2nd LIFE-EDESIA workshop

The role of *in vitro* functional assays for the assessment of Endocrine Disruptors (EDCs)

July 20th - 22nd, 2015

Hotel Belvedere, Meeting room, Ranco (VA), Italy

Organized by:

Istituto Superiore di Sanità (ISS), Rome, Italy

In collaboration with:

the Italian Platform of Alternative Methods (IPAM)

the Center for Alternatives to Animal Testing (CAAT) - Europe
WELCOME ADDRESS

Dear Colleagues,

it is a great pleasure to welcome you in Ranco (VA), Italy, at the 2\textsuperscript{nd} LIFE-EDESIA workshop on “The role of in vitro functional assays for the assessment of Endocrine Disruptors (EDCs)”, organized by the beneficiaries of the LIFE-EDESIA project “Endocrine Disruptors in silico/in vitro – Evaluation and substitution for industrial applications” (LIFE12 ENV/IT/000633) in collaboration with the Italian Platform of Alternative Methods (IPAM) and the Center for Alternatives to Animal Testing (CAAT).

The interest in alternative in silico and in vitro methods for toxicity testing is devoted to the processes of screening and prioritization of chemicals but also to exploiting their potential in hazard characterization. In this context, Endocrine Disruptors (or EDCs) represent a priority issue, whose relevance is highlighted by EDC inclusion among the Substances of Very High Concern (SVHC) within the REACH Regulation (REACH, EC No 1907/2006).

Within LIFE-EDESIA project, alternative in silico and in vitro methods are applied in searching for plasticizers’ alternative having no or less adverse effects in terms of endocrine disrupting activity(ies). The in vitro LIFE-EDESIA approach intends making a step forward compared to the purely mechanism-based assays for EDCs, since it aims to take advantage to currently used clinical biomarkers to be applied in toxicology as biomarkers of effect in cell-based functional assays.

LIFE-EDESIA project members are grateful to the Italian Platform on Alternative Methods (IPAM) and to the Center for Alternative to Animal Testing (CAAT) to cooperate in the organization of this 2\textsuperscript{nd} LIFE-EDESIA workshop, with the purpose to have a fruitful debate on different views on the assessment of EDC-like effects within the international scientific community.

Finally, the involvement of all attendees is deeply acknowledged as essential for a constructive exchange of ideas on the workshop topic.

Have you a pleasant stay in Ranco,

Alberto Mantovani, LIFE-EDESIA project coordinator

Stefano Lorenzetti, LIFE-EDESIA project manager
AIMS OF THE WORKSHOP

The 2nd LIFE-EDESIA workshop on “The role of in vitro functional assays for the assessment of endocrine disruptors/EDCs” will focus on the combined employment of human cell cultures and clinical biomarkers in the field of endocrine disruption with the main goal to characterize EDC adverse effects at the cellular level; this achievement will promote the assessment and validation of alternative methods to the animal experimentation in agreement with the requirements of directive 2010/63/EU and REACH policy as well as reinforce the use of cell-based bioassays in toxicology, environmental sciences and biomedical research beyond their current application for mechanistic purposes.

The 2nd LIFE-EDESIA workshop will be open only to invited scientists and experts (max 25 persons), from academy and industry, regulatory agencies and no-profit organizations to discuss current main topics on the identification and characterization of EDC-like activities by whole (in vitro) cellular endpoints compared to mechanistic-based approach (e.g., gene reporter assays) to define (dis)advantages of different screening approach in the field of EDC toxicology.

Within the LIFE-EDESIA project, we are taking advantages from currently used clinical biomarkers as cell-specific biomarkers of effect to be applied in toxicology in cell-based functional assays. We aim to discuss the LIFE-EDESIA approach within the frame of the current views about an “Adverse Outcome Pathway” and in comparison with the EU and USA approaches to look for EDC adverse effects.

The 2nd LIFE-EDESIA workshop is divided in a talk introductory session (1st day), followed by a whole 2nd day of discussion in two working groups about (A) “Building Adverse Outcome Pathways for EDCs: the concept of adversity and the use of biomarkers (anchoring adversity to biomarker of effects)” and (B) “EDCs: from screening to testing to assessment”. The 3rd day will be devoted to discuss the exchange of ideas of the working groups and then on how to transfer such ideas to the scientific community to move a step forwards in the field of EDC toxicology.

Alberto Mantovani, LIFE-EDESIA project coordinator

Stefano Lorenzetti, LIFE-EDESIA project manager
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Acknowledgements

The organization of the LIFE-EDESIA workshops is made possible by the support of the LIFE+ programme (LIFE12 ENV/IT/000633) and the 2nd LIFE-EDESIA workshop also thanks to the contribution of both the Italian Platform of Alternative Methods (IPAM; www.ipam.it) and the Center for Alternatives to Animal Testing (CAAT) – Europe (http://cms.uni-konstanz.de/leist/caat-europe/).
PROGRAMME DAY 1 - July 20th, 2015 - Monday

LECTURES on workshop topics

14.00  Definition of adverse effects in EDC toxicology
   Francois Busquet (CAAT-EU)

14.30  Specific aspects of pharmaco- and toxico-dynamics related to EDCs
   Scott Belcher (Cincinnati Univ., USA)

15.00  Use of High Throughput Assays and Predictive Models by the U.S. Environmental Protection Agency’s Endocrine Disruptor Screening Program
   David Dix (US EPA, USA)

15.30  Development of international performance standards and performance-based test guidelines for nuclear receptor transactivation assays
   Anne Milcamps (JRC-IHCP, I)

16.00  The development of in vitro assays for EDCs: the industry perspectives
   Bart van der Burg (BDS, NL)

16.30  coffee break

17.00  Assessment of potential endocrine disrupting effects in the EU Pesticides Peer Review
   Daniele Court Marques (EFSA, I)

17.30  Adverse Outcome Pathways: a step toward Integrated Approaches for Testing and Assessment
   Thomas Hartung (CAAT-USA)

18.00  LIFE-EDESIA: the role of functional assays to implement the substitution principle for EDCs
   Stefano Lorenzetti (ISS, I)

18.30  Discussion

19.00  Establishment of Working Groups participants, chairs and rapporteurs.

19.30  Day 1 end

20.30  workshop dinner
PROGRAMME DAY 2 - July 21st, 2015 - Tuesday

WORKING GROUPS

09.30 - 12.30 Working Group discussion

A. Building Adverse Outcome Pathways for EDCs: the concept of adversity and the use of biomarkers (anchoring adversity to biomarker of effects)

B. EDCs: from screening to testing to assessment

12.30 workshop lunch

14.30 - 17.30 Working Group discussion

18.00 Day 2 end

20.30 workshop dinner

PROGRAMME DAY 3 - July 22nd, 2015 - Wednesday

WORKING GROUPS

09.30 - 09.55 Working Group Report A

09.55 - 10.20 Working Group Report B

10.20 - 10.45 Questions on Reports A-B

chair: A. Mantovani, T. Hartung

10.45 coffee break

11.00 -13.00 Discussion on the Content and Structure of a WS Consensus Statement & on the WS draft paper

chair: S. Lorenzetti, C. Rovida

13.00 Concluding remarks

13.15 Day 3 end
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Francois BUSQUET

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Short Curriculum vitae

Francois Busquet is a French toxicologist with a PhD in life sciences. He has worked for the last 10 years in the field of toxicology/safety testing which covered experiences in the private sector, within EU institution and currently for academia. His specialties cover 1) alternatives to animal testing 2) regulatory toxicology 3) ecotoxicology and 4) zebrafish. From 2005 to 2008, during his PhD at Merck KgaA (Germany), he developed an alternative test method with zebrafish eggs for human reproductive toxicity studies. The German regional government (Hessen) acknowledged his work with an animal welfare prize in 2008.

