J. 2021 Apr; 19(4): e06548. Published online 2021 Apr 30. doi: 10.2903/j.efsa.2021.6548
PMCID: PMC8085978

Article PubReader PDF–5.6M Cite

Safety and efficacy of turmeric extract, turmeric **oil**, turmeric oleoresin and turmeric tincture from *Curcuma longa* L. rhizome when used as sensory **additives** in **feed** for all animal species

EFSA Panel on **Additives** and Products or Substances used in Animal **Feed** (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Mojca Kos Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Paul Brantom, Andrew Chesson, Johannes Westendorf, Lucilla Gregoretti, Paola Manini, Birgit Dusemund

EFSA J. 2020 Jun; 18(6): e06146. Published online 2020 Jun 12. doi: 10.2903/j.efsa.2020.6146
PMCID: PMC7448085

Article PubReader PDF–6.6M Cite

Safety and efficacy of essential **oil**, oleoresin and tincture from *Zingiber officinale* Roscoe when used as sensory **additives** in **feed** for all animal species

EFSA Panel on **Additives** and Products or Substances used in Animal **Feed** (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Mojca Kos Durjava, Maryline Kouba, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Paul Brantom, Andrew Chesson, Johannes Westendorf, Lucilla Gregoretti, Paola Manini, Birgit Dusemund

EFSA J. 2020 Jun; 18(6): e06147. Published online 2020 Jun 5. doi: 10.2903/j.efsa.2020.6147
PMCID: PMC7448036

Article PubReader PDF–3.3M Cite

Safety and efficacy of a **feed** additive consisting of expressed mandarin **oil** from the fruit peels of *Citrus reticulata* Blanco for use in all animal species (FEFANA asbl)

EFSA Panel on **Additives** and Products or Substances used in Animal **Feed** (FEEDAP), Vasileios Bampidis, Giovanna Azimonti, Maria de Lourdes Bastos, Henrik Christensen, Maryline Kouba, Mojca Fašmon Durjava, Marta López-Alonso, Secundino López Puente, Francesca Marcon, Baltasar Mayo, Alena Pechová, Mariana Petkova, Fernando Ramos, Yolanda Sanz, Roberto Edoardo Villa, Ruud Woutersen, Paul Brantom, Andrew Chesson, Johannes Westendorf, Paola Manini, Fabiola Pizzo, Birgit Dusemund

EFSA J. 2021 Jun; 19(6): e06625. Published online 2021 Jun 10. doi: 10.2903/j.efsa.2021.6625
PMCID: PMC8190682

Article PubReader PDF–2.8M Cite

Risk for animal and human health related to the presence of dioxins and dioxin-like PCBs in **feed** and food

EFSA Panel on Contaminants in the Food Chain (CONTAM), Helle Katrine Knutsen, Jan Alexander, Lars Barregård, Margherita Bignami, Beat Brüschweiler, Sandra Ceccatelli, Bruce Cottrill, Michael Dinovi, Lutz Edler, Bettina Grasl-Kraupp, Christer Hogstrand, Carlo Stefano Nebbia, Isabelle P Oswald, Annette Petersen, Martin Rose, Alain-Claude Roudot, Tanja Schwerdtle, Christiane Vleminckx, Günter Vollmer, Heather Wallace, Peter Fürst, Helen Håkansson, Thorhallur Halldorsson, Anne-Katrine Lundebye, Raimo Pohjanvirta, Lars Rylander, Andrew Smith, Henk van Loveren, Ine Waalkens-Berendsen, Marco Zeilmaker, Marco Binaglia, José Ángel Gómez Ruiz, Zsuzsanna Horváth, Eugen Christoph, Laura Ciccolallo, Luisa Ramos Bordajandi, Hans Steinkellner, Laurentius (Ron) Hoogenbo

EFSA J. 2018 Nov; 16(11): e05333. Published online 2018 Nov 20. doi: 10.2903/j.efsa.2018.5333
PMCID: PMC7009407

Article PubReader PDF–34M Cite

# Essential Oil Composition and Biosynthesis

EOs contain various compounds, including

1. terpenes,
2. terpenoids,
3. phenylpropenes, and
4. phenolics

that all contribute to the specific and often unique aromatic and bioactive properties of a range of herbs and spices .

*The chemical name, ID or CAS-number of the test compound was given.*

Species differences in metabolism

Electramed to identify with AI deep learning accurately chemical synonyms in relevant papers

GIVIMP & SciRAP AI tool

European Commission

Karolinska Institutet

POLITECNICO MILANO 1863

*Sandra Coecke* *Anna Beronius* *Giacomo Miolo & Giulio Mantoan & Carlotta Orsenigo*

**Computer Science > Computation and Language**

*[Submitted on 19 Apr 2021]*

**ELECTRAMed: a new pre-trained language representation model for biomedical NLP**

Giacomo Miolo, Giulio Mantoan, Carlotta Orsenigo

The overwhelming amount of biomedical scientific texts calls for the development of effective language models able to tackle a wide range of biomedical natural language processing (NLP) tasks. The most recent dominant approaches are domain-specific models, initialized with general-domain textual data and then trained on a variety of scientific corpora. However, it has been observed that for specialized domains in which large corpora exist, training a model from scratch with just in-domain knowledge may yield better results. Moreover, the increasing focus on the compute costs for pre-training recently led to the design of more efficient architectures, such as ELECTRA. In this paper, we propose a pre-trained domain-specific language model, called ELECTRAMed, suited for the biomedical field. The novel approach inherits the learning framework of the general-domain ELECTRA architecture, as well as its computational advantages. Experiments performed on benchmark datasets for several biomedical NLP tasks support the usefulness of ELECTRAMed, which sets the novel state-of-the-art result on the BC5CDR corpus for named entity recognition, and provides the best outcome in 2 over the 5 runs of the 7th BioASQ-factoid Challange for the question answering task.

molecules

MDPI

Review

# Essential Oils as Feed Additives—Future Perspectives

Zora Dajić Stevanović [1], Jasna Bošnjak-Neumüller [2], Ivana Pajić-Lijaković [3], Jog Raj [2,*] and Marko Vasiljević [2]

[1] Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade, Serbia; dajic@agrif.bg.ac.rs
[2] PATENT CO DOO, Vlade Cetkovica 1A, 24211 Misicevo, Serbia; jasna.bosnjak@patent-co.com (J.B.-N.); marko.vasiljevic@patent-co.com (M.V.)
[3] Department of Chemical Engineering, Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, 11000 Belgrade, Serbia; iva@elab.tmf.bg.ac.rs
* Correspondence: jog.raj@patent-co.com

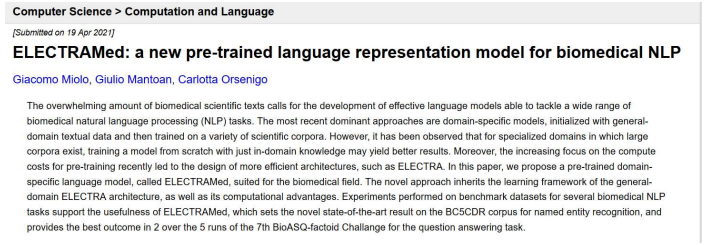
Academic Editor: Marcello Iriti

Received: 11 June 2018; Accepted: 10 July 2018; Published: 14 July 2018

check for updates

- EOs predominantly contain monoterpenoids (C10) and sesquiterpenoids (C15) (Drug relevance) relevant
- Apart from terpene compounds (mono-, sesqui-, and diterpenes),
    - **EO: contain alcohols, esters, aldehydes, acids, ketones, epoxides, amines, and sulfides**
- Isoprenoids or terpenoids, the main compounds of essential oils, are formed by combining of isoprene units (C5H8), which further build monoterpenes (C10), sesquiterpenes (C15), and diterpenes (C20) of two, three, or four isoprene units, respectively.
- The basic carbon terpene skeleton is additionally modified by isomerization, oxidation, reduction, and conjugation, leading to a range of different terpenoid compounds.
- Monoterpenes include hydrocarbons aldehydes, ketones, alcohols, ethers, and lactones, whereas the sesquiterpenes exhibit a high range of structures with more than 100 different skeletons.
- Terpenoids are formed by multiple biosynthetic pathways where two main precursors, isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP), are formed by two independent reaction chains of a plant cell.
- The acetate-mevalonate pathway of a cytoplasm, starting with the condensation of acetyl-CoA, results in the creation of sesquiterpenoids, whereas the plastidial methylerythritol phosphate (MEP) pathway that uses pyruvate and glyceraldehydes 3-phosphate results in the synthesis of isoprene, monoterpenes, and diterpenes.
- Many of resulting monoterpenes (e.g., limonene, thymol, carvacrol, *p*-cymene, γ-terpinene, and menthol) and sesquiterpenes (e.g., caryophyllene, cadinene, humulene, germacrene, and zingiberene) have a cyclic structure.
- However, the complex route that evolved for terpene biosynthesis in plants has been reported, where monoterpenes are synthesized in plastids and the cytosol by canonical monoterpene synthases, in addition to existence of a terpene synthase-independent pathway.
- High variability in the chemical structure of terpenoid compounds is a consequence of the diversity of terpene synthases, which can convert a phenyl diphosphate into different products through a range of reaction cycles.
- Aromatic compounds of essential oils, which are less reported than the terpenoids, are synthetized by a separate shikimate pathway.