From 2009 to 2012, he worked at the European Commission at the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM) of the Institute for Health and Consumer Protection, Joint Research Centre (Ispra, Italy). He was coordinating with his supervisor the international validation study of the Zebrafish egg test for chemicals environmental toxicity for the OECD. The test guideline is now adopted since July 2013 (TG236).

Since 2012, Francois Busquet is working for CAAT (Center for Alternatives to Animal Testing) which is a joint venture between University of Konstanz (Germany) and Johns Hopkins University (USA). He is responsible for the Policy Program in Brussels. His role is to foster the novel approaches and tools on alternatives to animal testing at the European Parliament, Member States, to stakeholders level and to the national regulatory agencies. In 2014, the policy program was the recipient of the Lush prize for lobbying.

Since July 2014, he is an ecotoxicologist consultant for REACH mastery which registers dossier for ECHA. He is a member of the french society of toxicology since December 2014 as well as scientific collaborator for the "Universite Libre de Bruxelles - ULB” on alternatives to animal testing. Last but not least, he holds the position of secretary for ECOPA (European COnsensus Platform for Alternatives approaches).
Abstract

Definition of adverse effects in EDC toxicology

When it comes to endocrine disruptors (EDs), consensus has been reached in European Union (EU) about its definition (WHO, IPSC) and the need to observe adverse effects. Beyond these two aspects, legislative work (EU interim criteria, EU EDs impact assessment), public debate (bisphenol A, phthalates) and scientific discussion (low-dose, monotonic responses) are still ongoing. Criteria’s definition to sort out suspected compounds are crystallizing all the attention since it is required in specific EU legislations (i.e. plant protection products, cosmetics regulation, REACH or Biocides). Even if the criteria indeed deserve such a special focus, the tools to derive the results are not less important since they will “filter” out the good, the bad and the ugly ones. Therefore, special attention should be dedicated to these tools and assess reliability, relevance and reproducibility. Otherwise, the motto “trash in, trash out” will not be avoided even if adequate criteria are in place. Keeping this in mind, this presentation intends to set the stage in identifying common challenges to be tackled by the scientific community when it comes to the use of in vitro tools and how to connect the dots to extrapolate in vitro data for “macro” or adverse effects. A quick overview of the current tools and methods (e.g. OECD conceptual framework, adverse outcome pathways, US EPA endocrine disrupter screening program (tier 1) and TiPED) will be given to update the audience on trends and work in progress for regulatory purposes.
Scott BELCHER

*Prof. of Pharmacology and Cell Biophysics, College of Medicine at the University of Cincinnati*

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**Short Curriculum vitae**

His research interests center around understanding the molecular, cellular, and physiological mechanisms that regulate the developmental actions of estrogens and endocrine disruptors in the heart and developing brain. Current research is primarily focused on mechanisms involving estrogen receptor beta action that influence development of the cerebellum, cardiac pathology and estrogen-responsive growth of childhood brain tumors.

**Education/Training:**

1990-93  PhD: University of Texas Southwestern, Dallas, TX  
Mitochondrial Molecular Genetics

1993-97  Post Doc: Yale University School of Medicine, New Haven, CT  
Developmental Neuroscience

**Honors, Awards and other activities related to EDCs**


2010-2011  Member - Expert Committee – World Health Organization/Food and Agriculture Organization of the United Nations (FAO) – Expert Review of Toxicology and Health aspects of Bisphenol A.

03/2008  Lead Discussant - US EPA – FIFRA Scientific Advisory Panel - Endocrine Disruptor Screening Program Tier-1 Battery

01/2008  Peer-Review Consultant - US EPA - Endocrine Disruptor Screening Program Tier-1 Battery: Aromatase Assay

11/2007  Chair – In vitro Mechanism Focus Group - Chapel Hill Expert panel on BPA’s Risk to Human Health

**Editorial Boards (current):**

Abstract

The Role of Coregulators and Rapid Nongenomic Mechanisms as Modifiers of Estrogen Receptor Mediated Endocrine Disruption

The actions of endocrine active ligands (i.e. endogenous hormones, pharmaceuticals or environmental ligands) are most often defined as acting through “classical” nuclear receptor mechanisms that involve ligand-dependent nuclear receptor binding at promotors of responsive genes. During the past 25 years increased understanding the mechanisms involved in hormone actions have defined transcriptional coregulators as the primary modifiers of nuclear receptor function [1, 2], and rapid nongenomic signaling mechanism as playing a key role in physiological responses to hormones and EDCs [3, 4]. The role of rapid nongenomic actions of estrogen, BPA and other EDCs in developing neurons will be used to illustrate the dynamic role these mechanisms can have in modifying toxicodynamics and physiological outputs of exposures [5-8]. The importance of including this expanded mechanistic understanding into high-throughput screening, cell-based assays, computational models and adverse outcome pathways used to identify EDCs will be highlighted.

References.
Since May of 2013, Dr. David Dix has served as Director of the Office of Science Coordination and Policy (OSCP) of the U.S. Environmental Protection Agency (EPA) in Washington DC. OSCP provides coordination, leadership, peer review, and synthesis of science and science policy for EPA’s Office of Chemical Safety and Pollution Prevention, assuring sound scientific decisions and coordinating emerging exposure and hazard assessment topics, such as endocrine disrupting chemicals. Prior to joining OSCP, Dr. Dix was Deputy and then Acting Director of EPA’s National Center for Computational Toxicology in Research Triangle Park, NC, where he led development of high throughput decision support tools for chemical exposure, hazard and risk. Dr. Dix joined EPA’s Office of Research and Development in 1995 as a Research Biologist, leading studies in reproductive, genomic and computational toxicology. He is an Adjunct Professor in the Department of Environmental Sciences and Engineering at the University of North Carolina at Chapel Hill. Dr. Dix earned a B.S. in Biological Sciences from the University of Illinois, a Ph.D. in Physiology from Rush University in Chicago, followed by postdoctoral training at the U.S. National Institute of Environmental Health Sciences. He has published over 120 scientific articles, reviews, reports and book chapters; serves on several journal Editorial Boards; and has represented EPA at a wide range of national and international meetings.
Abstract

Use of High Throughput Assays and Predictive Models by the U.S. Environmental Protection Agency’s Endocrine Disruptor Screening Program

The U.S. Environmental Protection Agency’s (EPA) Endocrine Disruptor Screening Program (EDSP) is incorporating an alternative scientific approach to screen chemicals for their ability to interact with the endocrine system. This shift will improve the Agency’s ability to fulfill its statutory mandate to screen pesticide chemicals and other substances for their ability to cause adverse effects by their interaction with the endocrine system. The approach incorporates validated high throughput assays and a predictive model and, based on current research, can serve as an alternative for some of the current assays in the Endocrine Disruptor Screening Program (EDSP) Tier 1 battery. EPA has partial screening results for over 1800 chemicals that have been evaluated using high throughput assays and a predictive model for the estrogen receptor pathway. In the future, EPA anticipates that additional alternative methods will be available for EDSP chemical screening based on further advancements of high throughput assays and predictive models for other endocrine pathways. Use of these alternative methods will accelerate the pace of screening, decrease costs, and reduce animal testing. In addition, this approach advances the goal of providing sensitive, specific, quantitative, and efficient screening using alternative test methods in the Tier 1 battery to protect human health and the environment. The application of these alternative, innovative tools for screening chemicals for endocrine bioactivity represents the first step in a paradigm shift for chemical safety testing, and the first systematic application of EPA’s ToxCast data in an EPA regulatory program.