# Technologies to harmonize evaluations


Computer Science > Computation and Language

[Submitted on 19 Apr 2021]

**ELECTRAMed: a new pre-trained language representation model for biomedical NLP**

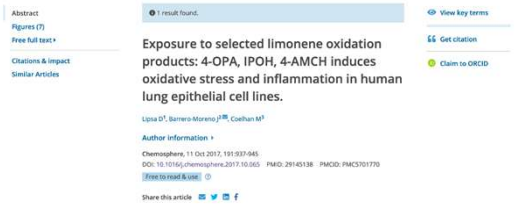
Giacomo Miolo, Giulio Mantoan, Carlotta Orsenigo

The overwhelming amount of biomedical scientific texts calls for the development of effective language models able to tackle a wide range of biomedical natural language processing (NLP) tasks. The most recent dominant approaches are domain-specific models, initialized with general-domain textual data and then trained on a variety of scientific corpora. However, it has been observed that for specialized domains in which large corpora exist, training a model from scratch with just in-domain knowledge may yield better results. Moreover, the increasing focus on the compute costs for pre-training recently led to the design of more efficient architectures, such as ELECTRA. In this paper, we propose a pre-trained domain-specific language model, called ELECTRAMed, suited for the biomedical field. The novel approach inherits the learning framework of the general-domain ELECTRA architecture, as well as its computational advantages. Experiments performed on benchmark datasets for several biomedical NLP tasks support the usefulness of ELECTRAMed, which sets the novel state-of-the-art result on the BC5CDR corpus for named entity recognition, and provides the best outcome in 2 over the 5 runs of the 7th BioASQ-factoid Challange for the question answering task.

**Example of SciRap reporting criterion**

*The chemical name, ID or CAS-number of the test compound was given.*

**Human Readable PubMed Article** → **Expert Inputs the ID of Article** → **Machine Readable Article** → **Text fed into the models** → **Output displayed in the Web Application**


Abstract
Figures (7)
Free full text
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1 result found.

Exposure to selected limonene oxidation products: 4-OPA, IPOH, 4-AMCH induces oxidative stress and inflammation in human lung epithelial cell lines.

Lipsa D[1], Barrero-Moreno J[2], Coelhan M[3]

Author information

Chemosphere, 11 Oct 2017, 191:937-945

DOI: 10.1016/j.chemosphere.2017.10.065 PMID: 29145138 PMCID: PMC5701770

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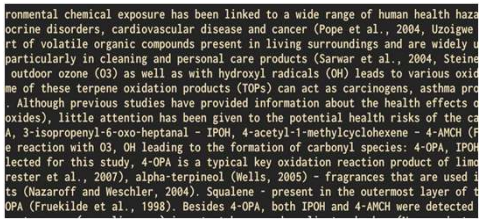
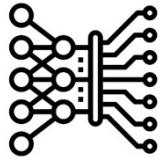
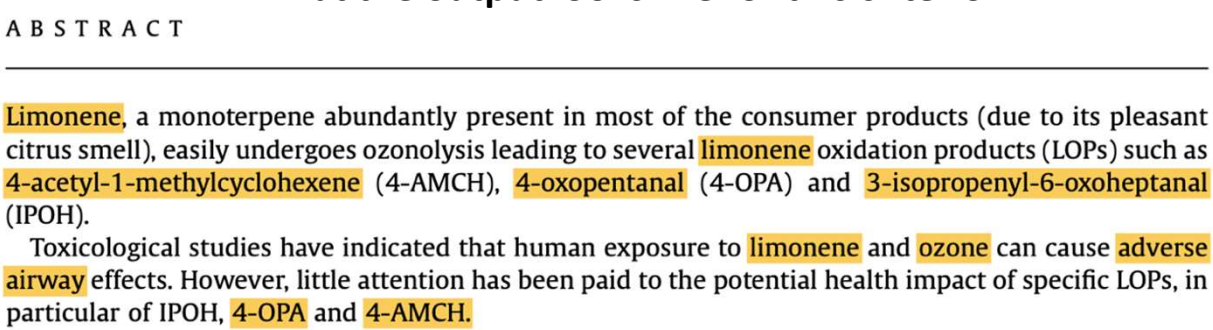

ronmental chemical exposure has been linked to a wide range of human health haza
ocrine disorders, cardiovascular disease and cancer (Pope et al., 2004, Uzoigwe
rt of volatile organic compounds present in living surroundings and are widely u
particularly in cleaning and personal care products (Sarwar et al., 2004, Steine
outdoor ozone (O3) as well as with hydroxyl radicals (OH) leads to various oxid
me of these terpene oxidation products (TOPs) can act as carcinogens, asthma pro
. Although previous studies have provided information about the health effects o
oxides), little attention has been given to the potential health risks of the ca
A, 3-isopropenyl-6-oxo-heptanal - IPOH, 4-acetyl-1-methylcyclohexene - 4-AMCH (F
e reaction with O3, OH leading to the formation of carbonyl species: 4-OPA, IPOH
lected for this study, 4-OPA is a typical key oxidation reaction product of limo
rester et al., 2007), alpha-terpineol (Wells, 2005) - fragrances that are used i
ts (Nazaroff and Weschler, 2004). Squalene - present in the outermost layer of t
OPA (Fruekilde et al., 1998). Besides 4-OPA, both IPOH and 4-AMCH were detected



**What the output looks like for this criterion**


ABSTRACT

Limonene, a monoterpene abundantly present in most of the consumer products (due to its pleasant citrus smell), easily undergoes ozonolysis leading to several limonene oxidation products (LOPs) such as 4-acetyl-1-methylcyclohexene (4-AMCH), 4-oxopentanal (4-OPA) and 3-isopropenyl-6-oxoheptanal (IPOH).

Toxicological studies have indicated that human exposure to limonene and ozone can cause adverse airway effects. However, little attention has been paid to the potential health impact of specific LOPs, in particular of IPOH, 4-OPA and 4-AMCH.

Lipsa et al. (2016) Inflammatory effects induced by selected limonene oxidation products: 4-OPA, IPOH, 4-AMCH in human bronchial (16HBE14o-) and alveolar (A549) epithelial cell lines. Toxicol Lett 262:70-79. https://doi.org/10.1016/j.toxlet.2016.08.023

# The case of Alpha-Pinene

## αPN rates among the most important monoterpenes of human exposure

EFSA (2011) EFSA panel on food contact materials, enzymes, flavourings and processing aids (CEF). Consideration of aliphatic and alicyclic and aromatic hydrocarbons evaluated by JECFA (63rd meeting) structurally related to aliphatic and aromatic hydrocarbons evaluated by EFSA in FGE.25Rev2. EFSA J 9(6:2178):69. doi:10.2903/j.efsa.2011.2178

EFSA Journal

SCIENTIFIC OPINION

ADOPTED: 1 December 2015

PUBLISHED: 05 January 2016

*doi:10.2903/j.efsa.2016.4339*

**Safety and efficacy of eight compounds belonging to chemical group 31 (aliphatic and aromatic hydrocarbons) when used as flavourings for all animal species and categories**

**EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)**

The FEEDAP Panel concluded that E-pinene, D-pinene, E-caryophyllene, myrcene, camphene, E-ocimene and δ-3-carene are safe at the proposed maximum dose level (5 mg/kg complete feed) for all animal species, except myrcene and β-ocimene when 4 mg/kg would apply for cats. For valencene, the calculated safe use level is 1.5 mg/kg complete feed for cattle, salmonids and non-food producing animals, and 1.0 mg/kg complete feed for pigs and poultry. No safety concern would arise for the consumer from the use of these compounds up to the highest safe levels in feeds. The Panel is unable to conclude on user safety in the absence of specific data.

Common Name: **alpha-PINENE**

Synonyms: 2-Pinene; Cyclic Dexadiene

Chemical Name: Bicyclo[3.1.1]Hept-2-ene, 2,6,6-Trimethyl-

Date: August 2008 Revision: April 2017

CAS Number: 80-56-8

RTK Substance Number: 0052

DOT Number: UN 2368

**EMERGENCY RESPONDERS >>>> SEE BACK PAGE**

### Description and Use

**alpha-Pinene** is an oily, colorless liquid with a *Turpentine*-like odor. It is used in the manufacture of *Camphor*, insecticides, solvents, plasticizers, perfumes, and synthetic pine oil. It is a major component of *Turpentine*.

### Hazard Summary

| Hazard Rating | NJDOH | NFPA |
|---|---|---|
| HEALTH | - | 1 |
| FLAMMABILITY | - | 3 |
| REACTIVITY | - | 0 |

FLAMMABLE
POISONOUS GASES ARE PRODUCED IN FIRE
CONTAINERS MAY EXPLODE IN FIRE

*Hazard Rating Key: 0=minimal; 1=slight; 2=moderate; 3=serious; 4=severe*

### Reasons for Citation

- **alpha-Pinene** is on the Right to Know Hazardous Substance List because it is cited by ACGIH, DOT and NFPA.
- This chemical is on the Special Health Hazard Substance List.

- **alpha-Pinene** can affect you when inhaled and by passing through the skin.
- Contact can irritate the skin and eyes.
- Inhaling **alpha-Pinene** can irritate the nose, throat and lungs.
- Exposure to **alpha-Pinene** can cause headache, nausea and vomiting.
- Very high exposure may affect the nervous system causing loss of coordination, dizziness, confusion, seizures and coma.
- **alpha-Pinene** may cause a skin allergy.
- **alpha-Pinene** may damage the kidneys.