References.

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Anne MILCAMPs

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Short Curriculum vitae

Expertise/experiences: molecular biology, microbiology, genetic engineering, validation of methods (GMO and in vitro methods), toxicology, HTS screening

Education/Training:

- PhD: University of Leuven – KU Leuven, Belgium
- Plant Biology

Honors, Awards and other activities

2011  Scientific officer at JRC Ispra EURL ECVAM (Systems toxicology unit)
2002-2011 Scientific officer at JRC Ispra at EURL GMFF (GMO unit)
1998-2002 Research Assistant at Michigan State University, US, Department of Biology
1993-1998 Research Assistant at Michigan State University, US, Plant Research Laboratory
Abstract

Development of international performance standards and performance-based test guidelines for nuclear receptor transactivation assays

Since 1996, OECD has given priority to the activities on endocrine disruption (ED) given the evidence and recognition of the impacts on human and animal health by chemicals that disturb the endocrine system. In terms of providing overview and guidance, Test Guidelines are being developed and a number of important documents have been established e.g. a Conceptual Framework for testing and assessment of EDs [1]. In vitro assays, and more precisely hormone receptor binding assays and transactivation assays, are recommended as important tools for the fast screening of putative EDs and for prioritisation purposes. The European Commission supports OECD by the development of such Test Guidelines.

OECD initiated some time ago the concept of Performance Based Test Guidelines where mechanistically and functionally similar methods can be included in the same Test Guideline. Performance Standards (including essential elements of the method, a set of Reference Chemicals, accuracy and reliability performance values) accompany such PBTG, based on the validated methods, and, are intended to facilitate the development and validation of new similar methods. A PBTG was established in 2012 for Estrogen Receptor Transaction Assays (ERTAs) [2]. The European Commission (EURL ECVAM) proposed in 2012 to develop a PBTG on Androgen Receptor Transaction Assays (ARTAs) and related Performance Standards. This was accepted at OECD and included in the work program. This PBTG aims at the inclusion of 3 ARTAs for which the validation is finished or ongoing. While EURL ECVAM will coordinate the validation of the AR-CALUX method, submitted by BioDetectionSystems (BDS, Netherlands), it will investigate simultaneously the validation data of the Japanese ARTA method [3] and follow closely the ongoing validation study of the Korean ARTA method.

The AR-CALUX method [4], using osteosarcoma cells transfected with the cDNA for a human androgen receptor and a luciferase reporter construct, was in-house assessed by BDS and submitted to EURL ECVAM for a validation. EURL ECVAM evaluated the method for completeness, clarity, and suitability to be implemented in a GLP environment. In parallel, the validation study was prepared by selecting suitable laboratories to participate from the recently established EU-Network for the Validation of Alternative methods (EU-NETVAL), by establishing a list of chemicals to be tested, by organising a group of experts to oversee the validation process. The validation process as well as the current state of play will be explained in more detail.

References.
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Short Curriculum vitae

Bart van der Burg got his PhD at the Faculty of Biology of the Utrecht University. Until 2002 he was a senior scientific staff member at the Hubrecht Institute of the Royal Netherlands Academy of Sciences in Utrecht, after which he joined BioDetection Systems as a Chief Scientific Officer and more recently Director of Innovation. He has ample experience in open innovation and as a leader of academic and industrial research groups of varying composition and has been coordinator of various large-scale collaborative research projects, including the FP7 ChemScreen project. He published more than 130 scientific publications that collectively were cited more than 8000 times (h-index=50). Focus is on development of novel mechanism-based test systems as alternatives to chemical analysis and animal experimentation.
Abstract

The development of in vitro assays for endocrine disrupting compounds (EDCs): an industry perspective

Industrial activities and particular those in small and medium enterprises (SMEs) form the backbone of the European economy, providing jobs and innovative products. Safety assessment is a critical issue in the chemical industry and reliable, well-defined and harmonized test methods are important to assure efficient chemical safety assessment. Careful test guideline development that are accepted preferably globally is an important step in this process. These processes are inherently time consuming and conservative in nature, since they need to support objective, precise and harmonized assessment of new industrial chemicals. This careful evaluation using traditional test models, i.e. animal models has a downside and led to a new degree of uncertainty, namely unacceptable delays in the number of chemicals having a complete safety assessment, which is corrected in part by REACH. Even more importantly, chemical risk assessment does not address their effects in mixtures, which is the most likely type of exposure. The latter issue is particularly relevant for the testing of endocrine disrupting compounds, known to address the same molecular targets, leading to profound mixture effects. It is also known that mixture effects need to consider natural background activity levels caused by endogenous hormones and natural compounds. New assay development is pertinent in this area and promising methods developed almost two decades ago are only now being considered for guideline inclusion. Clearly, there is room for improvement of these innovation routes, at the benefit of the competitiveness of innovative European SMEs involved in test development but also providing new opportunities to efficiently develop novel, “greener” products. While large companies may be able to accommodate the increased consumer demand for more safe and greener products, smaller enterprises have difficulties finding their way in this complicated area. Better collaboration between scientist, regulators and industries can be instrumental in more rapid innovation in safety assessment, each having their important role. The scientific community should try to avoid overselling problems associated with novel areas of concern, such as EDCs, to avoid propositions of too complicated and impractical novel testing procedures that may lead to a cost increase that affects the competitiveness of European industries. An important opportunity of the scientific community is to come to a consensus on central elements to be included in innovations in testing strategies, since without that regulators and industries will hesitate to change the current way of risk assessment. On the other hand, regulators and industry itself can be more proactive and more actively engaged in embracing novel in vitro technologies, since it is their combined responsibility to optimally protect consumers. In introducing new assays in the regulatory arena, it is essential to use established scientific insight in the mode of action of major endocrine systems known to be vulnerable to effects of EDCs, such as the estrogen-, androgen-, and thyroid receptor mediated signaling system, including some major enzymes and binding proteins. I will discuss some examples of straightforward and robust novel assays that focus on these central mechanisms [1-3], and the way how the results of these screening assays can be linked to adverse outcome pathways in order to predict complex toxicological endpoints such as reproductive toxicity [4-8]. These assays can also be used to assess the combined effect of chemical mixtures [9-12] and to measure background levels and chemical-exposure associated elevations in wildlife and humans [13-16], linking molecular initiating events in adverse outcome pathways to adversity in real-life situations.

References.


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Short Curriculum vitae

Danièle Court Marques has Portuguese and Swiss nationalities; she studied pharmaceutical Sciences at the Faculty of Pharmacy of Lisbon University (MSc), Phytopharmacology at the Institute Superior of Agronomy of Lisbon and an extended university degree in genetic toxicology and toxicogenomic at the Centre of Investigation in Human Molecular Genetics at the University Nova of Lisbon. She followed a postgraduate course on computer modelling of toxicokinetics and cellular signalling pathway at the Karolina Institute of Stockholm, Sweden. She worked for 13 years for the Portuguese ministry of Agriculture of Portugal in the area of pesticides risk assessment for human health, for national authorisations and participation to the European peer review, classification of pesticides, and risk assessment of operator, worker, bystander and residents exposure to pesticides. Since 2007, she works at the European Food Safety Authority, mammalian toxicology team of the pesticides Unit, dealing with the EU peer review of active substances used in plant protection products. She is a member of the European Chemical Agency expert group on endocrine disrupters. She participated in meetings and dialogues on endocrine disruption, which is one of the key elements of the pesticides risk assessment with the entry into force of Regulation (EC) 1107/2009.
Abstract

Assessment of potential endocrine disrupting effects in the EU Pesticides Peer Review

The assessment of endocrine disrupting (ED) properties of pesticide active substances (a.s.) has become one of the key element of the pesticides peer review according to Regulation (EC) 1107/2009 [1]. The approval criteria of a.s. included in plant protection products (PPP) establish that an a.s. shall be approved if it may be expected, in the light of current scientific and technical knowledge, that the PPP containing this a.s. and the residues consequent to PPP applications shall not have any harmful effects on human health, including that of vulnerable groups, or animal health, taking into account known cumulative and synergistic effects and shall not have any unacceptable effect on the environment. Criteria for the approval of a.s. regarding the impact on human health include that the a.s. cannot have ED properties that may cause adverse effect in humans, unless the exposure of humans is negligible. The Regulation calls for an agreement regarding specific scientific criteria for the determination of ED properties, pending the adoption of these criteria, interim measures were established based on the classification (carcinogenic category 2, toxic for reproduction category 2) and toxic effects on the endocrine organs to consider a substance as having ED properties. These measures however give room for some interpretation as the definition of endocrine organs is not yet agreed.

New data requirements associated to the PPP Regulation indicate the need of addressing the toxic mode of action through the generation of human metabolic profile, functional testing to clarify effects on the nervous, immune or endocrine systems with emphasis on effects accentuated over generations. Where there is evidence that the a.s. may have ED properties, additional information or specific studies are required to elucidate the mode/mechanism of action (MoA), and to provide sufficient evidence for relevant adverse effects. A search of the scientific peer-reviewed open literature on the a.s. and its relevant metabolites, dealing with side-effects on health, the environment and non-target species is mandatory.

In this context, the EFSA peer review of pesticide risk assessment performs an evaluation of the available evidence, in line with the opinion of the EFSA Scientific Committee on the hazard assessment of endocrine disruptors (EFSA SC, 2013) and testing strategies guidance developed by the OECD (OECD, 2012) regarding the oestrogen, androgen, thyroid and steroidogenesis pathways. Particular attention is given to adverse effects which may plausibly be linked to an ED MoA, available mechanistic studies and possible read-across from structure-related substances. The outcome of the scientific assessment is compared to the criteria in place for a hazard-based assessment and is used as well in the hazard characterisation and risk assessment to identify concerns. Recent examples of the EFSA peer review of pesticide risk assessment show that even in the case of data rich dossiers as is the case of pesticides, a firm conclusion is rarely achieved regarding ED properties and this is reflected in a number of data gaps and non-finalised ED assessments; further research and development of adverse outcome pathways in the field of endocrine disruption is seen as promising improvements in the risk assessment.

References.


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Short Curriculum vitae

Thomas Hartung, MD PhD, is Professor of Toxicology (Chair for Evidence-based Toxicology), Pharmacology, Molecular Microbiology and Immunology at Johns Hopkins Bloomberg School of Public Health, Baltimore, and University of Konstanz, Germany; he also is Director of their Centers for Alternatives to Animal Testing (CAAT, http://caat.jhsph.edu) with the portal AltWeb (http://altweb.jhsph.edu). CAAT hosts the secretariat of the Evidence-based Toxicology Collaboration (http://www.ebtox.com) and the industry refinement working group. As PI, he heads the Human Toxome project (http://humantoxome.com) funded as an NIH Transformative Research Grant. He is the former Head of the European Commission’s Center for the Validation of Alternative Methods (ECVAM), Ispra, Italy. He has authored more than 430 scientific publications.
Abstract

Adverse Outcome Pathways: a step toward Integrated Approaches for Testing and Assessment

Despite the fact that toxicology uses many stand-alone tests, a systematic combination of several information sources very often is required: Examples include: when not all possible outcomes of interest (e.g., modes of action), classes of test substances (applicability domains), or severity classes of effect are covered in a single test; when the positive test result is rare (low prevalence leading to excessive false-positive results); when the gold standard test is too costly or uses too many animals, creating a need for prioritization by screening. Similarly, tests are combined when the human predictivity of a single test is not satisfactory or when existing data and evidence from various tests will be integrated. Increasingly, kinetic information also will be integrated to make an in vivo extrapolation from in vitro data.

Integrated Testing Strategies (ITS) or Integrated Approaches for Testing and Assessment (IATA) offer the solution to these problems. ITS have been discussed for more than a decade, and some attempts have been made in test guidance for regulations. Despite their obvious potential for revamping regulatory toxicology, however, we still have little guidance on the composition, validation, and adaptation of ITS for different purposes. Similarly, Weight of Evidence and Evidence-based Toxicology approaches require different pieces of evidence and test data to be weighed and combined.

ITS also represent the logical way of combining pathway-based tests, as suggested in Toxicology for the 21st Century. In recent years a number of Tox-21c technologies, have emerged: Stem cell technologies make human cells of high quality and individual donors available; organo-typic cultures improve the physiological relevance of in vitro systems; quality assurance and standardization of cell cultures is improving; miniaturized models accommodate the small amounts of substance available at early stages of development as well as robotized testing of many candidates; high-content measurements such as various omics and high-content imaging techniques allow wholesome phenotyping of reactions stretching to the molecular level, i.e. the Adverse Outcome Pathways (AOP) or Pathways of Toxicity (PoT). The AOP lend themselves as a tool to construct ITS / IATA covering the underlying mechanism. The presentation describes the state of the art of ITS and makes suggestions as to the definition, systematic combination e.g. by machine learning approaches, and quality assurance of ITS.

References.

Stefano LORENZETTI

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**Short Curriculum vitae**

Stefano Lorenzetti, graduated in Biological Sciences (1991) and in Human Nutrition Sciences (2003), since 2006 is a staff researcher at the Dpt. of Food Safety and Veterinary Public Health of the Italian National Health Institute - ISS (Istituto Superiore di Sanità) in Rome. He has been lecturer (2006-09) in Molecular Biology and Toxicology at the Faculty of Medicine of University *Tor Vergata* of Rome at the post-graduated course of Sciences of Nutrition. Since January 2012, he has been appointed as a member of the Italian national expert group of “Alternative methods to animal experimentation”.

SL research is focused on the set up of *in vitro* alternative methods to animal experimentation to study the role of endocrine disruptors/EDCs or Endocrine Active Substances/EASs such as bioactive compounds of plant origin (*e.g.* polyphenols) and environmental and dietary contaminants (*e.g.*, plasticizers, pesticides and biocides).

SL participated to different international projects on the hazard and risk characterization of environmental and dietary contaminants such as: the EU Integrated Project ReProTect (“Development of a novel approach in hazard and risk assessment or reproductive toxicity by a combination and application of *in vitro*, tissue and sensor technologies”; 2006-2010) to develop an integrated *in vitro* approach linking toxicogenomics to clinical biomarkers (phenotypic anchoring), the NIH-ISS project “Tackling rare diseases yet lacking diagnosis and/or prognosis: a pilot project integrating data collection and experimental studies” (2007-10) linking *in utero* DEHP exposure to liver programming and hepatoblastoma; the Italian Health Ministery granted project “*In vitro* alternative methods: male reproductive prostate-mediated toxicity of potential SVHC “ (2012-13).