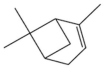
WHO FOOD ADDITIVES SERIES: 54

Safety evaluation of certain food additives

Prepared by the
Sixty-third meeting of the Joint FAO/WHO
Expert Committee on Food Additives (JECFA)

*Table 1.* (contd)

| Flavouring agent | No. | CAS No. and structure |
|---|---|---|
| α-Pinene | 1329 | 80-56-8 |



# The case of Alpha-Pinene (cont.)

Industrial Crops & Products 124 (2018) 643–652

Contents lists available at ScienceDirect

Industrial Crops & Products

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

## Differences in essential oil yield, composition, and bioactivity of three juniper species from Eastern Europe

T. Radoukova[a], V.D. Zheljazkov[b,*], I. Semerdjieva[c], I. Dincheva[d], A. Stoyanova[e], M. Kačániová[f,g], T. Marković[h], D. Radanović[h], T. Astatkie[i], I. Salamon[j]

https://www.sciencedirect.com/science/article/pii/S0926669018307064

Toxicology and Applied Pharmacology 418 (2021) 115496

Contents lists available at ScienceDirect

Toxicology and Applied Pharmacology

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

## Toxicokinetic evaluation of the common indoor air pollutant, α-pinene, and its potential reactive metabolite, α-pinene oxide, following inhalation exposure in rodents

Suramya Waidyanatha[a,*], Michael Hackett[b], Sherry R. Black[c], Mathew D. Stout[a], Timothy R. Fennell[c], Melanie R. Silinski[c], Scott L. Watson[c], Joseph Licause[c], Veronica G. Robinson[a], Barney Sparrow[b], Reshan A. Fernando[c], Stephen Cooper[c], Cynthia V. Rider[a]

[a] Division of the National Toxicology Program, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA
[b] Battelle, Columbus, OH, USA
[c] RTI International, Research Triangle Park, NC, USA

https://pubmed.ncbi.nlm.nih.gov/33744279/

Phytotherapy Research

Research Article

## Daily Inhalation of α-Pinene in Mice: Effects on Behavior and Organ Accumulation

Tadaaki Satou, Hikaru Kasuya, Kazumi Maeda, Kazuo Koike

First published: 26 December 2013 | https://doi.org/10.1002/ptr.5105 | Citations: 28

Volume 28, Issue 9
September 2014
Pages 1284-1287

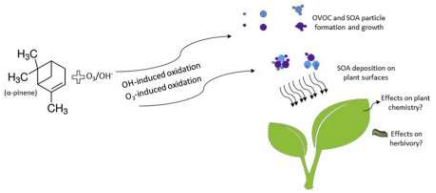
Related Information

Arch Toxicol (2017) 91:677–687
DOI 10.1007/s00204-015-1656-9

TOXICOKINETICS AND METABOLISM

## Human metabolism of α-pinene and metabolite kinetics after oral administration

Lukas Schmidt[1] · Thomas Göen[1]




https://www.industrialchemicals.gov.au/sites/default/files/Alpha-pinene_Human%20health%20tier%20II%20assessment.pdf

Environmental Pollution
Volume 263, Part B, August 2020, 114437

## Deposition of α-pinene oxidation products on plant surfaces affects plant VOC emission and herbivore feeding and oviposition ☆

Open Access Review

## Therapeutic Potential of α- and β-Pinene: A Miracle Gift of Nature

https://www.mdpi.com/2218-273X/9/11/738

by Bahare Salehi[1], Shashi Upadhyay[2], Ilkay Erdogan Orhan[3,*], Arun Kumar Jugran[4,*], Sumali L.D. Jayaweera[5], Daniel A. Dias[5], Farukh Sharopov[6], Yasaman Taheri[7], Natália Martins[8,9], Navid Baghalpour[7], William C. Cho[10,*] and Javad Sharifi-Rad[11,*]

MOLECULAR ECOLOGY

Volume 29, Issue 9
May 2020
Pages 1674-1683

ORIGINAL ARTICLE

## Strategies in herbivory by mammals revisited: The role of liver metabolism in a juniper specialist (*Neotoma stephensi*) and a generalist (*Neotoma albigula*)

Teri J. Orr, Smiljka Kitanovic, Katharina M. Schramm, Michele M. Skopec, P. Ross Wilderman, James R. Halpert, M. Denise Dearing

First published: 04 April 2020 | https://doi.org/10.1111/mec.15431

Related Information

Metrics

Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology
Volume 124, Issue 3, November 1999, Pages 239-246

## Induction of xenobiotic metabolising enzymes in the common brushtail possum, *Trichosurus vulpecula*, by *Eucalyptus* terpenes

Georgia J Pass[a], Stuart McLean[a], Ieva Stupans[b]

Article | Open Access | Published: 06 February 2019

## The cytochrome P450 CYP6DE1 catalyzes the conversion of α-pinene into the mountain pine beetle aggregation pheromone *trans*-verbenol

Christine C. Chiu, Christopher I. Keeling & Joerg Bohlmann

Scientific Reports 9, Article number: 1477 (2019) | Cite this article
1491 Accesses | 15 Citations | 13 Altmetric | Metrics

https://www.nature.com/articles/s41598-018-38047-8

## IMAP Group Assessment Report, Australia , 2020

This group assessment contains chemicals related to alpha-pinene. Three of the chemicals in this group are: alpha-pinene(unspecified isomer) (CAS No. 80-56-8), the (1S,5S)- or (-)-alpha-pinene (CAS No. 785-26-4) isomer and the (1R,5R)- or (+)-alpha-pinene isomer (CAS No. 7785-70-8). They are closely structurally-related and are expected to have similar toxicologicalproperties. The chemicals are naturally-occurring and the racemic mixture of both enantiomers does not occur in nature. In thisassessment, 'alpha-pinene', refers to the unspecified isomer, unless stated otherwise.This assessment also includes the chemical 'oil of turpentine, alpha-pinene fraction' (CAS No. 65996-96-5). This chemical is thedistillation fraction of turpentine oil containing >80 % alpha-pinene . While this fraction is expected to also contain small amountsof the other terpene hydrocarbons in turpentine (beta-pinene, delta-3-carene, camphene, terpinolene, carene and limonene), itstoxicological profile is expected to be closely related to that of alpha-pinene (CAS No. 80-56-8)