SL is currently the project manager of the EU-granted LIFE-EDESIA project (“Endocrine Disruptors *in silico / in vitro* - Evaluation and Substitution for Industrial Applications”; LIFE12 ENV/IT/000633) and the scientific responsible of the national project PRO-HEALTH-Zinc (“Biomonitoring of zinc and selenium as biomarkers of clinical support to PSA measurement for the prediction of prostate diseases“).
Abstract

According to the requirements of the European Regulation REACH, the development of in vitro testing strategies should provide a cost-effective generation of comprehensive datasets on a large number of chemicals. Screening tools (1-9) based on non animal, cell-based assays are under development taking advantage of the current knowledge about known endocrine-dependent subcellular mechanisms (e.g., ligand binding to nuclear receptors) and/or their cellular effects (1, 5, and refs therein).

The predictive value of molecular or biochemical changes needed to produce a specific adverse effect require the assessment of cell-specific downstream response(s), a pivotal intermediate event within an Adverse Outcome Pathway (AOP) that links a cell property to a measurable toxicological endpoint (1, 5, and refs therein).

Several in vitro assays are based on human cell lines representative of endocrine-targeted tissues (e.g., prostate) and on functional biomarkers of clinical relevance (e.g., PSA secretion in human prostate epithelial cells). The implementation of such functional biomarkers in the AOP context will be discussed also comparing the different “adverse effect” assessed by the LIFE-EDESIA functional assays vs mechanistic-based approach (1, and refs therein).

References.
Dr. Paloma Alonso-Magdalena, BSc, PhD, graduated from University of Oviedo (Spain) and completed her PhD at Miguel Hernandez University (Spain). After a postdoctoral training in the Department of Nutrition at Karolinska Institutet, Stockholm, (Sweden) about the physiology of estrogen receptor ERβ, she is now an Assistant Professor of Nutrition and principal investigator at Miguel Hernández University of Elche, Alicante, Spain. She is particularly interested in understanding which is the role of the endocrine disruptors in the etiology of type 2 diabetes. Her research interest also includes the role of estrogens and their receptors in the physiology of the endocrine pancreas, and how estrogens influence the plasticity of the islets of Langerhans during the adaptation to pregnancy and obesity.

Short Curriculum vitae

Dr. Paloma Alonso-Magdalena, BSc, PhD, graduated from University of Oviedo (Spain) and completed her PhD at Miguel Hernandez University (Spain). After a postdoctoral training in the Department of Nutrition at Karolinska Institutet, Stockholm, (Sweden) about the physiology of estrogen receptor ERβ, she is now an Assistant Professor of Nutrition and principal investigator at Miguel Hernández University of Elche, Alicante, Spain. She is particularly interested in understanding which is the role of the endocrine disruptors in the etiology of type 2 diabetes. Her research interest also includes the role of estrogens and their receptors in the physiology of the endocrine pancreas, and how estrogens influence the plasticity of the islets of Langerhans during the adaptation to pregnancy and obesity.
EDC scientific interests

We are interested in the role that endocrine disruptor chemicals (EDCs) have in the etiology of diabetes and obesity. We work to understand the mechanisms of action and signaling pathways that mediate the low dose effects of EDCs on the endocrine pancreas and the consequences in glucose and lipid metabolism. We investigate the role of estrogen receptors in low dose effects of EDCs with estrogenic activity and how they affect insulin and glucagon biosynthesis and release.

In addition, we study how exposure to EDCs at different times during life affects insulin sensitivity and the function of the endocrine pancreas. We have interest in how EDCs exposure during pregnancy affects offspring and maternal glucose metabolism later in life.

Abstract

The role of in vitro functional assays for the assessment of endocrine disruptors in metabolic disorders

Metabolic disorders remain the leading causes of morbidity and mortality in the modern world. Diabetes mellitus is on the rise, which makes it one of the most important public health challenges. In the etiology of this disorder there is a strong genetic component; however, its rapidly increasing incidence seems difficult to explain just as a result of genetic changes, which comes to emphasize the important contribution of the environmental factors. Of note, novel environmental factors such as endocrine disruptor chemicals (EDCs) are emerging as important key players. Studies in animal models as well as human epidemiological studies have shown that some of these compounds can have diabetogenic behaviour.

Glucose homeostasis is precisely regulated by the hormones insulin and glucagon hormones, which are released by pancreatic β- and α-cells, respectively. Alterations in both type cells are crucial in the pathophysiological events related to the development of diabetes. In order to reveal the potential detrimental effects on glucose metabolism of endocrine disruptors, as well as mixtures of them, it results necessary to develop rapid and reliable in vitro models for testing endocrine-related endpoints. This could greatly reduce the need for compound testing in animals. As alternative insulin containing beta cell lines, including MIN6 and INS1E, and glucagon secreting alpha cell lines, like TC1-9, resemble quite well the physiology of beta and alpha cells respectively. For these reasons, we believe that the use of these cell lines in combination with the primary cultures of pancreatic islets would be useful to investigate if pancreatic function and thus, glucose metabolism, could be impaired because of a direct action of some of these compounds. Endpoints measured in these cells would include insulin and glucagon secretion, cell division, apoptosis, Ca²⁺ signalling, cell metabolism and mitochondrial function. In addition, mechanistic studies could be performed. This information will be of special interest in order to elucidate the potential impact of some EDCs for metabolic disruption.
Remi BARS

Responsable Toxicologie Recherche chez Bayer Pharmaceuticals

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Short Curriculum vitae

Remi Bars studied pharmacy and toxicology at the University of Paris XI. He did a PhD and a post-doc in the field of non-genotoxic liver carcinogens. He worked as a regulatory toxicologist for many years in the agrochemical industry. He is currently heading a research group at Bayer CropScience and is actively involved in mechanistic investigation and developing screening methods. Remi Bars is a member of the ECETOC Scientific Committee (European Centre for Ecotoxicology and Toxicology of Chemicals) and is currently part of the ECHA endocrine expert group to identify chemicals having endocrine disrupting properties.
Emilio BENFENATI
Istituto di Ricerche Farmacologiche “Mario Negri” (IRFMN)

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Short Curriculum vitae

Dr. Emilio Benfenati is head of the Laboratory of Environmental Chemistry and Toxicology at the Mario Negri Institute, Milan, Italy, since 1997. In the laboratory about 35 researchers are active. Previously he has been researcher at Stanford University, California, USA (in that period he also had a collaborative research at the Berkeley University, California, USA). He coordinated 16 European projects (including CAESAR, CALEIDOS, ANTARES, PROSIL, ToxBank, IMAGETOX) and participated to 22 others. Many of them are on toxicity and environmental modelling. His research activities include: toxicity and environmental modelling, molecular descriptors, QSARs, toxicity prediction, environmental management, characterisation and assessment of contaminants, risk assessment; development of QSAR models; analysis of environmental and food samples for pollutants such as dioxins, PCB, PAH, pesticides, endocrine disruptors, industrial pollutants; environmental assessment. He is author or co-author of about 300 papers in international journals and edited a few books.
Francesca CALONI

Prof. of Veterinary Toxicology, Department of Health, Animal Science and Food Safety, University of Milan

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Short Curriculum vitae

Francesca Caloni holds a Degree in Veterinary Medicine (1990) and a PhD in Veterinary Pharmacology & Toxicology (1995). After a Post-Doctorate Fellowship (1996-1998 at Univ. of Milan, Inst. of Veterinary Pharmacology & Toxicology), she first got a Researcher position (1999-2011, Univ. of Milan, Veterinary Medicine Faculty, Dpt. of Veterinary Sciences and Technologies for Food Safety) and then Associate Professor (2011-present) at Univ. of Milan, Dpt of Health, Animal Science and Food Safety (VESPA). Author and co-author of several publications. Member of scientific committees of National and International Congresses. Participant as Speaker and Chair in numerous conferences and meetings.

Teaching activity

2000-2004 Course: Food Toxicology in Domestic Animals, Degree Veterinary Medicine
2001-2004 Course: Applied Toxicology for the Protection of Wild Fauna and ‘Organic’ Farming, Degree Veterinary Medicine
2004-2006 Course: Reproductive Toxicology of Companion Animals, Degree Veterinary Medicine
2004-2015 Course: Applied Toxicology for the Protection of Fauna, Degree Veterinary Medicine
2002-2011 Course: Veterinary Toxicology, Specialization: Organic Animal Production Degree Farming & Animal Well-being
2010-2015 Course: Alternative Methods for Animal Testing and Toxicology Master in Veterinary Biotechnologies
2012-2015 Course: Food Toxicology in Domestic Animals –PAAS: Animal Production, Food, Health
2011-2015 Course: Veterinary Toxicology-Degree Veterinary Medicine

Scientific Activity

In vitro toxicological studies using species-specific models to evaluate absorption, bioavailability, metabolism, endocrine disruptor effects of xenobiotics. Epidemiological studies on poisoning in animals.

2005-2011 Board Member Italian Association of In Vitro Toxicology CELLTOX
2008-present Board Member European Society of Toxicology in Vitro, ESTIV
2009-Nominated Expert EFSA FEEDAP (from 06/2009 to 01/2010)
2011-present Working Group Expert Italian Reference Center for Alternative Methods, Welfare and Care of Laboratory Animals
2012-present Board Member Italian Platform of Alternative Methods (IPAM)
2013-present Member of the Advisory board of Center for Alternative to Animal Testing-EU (CAAT-EU)
2015-present President of Italian Association of In Vitro Toxicology CELLTOX
EDC scientific interests

Endocrine Disruptor Chemicals exposures in the EU are likely to contribute substantially to disease and dysfunction across the life course with costs in the hundreds of billions of Euros per year (Trasandel et al., 2015).

Endocrine Active Substances (EAS) (EFSA, 2013) are defined substances that have the inherent ability to interact or interfere with one or more components of the endocrine system, causing a biological effect, but not necessarily cause adverse effects. Generally, but not always, transient changes, minor and minor to the molecular level can be considered adaptive. A prerequisite for an EAS to be considered an Endocrine Disruptor (ED), that is, an endocrine disrupter of natural origin or synthetic, Endocrine Disruptors Chemical, is associated with identification, following exposure, of an adverse effect. The mechanisms of action with measurable toxicity tests are attributable to effects EATS (estrogen, androgen, thyroid, steroidogenesis) or not EATS.

According with the OECD conceptual framework (CF) for the testing and assessment of EDs, information on ED activity can be obtain from existing data, read across, in silico tools, (level 1), in vitro (level 2) and in vivo (level 3) screening assays for evaluation of mechanism of action or pathway, and in vivo assays for the evaluation of adverse pathological and functional effects (Worth et al., 2014).

Granulosa cells (GC), derived from different species are widely used as an in vitro model to study the effects of xenobiotics as an innovative approach in reproductive toxicology research (Petro et al., 2012). ED effects have been investigated with bovine and swine granulosa cells (Caloni et al., 2009; Spicer and Aad, 2007; Pizzo et al., 2015) on cell proliferation, steroidogenesis (estradiol/progesterone production), CYP19A1 (aromatase enzyme) and CYP11A1 (P450 side-chain cleavage enzyme) gene expression. ED activity (EATS) can be assessed also by the evaluation of the interaction with specific proteins (cingulin, tight-junction protein-1 (i.e., ZO-1), junctional adhesion molecule (JAM)-2 and claudin-2) that play critical roles in gap junctions and paracellular permeability in ovarian cells.

References.
Pietro COZZINI

Prof. of Chemistry and Molecular Modelling, Department of Food Chemistry, University of Parma

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Short Curriculum vitae

Pietro Cozzini, associate professor of Chemistry and of Molecular Modelling, Department of Food Chemistry, University of Parma. Head and cofounder of Molecular Modelling Lab in Institute of Structural Chemistry in 1989, then in Dept. Of General Chemistry and now in Food Chemistry Dept. Member of the editorial board of Nuclear Receptor Research, referee for ACS, RSC, Elsevier, etc. He has collaboration with Virginia Commonwealth University, University of Barcelona, Istituto Superiore di Sanità, Molecular Simulation INC. His main interest is the development of computational methods to study biomolecular association, Protein-Ligand-Water, Protein-Protein, Protein-DNA.

In particular, one of the most important project is to study, using in silico/in vitro experiments, the great family of Nuclear Receptor because of they are involved in many diseases. Computational methods developed are applied to discover new lead compounds or new possible pollutants in food.
Abstract

In silico funnel: a mandatory path to reduce in vitro and in vivo tests

The food we buy everyday is in contact with packaging, packaging machines, earth and with other contaminated food. Molecules generally defined as endocrine disruptors or xenoestrogens are ubiquitous in our life. In particular chemicals coming as industrial residues like bisphenols A and S (BPA) are well known as endocrine disruptors and may be found in many everyday products, including plastic bottles, metal food cans, detergents, flame retardants, food, toys, cosmetics, and pesticides.

The idea to substitute in vivo test with in vitro test for the complete set of chemicals in use, is an impossible dream because of the huge number of chemicals to be considered.

Nowadays we are forced to have predictive methods in chemistry, medicinal chemistry, food science, etc. if we want to be fast and cheap, to save money, time or to save animals life.

The unique alternative is to use several computational predictive methods to reduce dramatically the number of molecules really predicted as interactors toward a large set of receptors. Thus, it is possible to reduce the number of in vitro tests for the chemicals which have a realistic probability to be considered dangerous.

The “in silico funnel” proposed is a particular computational methodology that uses virtual screening, docking and scoring techniques well known in medicinal chemistry.

The general assumption is that from a pure chemical point of view there is no differences between pharmacological molecules, mycotoxins, nutraceuticals, pollutants, food additives, etc. Therefore we can today use in silico methods to face up food safety problems.

References.