ELSEVIER

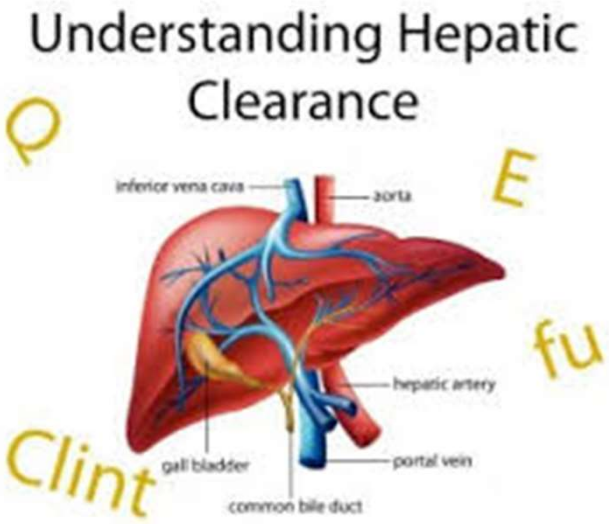
Toxicology in Vitro

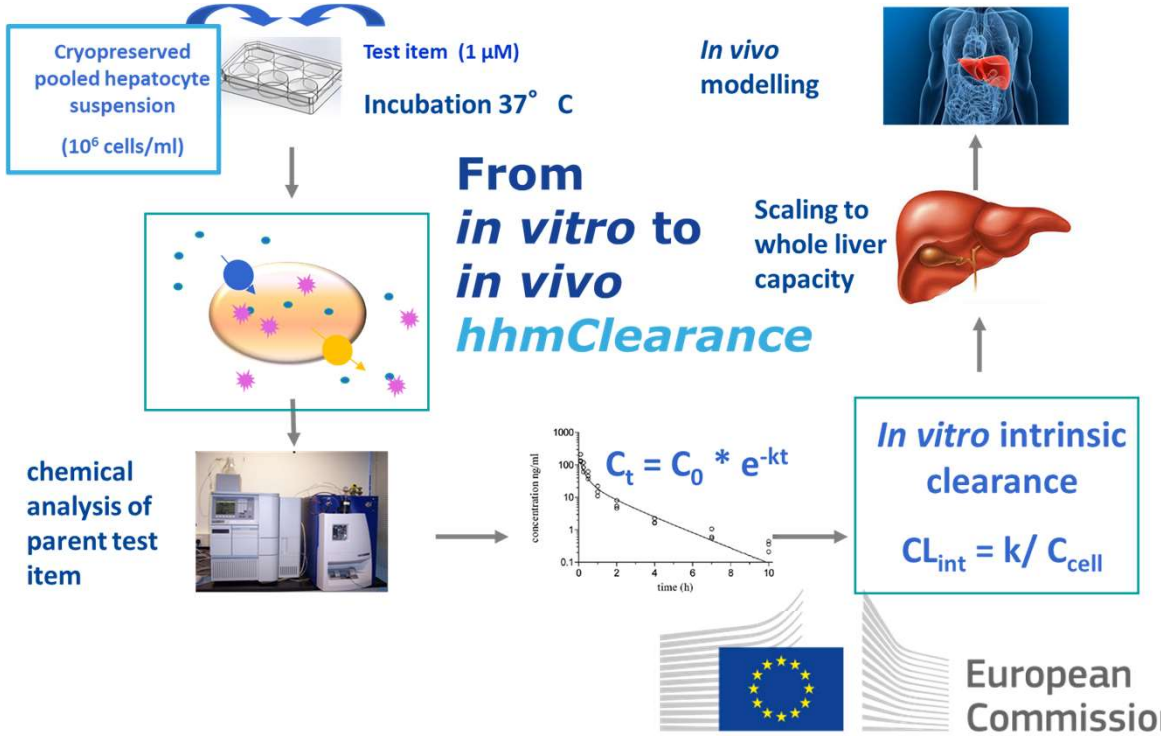
Volume 53, December 2018, Pages 233-244

# 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

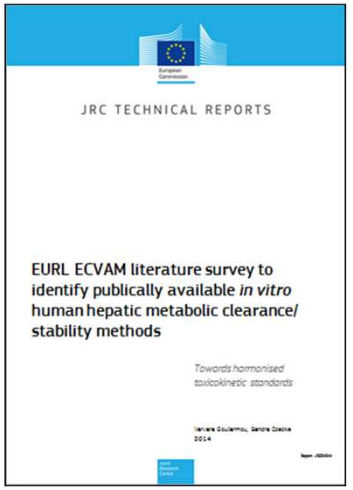


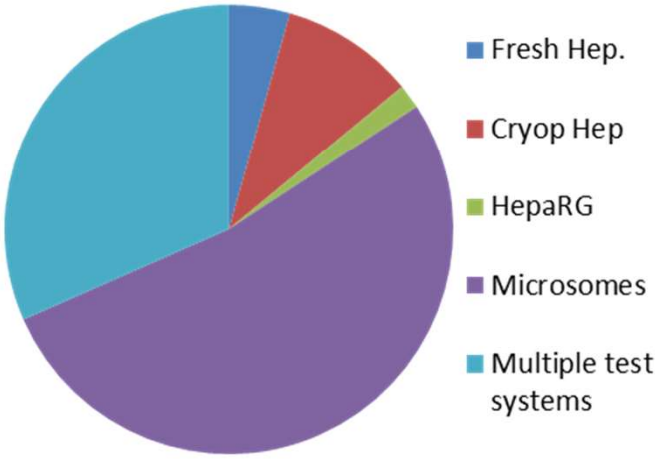
# 2015 - Literature search and call for clearance methods

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

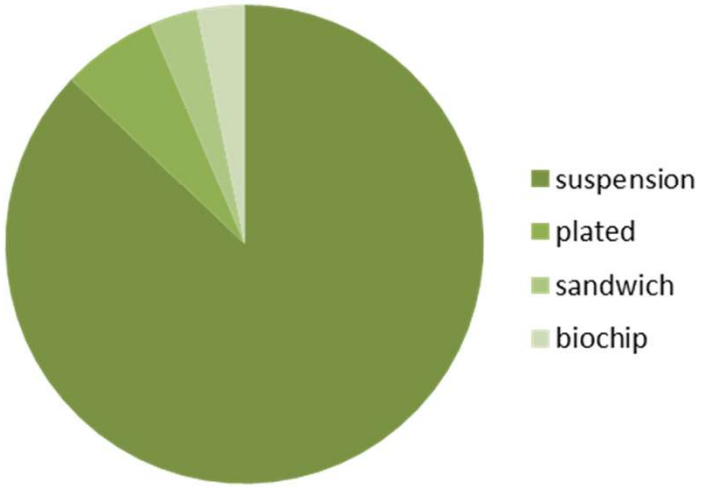
**Inclusion** of **115** published studies

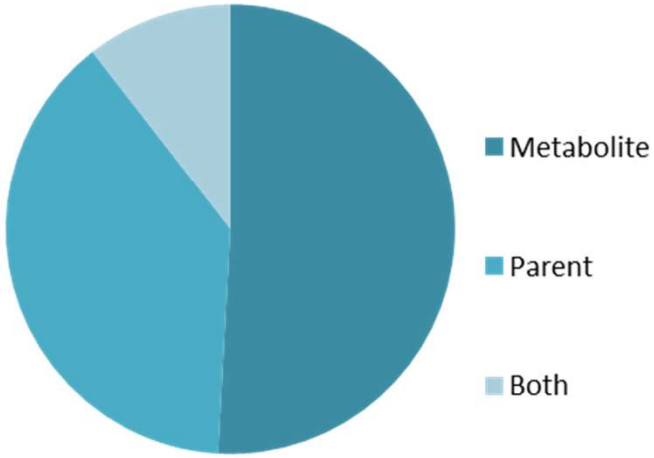

**Test system**




**Test system configuration**

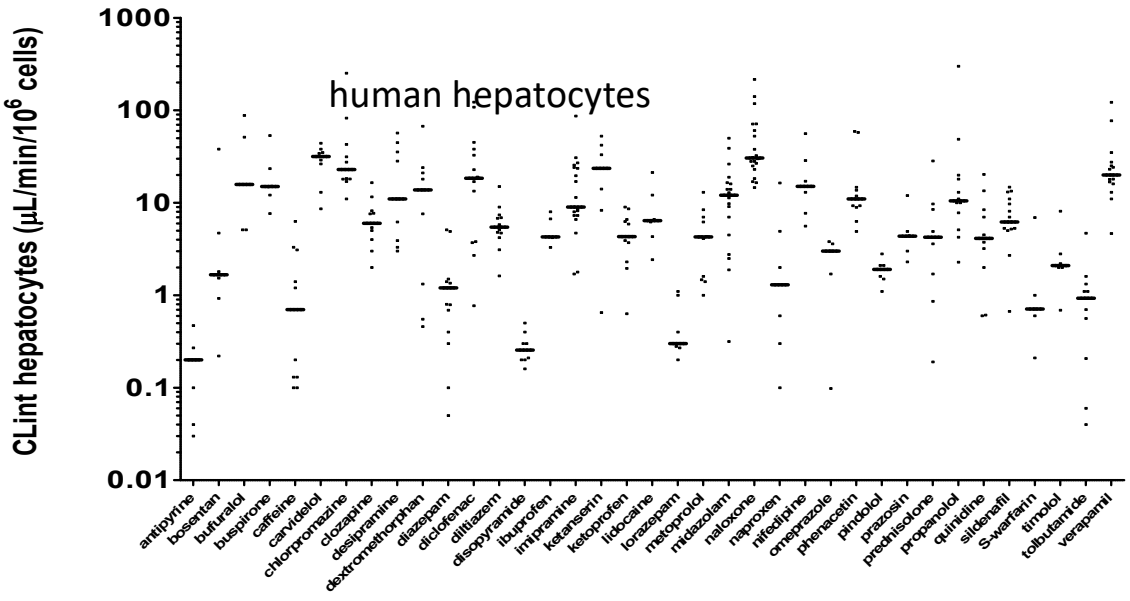


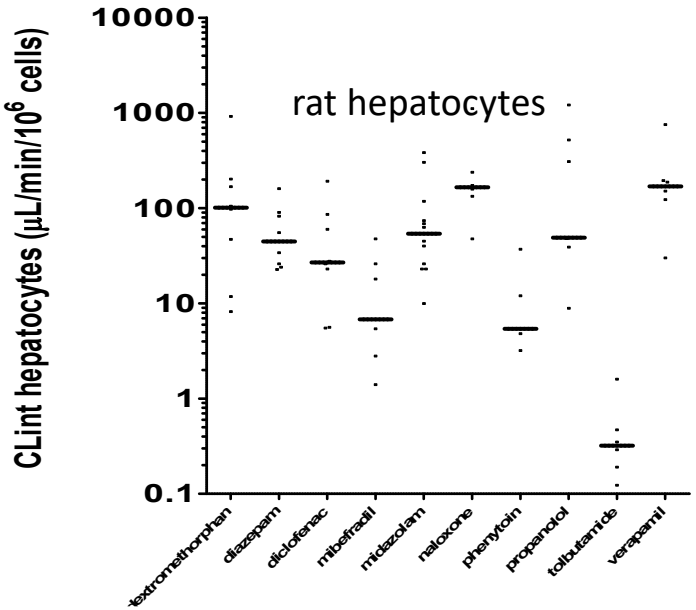

**Measured parameter**



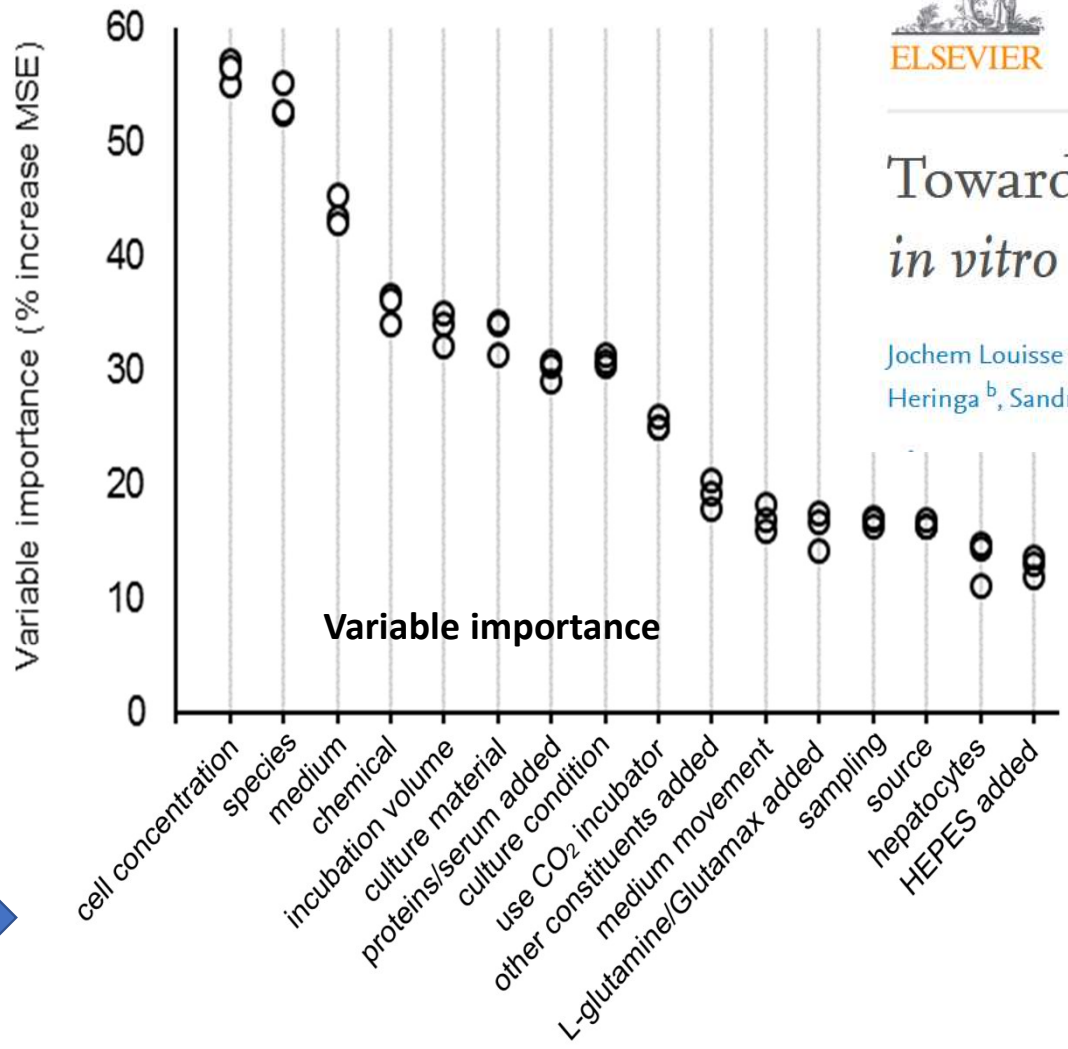
GIVIMP OECD Guidance Document on Good *In Vitro* Method Practices

- 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)



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




**Variable importance**

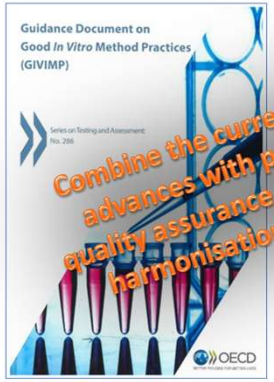
ELSEVIER

Toxicology in Vitro

Volume 63, March 2020, 104722

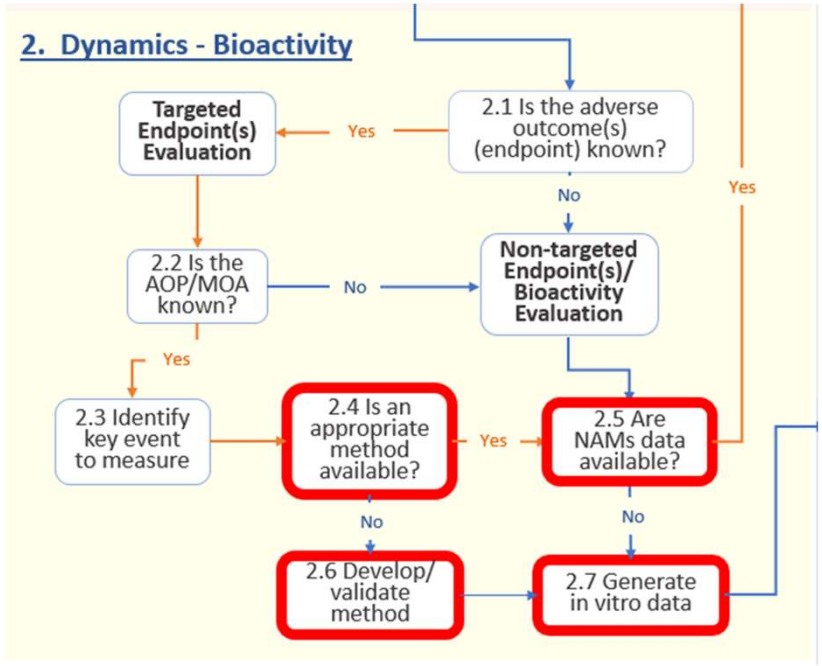
# Towards harmonization of test methods for *in vitro* hepatic clearance studies

Jochem Louisse [a], Martin Alewijn [a], Ad A.C.M. Peijnenburg [a], Nicole H.P. Cnubben [b], Minne B. Heringa [b], Sandra Coecke [c], Ans Punt [a]

# 4. An example of standardisation of in vitro mechanistic metabolism methods:

## CYP induction validation study




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)

# 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

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

VUB VRIJE UNIVERSITEIT BRUSSEL

Rogiers Vera

Tamara Vanhaecke

ATERA

Erwin Roggen

Sonja Beken

Michael Cunningham

Judy Strickland

Warren Casey

UNIVERSITY OF OULU

Olavi Pelkonen

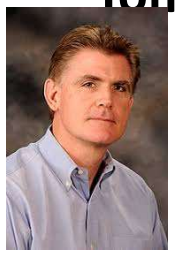
NTP National Toxicology Program

NIH NIEHS

NIH National Institutes of Health

NICEATM

KAROLINSKA INSTITUTET ANNO 1810



Magnus Ingelman-Sundberg

Tommy B Andersson

Armin Kern

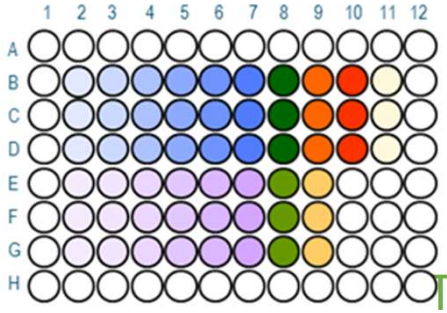
BAYER

Momoko Sunouch

JaCVAM

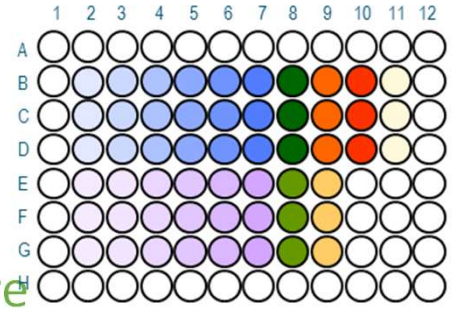
NIHS

## Cell culture




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

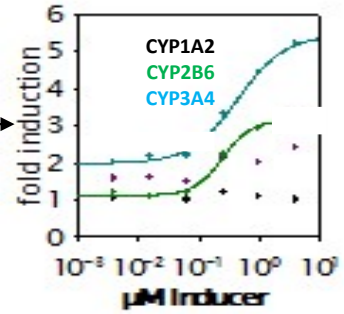
CYP
Induction
48-72h
Test item exposure




+ enzymatic probe substrates

## Analytics (LC-MS/MS)

metabolites measurement





| 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 |


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

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.

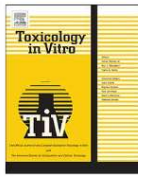
BLR based on concordance of predictions obtained for one particular batch across the three laboratories and for 12 (PHH)/10 (HepaRG cells) test items.

Toxicology in Vitro 60 (2019) 212–228

Contents lists available at ScienceDirect

Toxicology in Vitro

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




## Validation of *in vitro* methods for human cytochrome P450 enzyme induction: Outcome of a multi-laboratory study

Camilla Bernasconi[a], Olavi Pelkonen[b,i], Tommy B. Andersson[c,d], Judy Strickland[e], Iwona Wilk-Zasadna[a], David Asturiol[a], Thomas Cole[a], Roman Liska[a], Andrew Worth[a], Ursula Müller-Vieira[f], Lysiane Richert[g], Christophe Chesne[h], Sandra Coecke[a,*]

[a] *European Commission, Joint Research Centre (JRC), Ispra, Italy*

# The human CYP induction in vitro method: predictivity

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

| | 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 |

International Journal of *Molecular Sciences*

MDPI

*Project Report*

**The GOLIATH Project: Towards an Internationally Harmonised Approach for Testing Metabolism Disrupting Compounds**

Juliette Legler [1,*], Daniel Zalko [2], Fabien Jourdan [2], Miriam Jacobs [3], Bernard Fromenty [4], Patrick Balaguer [5], William Bourguet [6], Vesna Munic Kos [7], Angel Nadal [8], Claire Beausoleil [9], Susana Cristobal [10], Sylvie Remy [11], Sibylle Ermler [12], Luigi Margiotta-Casaluci [12], Julian L. Griffin [13], Bruce Blumberg [14], Christophe Chesné [15], Sebastian Hoffmann [16], Patrik L. Andersson [17], Jorke H. Kamstra [1] and on behalf of the GOLIATH Consortium