Short Curriculum vitae

Isabella De Angelis (IDA), graduated in Biological Sciences, is researcher at the Department of Environment and Primary Prevention of the Italian Institute of Health. Her research interests are focused on the use and validation of in vitro models to investigate mechanisms of toxic action. In particular, she is interested in chemicals and nanoparticles absorption processes through in vitro intestinal barrier. IDA has coordinated and participated in National and European projects on in vitro toxicology and on regulatory acceptance of in vitro methods. Convinced of the importance of 3Rs culture dissemination, she regularly lectures in courses and meeting on alternative methods. She is member of the coordination group of the Italian National Reference Centre for Alternative Methods, and, since 2011, head of the Italian Delegation at the OECD Working Party of Manufactured Nanomaterials. IDA was president of CELLTOX, the Italian Association of Toxicology in Vitro (2006-2011) and since 2012 she is president of the Italian Platform for Alternative Methods (IPAM).
EDC scientific interests

The establishment of National Platforms for alternative methods has been promoted by a large group of participants in the "Third World Congress on Alternatives and Animal Use in the Life Sciences", held in Bologna in 1999. In the final document drawn up at the end of the conference were formalized two important concepts:

1) To promote and facilitate implementation of alternative methods the establishment of National Platforms (PN) is needed in order to cover particularly relevant tasks as communication, information, promotion, and training in relation to ethical and scientific aspects of alternative methods;

2) as parties interested in the use of alternative methods have been identified representative figures belonging to government institutions, industry, universities - research institutions, and organizations for animal welfare. The collaboration between these four figures allow to reach the PN goals with great efficacy, in particular the rapid implementation of validated methods in the national legislation.

According to this statement, IPAM, Italian Platform on Alternative Methods, was established on May 2003 by fourteen founding members belonging to the four areas of interest. It is a not-for-profit organization based on consensus, i.e. the agreement in opinions among the four parties. Its main objectives are to stimulate research into alternatives to animal experiments and contribute to their implementation (acceptance in practice).

In this perspective, IPAM takes part as stakeholder to LIFE-EDESIA project, strongly convinced to the relevance of the project approach for alternative methods implementations.
Matthew Dent joined Unilever’s Safety and Environmental Assurance Centre as a toxicologist in 2004. There he provides leadership in the area of toxicology risk assessment for both innovation and product stewardship, with a particular emphasis on developmental, reproductive, and endocrine toxicity. Matthew is also heavily involved in Unilever’s research into applying mechanistic approaches to non-animal safety assessments, working on case studies integrating in vitro data and computational models to construct prototype risk assessments for the p53 pathway and endocrine pathways. He also serves on several scientific working groups and task forces with groups such as ECETOC and Cosmetics Europe. Prior to working for Unilever, he directed reproductive and developmental toxicology studies at a major global contract research organization. Matthew gained his B.Sc. (Hons.) degree in Applied Biology (Toxicology) from De Montfort University, Leicester in 1997, and was awarded the International Diploma in Toxicology (I.D.T.) in 2006. He is a Eurotox registered toxicologist (E.R.T.) and a member of the British Toxicology Society and the British Society for Toxicologic Pathology.
**EDC scientific interests**

As a developmental and reproductive toxicologist, I have a keen interest in chemicals that have the potential to affect endocrine signalling. For some time Unilever has been actively researching new ways of assuring consumer safety without animals, using *in vitro* test data and computational modelling in a risk assessment context ([www.TT21C.org](http://www.TT21C.org)). The large number of *in vitro* screening assays for endocrine activity and the current heavy reliance on animal data to characterize the risks of endocrine active chemicals makes endocrine signalling a particularly interesting case study for non-animal mechanistic safety assessments. I am therefore currently involved in a project to consider how these types of information can be used to make decisions on consumer safety without the need to generate animal data.
Alberto MANTOVANI

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**Short Curriculum vitae**

- Date/place of Birth: Bologna, February 22nd 1956
- Degree in Veterinary Medicine (University of Bologna, December 1979); Master of Science in Veterinary Public Health (University of Edinburgh, September 1982)
- Staff scientist at the Italian National Health Institute (Istituto Superiore di Sanità, ISS) since December 1982; since 2008 director of then established Food and Veterinary Toxicology Unit at the ISS Dept of Food Safety and Veterinary Public Health
- Chair of the national pilot project on endocrine disruptors (ED) (http://www.iss.it/inte), (2000-3), of the national pilot project on ED biomarkers PREVIENI (http://www.iss.it/prvn) (2008-11) and of the LIFE-EDESIA project on the implementation of substitution principle for ED (EU LIFE programme, https://www.iss.it/life)
- International working groups on toxicological risk assessment: (EMA, 1994-9) Safety of veterinary drug residues; (OECD 2000-9) Working Group on ED Testing and Assessment; (European Commission, Environment and Health Action Plan; 2002-3) co-Chair of the Working Group on ED; EFSA Panel member: (Additives and Products or Substances Used In Animal Feed (FEEDAP); 2003-12, vice-chair in 2009-12, again in 2015-18); Plant Protection Products and their residues (PPR); (2012-5); Standing Working Group on Emerging Risks (2012-15)
**EDC scientific interests**

My interest on EDCs is shown by my CV. EDC are most interesting to me as a cross-cutting area: between medical research and toxicology, between molecular and cell biology and chemical testing, between basic science and risk analysis.

I am convinced EDCs are both a public health issue and an enticing model for other emerging risk assessment issues with a significant burden of uncertainties.
Daniele MARCOCCIA

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Short Curriculum vitae

Education/Training:
2012-15 PhD: University RomaTre of Rome, Italy Science and Biotechnology of Reproduction
2012 Degree in Medical Biotechnology at the University Tor Vergata of Rome, Italy

Research activities
- Assessment of the role of plant bioactives, plant extracts, pesticides and plasticizer on reproductive tissues (prostate and placenta) in alternative (to animal experimentation) in vitro methods by studying both gene expression profiling and protein secretion as molecular and cellular biomarkers of exposure and effect.
- Development of alternative to animal experimentation with in silico/in vitro methods
- Study in vivo of the environmental and dietary contaminant “di-(2-ethylhexyl)phthalate/DEHP” as a potential causative factor of hepatoblastoma (within the frame of a NIH-ISS project on rare diseases)
- assessment of the androgenic-like properties of the pesticide glufosinate ammonium and the NMDAR-agonist-like properties of the environmental and dietary contaminant semicarbazide/SEM

Publications related to the subject
EDC scientific interests

Daniele Marcoccia research interests are focused around understanding the androgen receptor molecular mechanisms and its interaction with endocrine disruptors (EDCs) and plant bioactives and extracts. In particular, the role of EDCs and natural chemicals on the cellular physiological mechanisms that regulate the human male reproductive system. Moreover, main interests are: i) the development of both in vitro and in silico alternative methods to animal testing for EDC assessment; and ii) the study of new biomarkers for prostate cancer (PCa).
Jane MUNCKE
Managing Director at Food Packaging Forum (FPF) Foundation

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Short Curriculum vitae

Jane Muncke is South African, German and Swiss. She holds a PhD in Environmental Toxicology from the Swiss Federal Institute of Technology Zurich (ETHZ) and an MSc in Environmental Science/Environmental Chemistry from ETHZ. During her graduate and postgraduate work she was trained in analytical chemistry and ecotoxicology at Eawag, the Swiss Federal Institute of Aquatic Science and Technology. Jane worked in the project management team of Novaquatis, an interdisciplinary research project on urban water management aiming at reducing nutrients and chemical pollutants in the aquatic environment, including endocrine disrupting chemicals. During her PhD, Jane developed a molecular-based screening for endocrine disrupting chemicals in zebrafish (Danio rerio) embryos and larvae. After post-doctoral work at Eawag in the area of endocrine disruption screening in wastewater effluent, Jane joined Emhart Glass, a supplier company to the container glass industry. In her role as Environmental Risk Assessment Specialist, she analysed scientific information on food packaging composition, migration and impacts on human and environmental health, with a special focus on emerging toxicological issues such as mixture toxicity, developmental exposures and low-dose effects of endocrine disruptors. Currently, Jane is Managing Director of the Food Packaging Forum Foundation (FPF), a charitable non-profit organization founded in 2012 and dedicated to science communication and stakeholder dialogue on the issue of food packaging and chemical health. FPF’s aim is to raise awareness for the issue of chemical migration from all food contact materials, and ultimately improve public health by primary disease prevention. She is a member of the Society of Toxicology, the American Chemical Society, and the Society for Environmental Toxicology and Chemistry (SETAC). Jane is married has two children and lives in Zurich, Switzerland.
EDC scientific interests