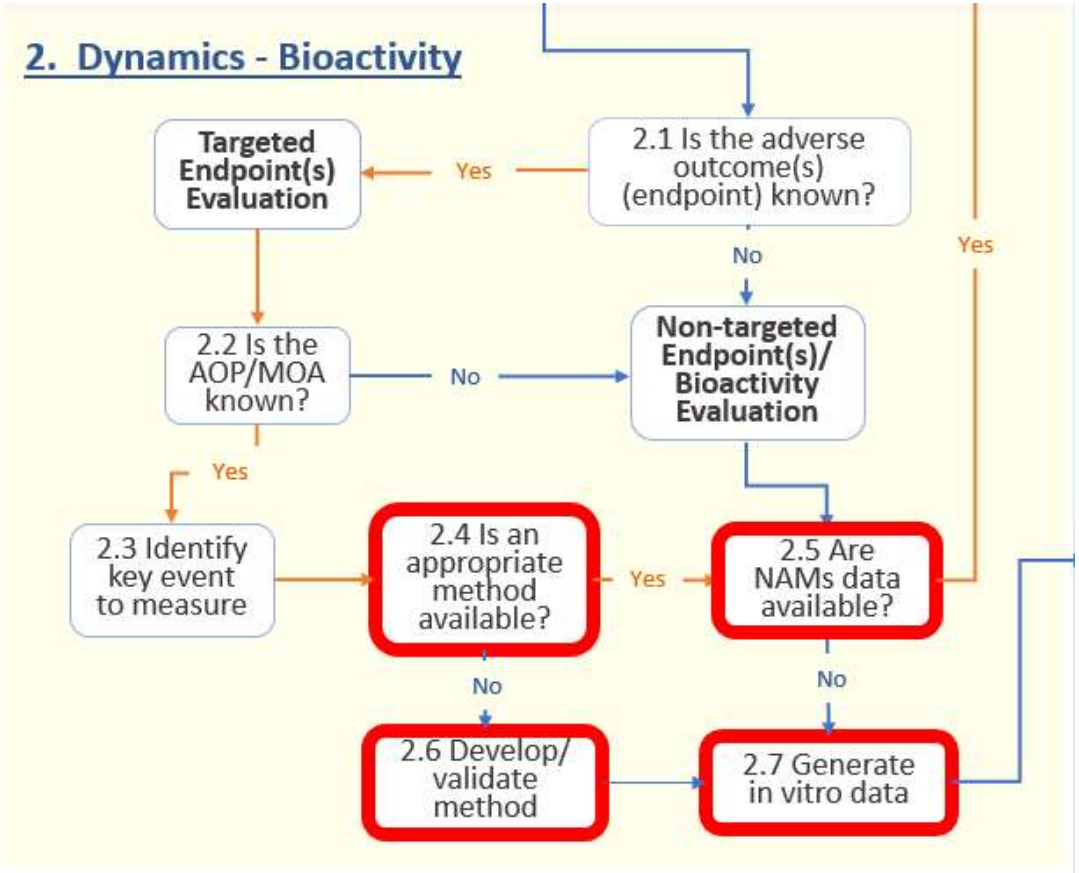
Extend applicability domain CYP induction method with industrial chemicals and pesticides

**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.

# 5. Current regulatory needs for *in vitro* metabolism methods




Framework for the Application of New Approach Methods: Metabolism considerations

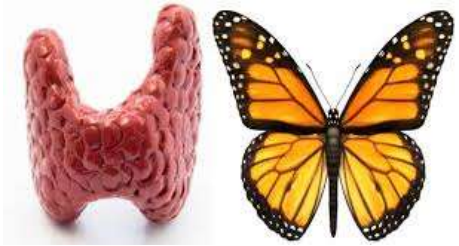
HORIZON 2020

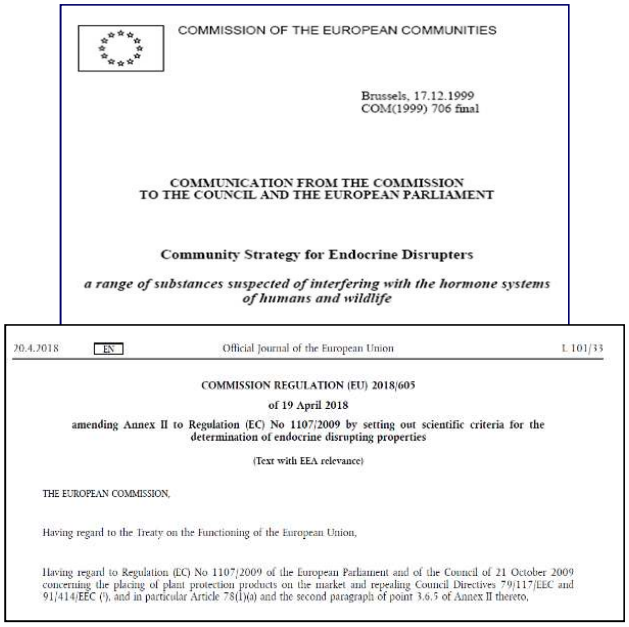
EUTOXRISK

An Integrated European 'Flagship' Programme Driving Mechanism-based Toxicity Testing and Risk Assessment for the 21st century – is a European collaborative project funded by the EU Framework Programme for Research and Innovation, Horizon 2020.

23-24 February
https://www.eu-toxrisk.eu/page/en/project-outreach/new-and-sevents.php

Large scale collaborative effect to tackle global thyroid disruption health burden using a combination of mechanistic *in vitro* methods





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***

20.4.2018 EN Official Journal of the European Union L 101/33

**COMMISSION REGULATION (EU) 2018/605**

**of 19 April 2018**

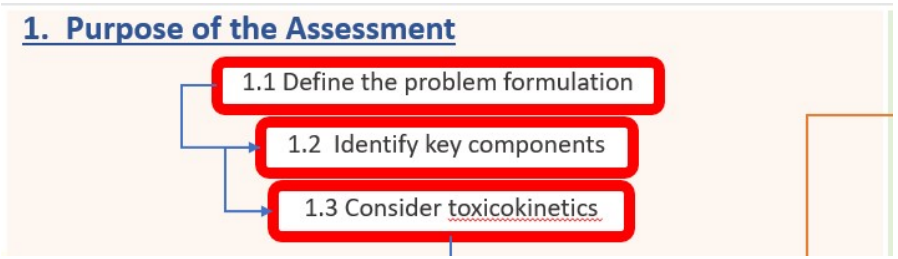
**amending Annex II to Regulation (EC) No 1107/2009 by setting out scientific criteria for the determination of endocrine disrupting properties**

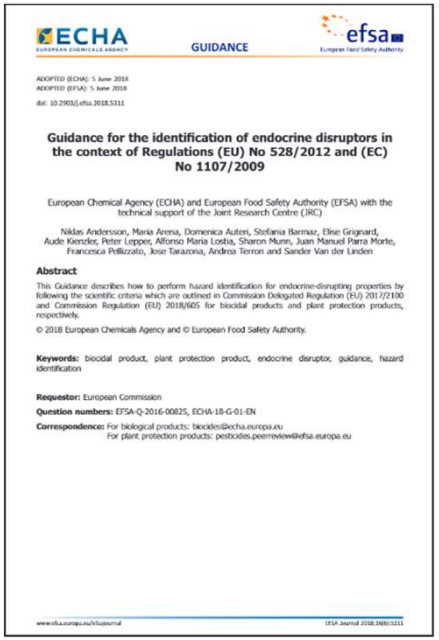
(Text with EEA relevance)

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 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 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 GUIDANCE efsa

**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)

**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.

© 2018 European Chemicals Agency and © European Food Safety Authority.

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

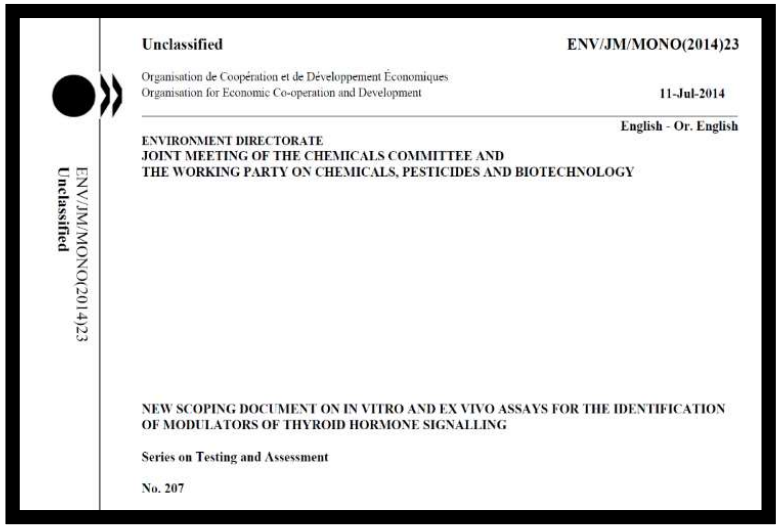
**Requestor:** European Commission

**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

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

OECD
BETTER POLICIES FOR BETTER LIVES



**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**

ENV/JM/MONO(2014)23
Unclassified

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

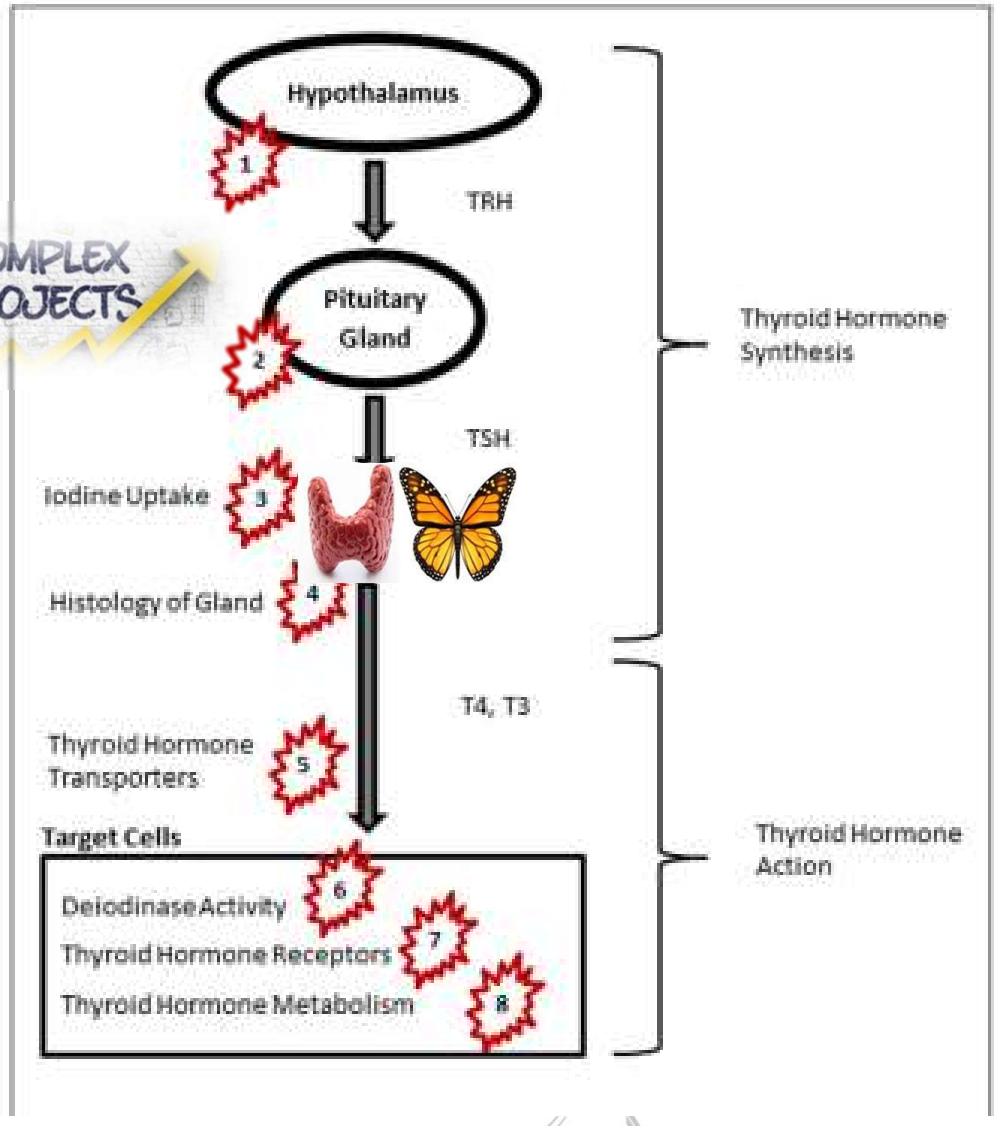
**Series on Testing and Assessment**

**No. 207**

European Commission

**TABLE 1** | Endocrine disrupting chemicals (EDCs) and target of action in the hypothalamus–pituitary–thyroid axis.

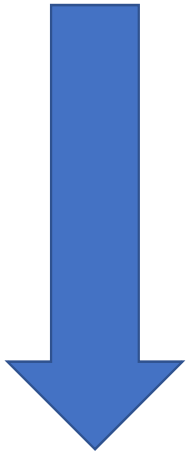
| Groups of EDCs | Target of action | |
|---|---|---|
| Polychlorinated biphenyls and polychlorinated dibenzodioxins (PCDD) | Thyroid hormone transportation<br>Thyroid hormone receptors | 5, 7 |
| Polybrominated diphenyl ethers | Thyroid hormone transporters<br>Deiodinase activity in the thyroid gland<br>Thyroid hormone receptors<br>Thyroid hormone metabolism | 5, 6, 7, 8 |
| Pesticides | Histology of thyroid gland<br>Thyroid hormone transportation<br>Thyroid hormone receptors | 4, 5, 7 |
| Perfluoroalkyl substances (PFASs) | Thyroid hormone transportation<br>Deiodinase activity in the thyroid gland | 5, 6 |
| Sodium iodide symporters (NIS) | Iodine uptake into the thyroid gland | 3 |
| Bisphenol A and other phenols | Expression of thyroid receptor genes in the pituitary<br>Thyroid hormone receptors | 2, 7 |
| Phthalates | Thyroid-releasing hormone receptor in the hypothalamus and pituitary<br>Thyroid-stimulating hormone receptor in the thyroid gland<br>Expression of genes related to thyroid hormone metabolism, synthesis, and transportation | 1, 2, 5, 8 |




Akhgar Ghassabian and Leonardo Trasande, Frontiers in Endocrinologie 2018

European Commission

## 14+ method developers




1. University of Pisa, Pisa, Italy.
2. Karolinska Institutet, Solna, Sweden.
3. OECD, Paris, France.
4. National Museum of Natural History, Paris, France.
5. Bayer AG, Wuppertal, Germany.
6. US Environmental Protection Agency, Durnham, USA.
7. Masaryk University, Brno, Czech
8. Republic. University of Antwerp, Antwerp, Belgium.
9. Charité-Universitätsmedizin, Berlin, Germany.
10. University of Catania, Catania, Italy.
11. Syngenta, Cambridge, United Kingdom.
12. German Federal Institute for
13. Risk Assessment, Berlin, Germany.
14. Health Canada, Ottawa, Canada

## 15+EU-NETVAL labs

https://ec.europa.eu/jrc/en/eurl/ecvam/alternative-methods-toxicity-testing/eu-netval

## 18+Methods

https://ec.europa.eu/jrc/en/eurl/ecvam/alternative-methods-toxicity-testing/eu-netval

The European Commission Joint Research Centre's European Union Reference Laboratory for alternatives to animal testing in collaboration with European Union Network of Laboratories for the Validation of Alternative Methods has launched a validation study to assess 17-mechanistic methods to detect chemicals that may interact with the thyroid hormone system.

FICAM


https://tsar.jrc.ec.europa.eu/

Thyroid method 2c: Tyrosine iodination using liquid chromatography

| | | | |
|---|---|---|---|
| **TM Status:** | Open | **Topic(s):** | Endocrine disruption |
| **Test Method Number:** | TM2019-06 (EU) | | |
| **Short Name of TM:** | TYRO-IOD | | |
| **Responsible Organisation:** | EURL ECVAM - European Union | | |

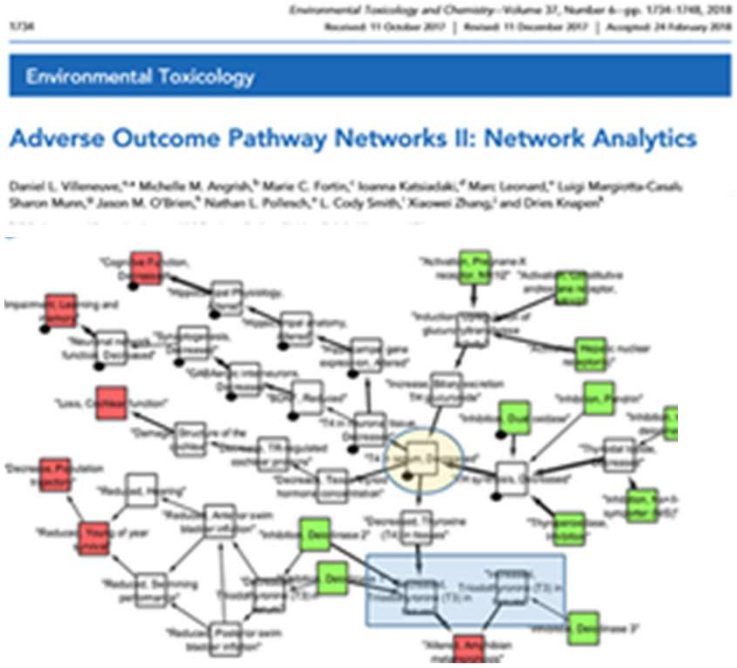
SUBMISSION Finalised | VALIDATION Ongoing | PEER-REVIEW Step not reached | RECOMMENDATION Step not reached | REGULATORY ACCEPTANCE/STANDARDS Step not reached

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

| | | | |
|---|---|---|---|
| **TM Status:** | Open | **Topic(s):** | Endocrine disruption |
| **Test Method Number:** | TM2019-07 (EU) | | |
| **Short Name of TM:** | NIS-SKR | | |
| **Responsible Organisation:** | EURL ECVAM - European Union | | |

SUBMISSION Finalised | VALIDATION Ongoing | PEER-REVIEW Step not reached | RECOMMENDATION Step not reached | REGULATORY ACCEPTANCE/STANDARDS Step not reached