- Endocrine disrupting chemicals (EDCs) are thought to be involved in the development of several/many chronic diseases affecting our global society today
- Especially during critical time windows of fetal development, pregnancy EDCs may cause disease later in life (including metabolic disruption, diabetes, obesity)
- Avoiding EDCs (especially during pregnancy) promises an important opportunity for prevention of chronic diseases
- Food contact materials (FCMs) are packaging, food storage, filling and processing equipment in direct contact with foodstuffs
- FCMs are made from different materials, but often the direct FCM layer is chemically not inert and can be a source of smaller chemicals migrating into foods, including EDCs
- My colleagues and my work has shown that more than 100 known or suspected EDCs are legally used in the manufacture of FCMs today though we do not have exact information on levels and applications for known or suspected EDCs in FCMs
- From my practical work in academic research I am familiar with in vitro and in vivo testing for EDCs in aquatic media (incl waste water effluent)
- I have worked with YES, HepG2 and zebrafish test systems (molecular, embryo morphological)
- I have also worked with transgenic zebrafish (using GFP or other colours, luciferase)
- Trained in animal experiments in general
- Experience in chemical fate modelling based on thermodynamic properties, I am interested in a holistic approach of chemical safety, focusing on source control rather than end-of-pipe measures e.g. hazard-based identification of EDCs and replacement by inherently non-hazardous alternatives
Short Curriculum vitae

Professional experience
Since 2000, I am research senior toxicologist for CEA (French Agency for Energy), experienced in cellular and molecular biology, with an enlarged vision of human biology. I am part of multiple collaborative projects aiming to identify toxic effects of metals, nanoparticles and chemicals. I am aware of alternative methods to animal experimentation and specialized in toxicogenomics. My core competency concerns omics analysis, from design to data mining, and especially biological analysis and identification of cellular effectors for further targeted physiological studies. From 1995 to 2000, I was project manager in the R&D department of a French private company specialized in biomedical diagnosis (Oncology, Endocrinology and Autoimmunity), with managerial experience. From 1985 to 1995, I was research junior scientist in the same company, in charge of the design of new immunoassays for medical analysis.

Education and training
- Post-doctoral position in Biophysics (UCSD, San Diego, USA)
- PhD in Chemistry and Master in Biochemistry (University of Sciences, Montpellier, France)
- Engineer in Organic Chemistry (National School of Chemistry, Montpellier, France)

Research activities
- Expert for chemical substances toxicity in the French National Agency for Food and Environment (ANSES)
- Member of a workshop about forecasting the health impact of exposure to substances used in new technologies
- Reviewer in peer review journals (Proteomics, BBRC, In Vitro Tox., BMC genomics, Particle and Fibre toxicology etc)
- Member of French Cellular Pharmaco-Toxicology Society
- I trained many students and interns throughout my career and I taught in a master course of Toxicology in the French

Last publications related to the subject
Dr Costanza Rovida works as Scientific Officer at CAAT Europe (Centre for Alternatives to Animal Testing) – University of Konstanz, Germany, to promote alternatives to animal testing at both scientific and political level, by organising Workshop and Symposia, by participating in meeting and International conferences and through publications on scientific journals. She is also part of REACH Mastery staff as Senior Regulatory Specialist where she has an active role in the preparation of REACH Registration dossiers, taking care in particular to the possible application of advanced testing strategies for the risk assessment of chemicals. In Italy, she is in the board of the Regional Chemistry Council and national expert for alternative methods. She is also an accredited stakeholder at ECHA (European Chemical Agency) and member of ESTAF (ECVAM Stakeholder Forum). She got her Master Degree in Organic Chemistry and a specialization in Analytical Chemistry. After many years of experience as chromatography specialist, she joined a biotech pharmaceutical company as project manager of the analytical department. In the period 2005-2008, Costanza Rovida worked at EURL-ECVAM taking part of a EU Integrated Project (Sens-it-iv) focused in the development of replacement methods for the evaluation of skin and respiratory sensitisation.

Costanza ROVIDA

University of Konstanz, CAAT (Center for Alternatives to Animal Testing) – Europe

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Short Curriculum vitae
In spite of the recognised high concern related to EDC, still there is no official methods or strategy for the definition of ED activity of chemicals and the relationship with human exposure. In the EU, many Regulations are referring to EDCs. In REACH (Regulation 1907/2006), ED activity is a requisite for entering into Annex XIV for authorisation. This list now contains 31 substances including 3 phthalates, which are officially considered EDCs. Approval criteria for basic substances in Regulation 1107/2009 on Plant Protection Products (PPP) includes the absence of “inherent capacity to cause endocrine disrupting” (Article 23). The same principle is repeated in Regulation 528/2012 on biocidal products. EDCs are also mentioned in Regulation 1223/2009 on cosmetic products even though without precise limitations.

The fact that there is no an official approach to identify EDC is confusing for the Authorities which must endorse the use of a specific substance. The recently approved OECD TG-443 on Extended One Generation Reproductive Toxicity considers some parameters for the assessment of ED activity. However, this is a long and very expensive test that cannot be performed on all circulating substances, which are estimated in the range of 80,000. Moreover, it is an animal method which does not fully answers questions about human relevance and dose-activity of EDCs. The OECD has approved many other in vitro assays, but there is no definition on how to combine them in an efficient strategy that should answer to questions about hazard exposure limits and the real risk to human health.

From the regulatory point of view, the scientific value of a new strategy should be combined with a formal acceptance derived from a validation process that should be optimised for the specific issue of identifying EDCs.

References.

2. Rovida et al. (2013). Alternative in vitro methods to characterize the role of Endocrine Active Substances (EASs) in hormone-targeted tissues. ALTEX. 30(2):253-5
Biography – Dr Andrew Worth MA, MSt, PhD
Dr Andrew Worth is a senior scientific officer at the European Commission’s Joint Research Centre (JRC), where he leads the Predictive Toxicology Group within the Systems Toxicology Unit of the JRC’s Institute for Health & Consumer Protection (IHCP). The JRC provides independent scientific and technical support to the European Commission and other policy makers in the EU, and is actively involved in the international scientific community.
Dr Worth has degrees in Physiological Sciences and in Linguistics from Oxford University, and a PhD in Computational Toxicology from Liverpool John Moores University. He has over 150 publications in the area of predictive toxicology, and has a particular interest in the development and assessment of computational methods and their application in the regulatory assessment of chemical safety. From January-June 2012, Dr Worth was a visiting scientist at the US Food and Drug Administration (FDA) within the FDA Center for Food Safety and Applied Nutrition (CFSAN). Dr Worth is a member of the editorial boards of Alternatives to Laboratory Animals (ATLA) and SAR and QSAR in Environmental Research (SQER).

JRC webpage:
https://eurl-ecvam.jrc.ec.europa.eu/laboratories-research/predictive_toxicology