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




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

European Commission

Framework for the Application of New Approach Methods: **Metabolism methods**

| 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) |

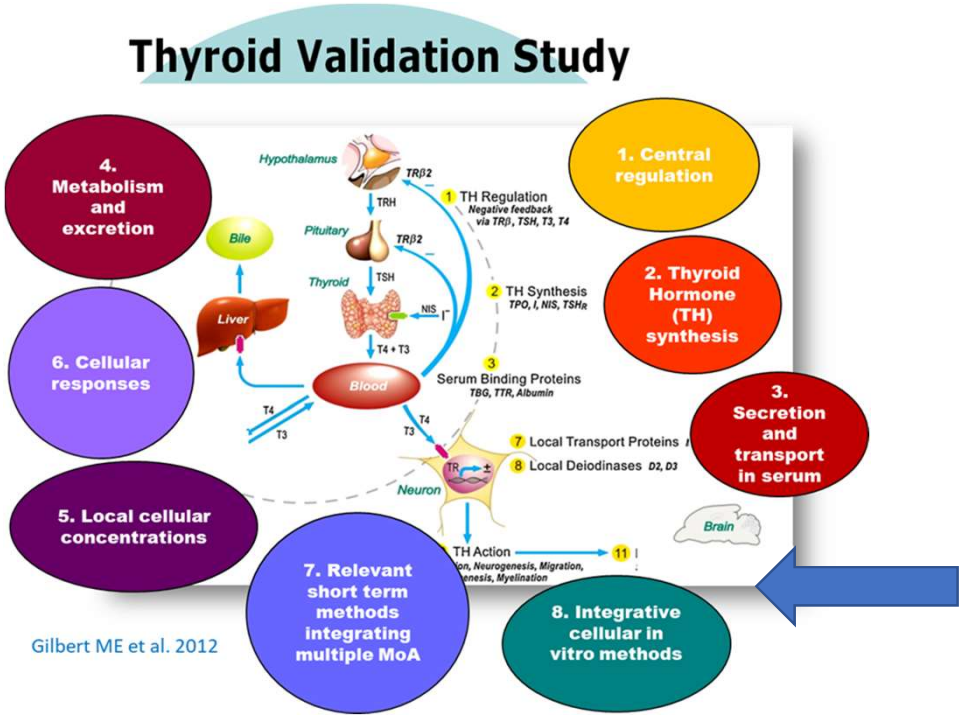
Hepa RG?

# Application of the framework in the thyroid validation study using New Approach Methods including **Metabolism methods**

**Amiodarone is an antiarrhythmic agent inducing adverse effects on the nervous system, among others.**

**Amiodarone** inhibits the monodeiodination (5-deiodinase activity) of T4.
This leads to a decrease in the generation of T3 from T4, a decrease in the clearance of reverse T3 (rT3) and consequently increased rT3 accumulation

**Amiodarone** is metabolized to desethylamiodarone by the cytochrome P450 (CYP450) enzyme group, specifically cytochrome P450 3A4 (CYP3A4) and CYP2C8. The CYP3A4 isoenzyme is present in both the liver and intestines




Gilbert ME et al. 2012

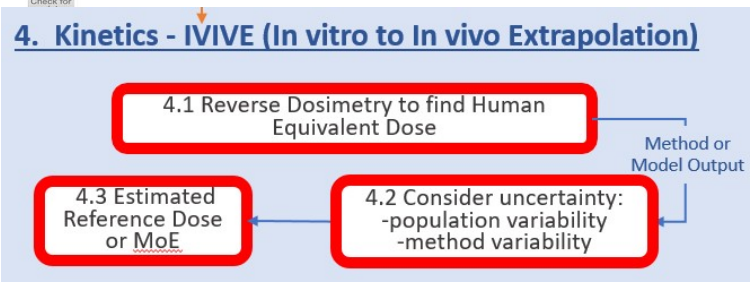
Archives of Toxicology
https://doi.org/10.1007/s00204-021-02989-2

ORGAN TOXICITY AND MECHANISMS

**Prediction of the dose range for adverse neurological effects of amiodarone in patients from an in vitro toxicity test by in vitro–in vivo extrapolation**

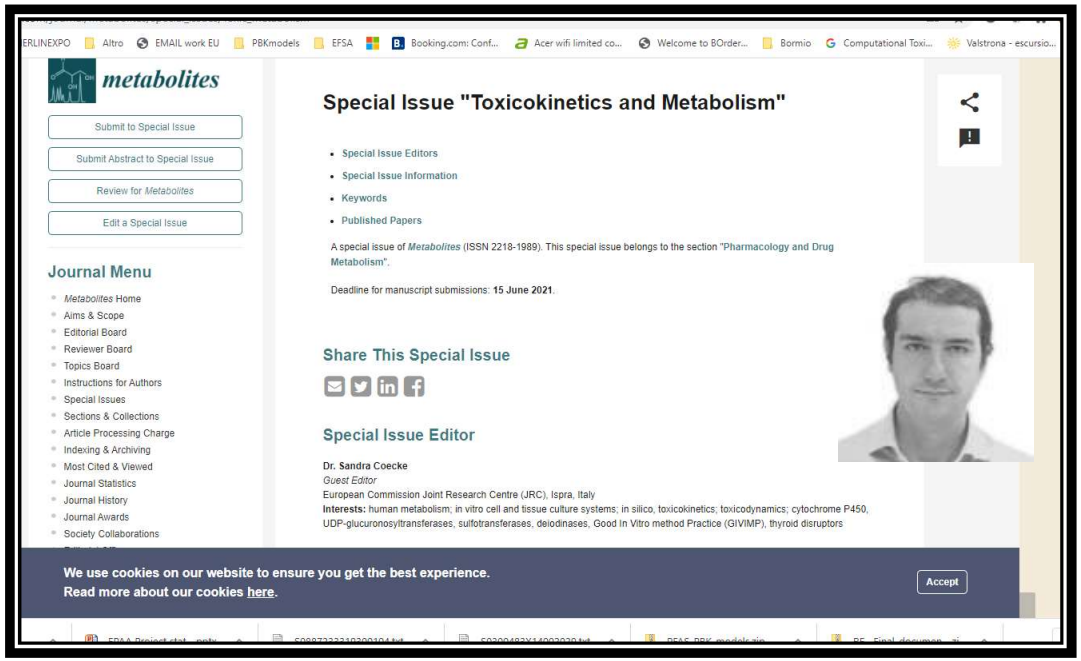
Engi Abd el-Hady Algharably[1] · Emma Di Consiglio[2] · Emanuela Testai[2] · Reinhold Kreutz[1,3] · Ursula Gundert-Remy[1]

Received: 31 August 2020 / Accepted: 21 January 2021

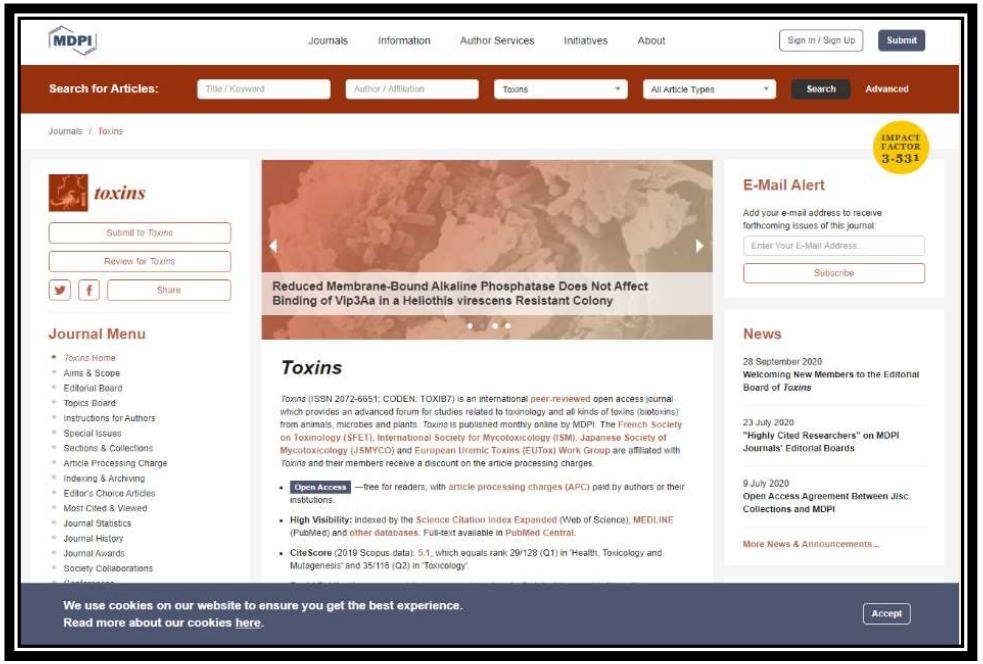



# Special issues in MDPI

SI: Toxicokinetics & Metabolism
Guest Editor:
Dr. Sandra Coecke EC JRC




SI: Computational Toxicology
Guest Editors:
Dr. Annie Jarabek US EPA
Dr. Peter Egeghy US EPA
Dr. Alicia Paini EC JRC



**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*

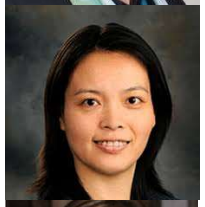
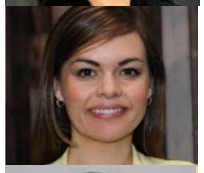


efsa
European Food Safety Authority

European Commission

Annual Meeting & ToxExpo
VIRTUAL EVENT • MARCH 2021



# WORKSHOP SESSIONS

A Future Framework for Application of *In Vitro* Metabolism and QIVIVE Models to Inform Risk Assessment

*Esther Haugabrooks, Sandra Coecke, Xiaoqing Chang, Kelly Magurany, Sue Marty, Rebecca Clewell*

***Monday 15 March 2021, 11.15 till 14.00 (US time)***

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