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ESTIV

The European Society of Toxicology *In Vitro*

Official Journal: Toxicology *In Vitro* (published by Elsevier Science)

Newsletter No. 38

July 2015

Editorial

Dear ESTIV members,

Welcome to the 38tht edition of the ESTIV newsletter that provides an update on the *in vitro* toxicology, information on activities in the filed undertaken in the last six months and those planned for the near future.

This issue includes information on many events including announcement of the ESTIV 2016 Congress, report from Annual INVITROM Symposium, reports from three workshops dedicated to Alternative Methods to Animal Testing for the Safety Assessment of Chemicals organised in Brazil, information about II International Conference on Alternatives to Animal Experimentation in Portugal, meeting reports from MOON LIVER workshop organised by CELLTOX in Italy and annual IVTIP meeting held in Denmark.

We are pleased to announce in this issue Professor Michael Balls as the recipient of the 2015 Björn Ekwall Memorial Award, which recognizes his pioneering work in promoting animal welfare and alternatives to animal testing – Congratulations!

I would like to thank everyone who contributed to this Newsletter and encourage those who have any news from the *in vitro* field, comments or questions about ESTIV and its activities to contact us directly at the e-mails given in this N-letter.

Best wishes and happy reading,
Helena Kandarova
Communications and Newsletter

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Message from the President

Dear members,

It is with great pleasure that we share with you the latest information and developments in the field of toxicology *in vitro* that Helena Kandarova has carefully prepared to all. She has been appointed as the new board member responsible for the ESTIV Newsletters, replacing Francesca Caloni whom I would like to send a special thanks for all the efforts undertaken in preparing the Newsletter from January 2011 up to now.

Of particular importance is the announcement of the upcoming ESTIV Congress which will be held on 17-20 October 2016 in Juin-les-Pins, France focusing on "*In Vitro* Toxicology for Human Safety Assessment".

In addition, it is my pleasure to congratulate Prof. Michael Balls for the 2015 Björn Ekwall Memorial Award, a well-deserved award for all the commitment and efforts he has pursued in fostering the acceptance and implementation of alternative methods to animal testing.

I hope you enjoy the reading of the newsletter and that you find useful information in it related to your activities!

With best regards,

Chantra Eskes
ESTIV President

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Announcement ESTIV 2016 Congress October 17-20, Juin-les-Pins, France

ESTIV, the French Society of Toxicology (SFT), and the Cell Pharmacology-Toxicology Society (SPTC) cordially invite you to attend the 19th International Congress on *In Vitro* Toxicology ESTIV2016, in Juan-les-Pins, France, 17-20 October 2016, focused on "*In Vitro* Toxicology for Human Safety Assessment". The ESTIV2016 congress will be a four-day event, with specific emphasis on how new technologies can strengthen the interpretation and application of *in vitro* methods in risk assessment.

ESTIV2016 scientific program will include the following sessions:

- Emerging technologies
- Extrapolation, modelling and biodistribution
- Systemic toxicity, Endocrine disruptors
- Biopharmaceuticals
- Cell bioengineering and self-assembly
- Regulatory updates (validation, integrated testing strategy, regulatory application)
- Mixtures
- A debate session on the "Application of IATA in a regulatory context: prescriptiveness versus flexibility and cost versus coverage"
- Two student sessions (a student career session and a young speaker session)

In addition, a pre-congress workshop on Carcinogenesis, and a post-congress Training course on lung, dermal and ocular *in vitro* toxicity will be organized. Together with the poster sessions, the ESTIV 2016 Congress will give ample opportunities for both young and senior scientists to interact in a friendly atmosphere, and to learn about shifts in research and new developments in the field of *in vitro* Toxicology, applied to human safety.

We look forward to welcome you next year in Juan-les-Pins, France on October 17-20, 2016.

The organizing committee

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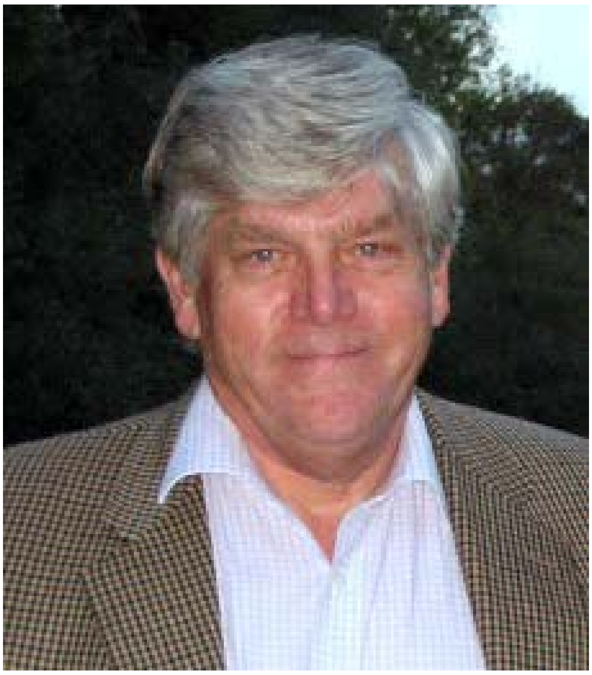
The Björn Ekwall Memorial Award 2015

Professor Michael Balls, from the University of Nottingham, United Kingdom, is the recipient of the Björn Ekwall Memorial Award for the year 2015 in recognition of his pioneering work in promoting animal welfare and alternatives to animal testing.

Michael Balls (born in 1938, in Norwich, Norfolk, UK) studied zoology at Oxford University, where he graduated in 1960, and obtained his DPhil in 1964. After post-doctoral research at the University of California, Berkeley, California, USA, and Reed College, Portland, Oregon, USA, he lectured in the School of Biological Sciences at the University of East Anglia, Norwich. In 1975, he moved to the University of Nottingham as Senior Lecturer in the Department of Human Morphology. In 1985, he became Reader in Medical Cell Biology, and in 1990 he was promoted to Professor of Medical Cell Biology. Since 1995, he has been an Emeritus Professor of the University of Nottingham.

In 1979, Michael Balls became a Trustee of the Fund for the Replacement of Animals in Medical Experiments (FRAME), and in 1981 he became Chairman of the Trustees and moved the charity from London to Nottingham. Under his leadership, FRAME became the most important organisation in the world for fighting for animal welfare and promoting alternatives to animal experimentation.

In 1983, Michael Balls became Editor of the peer-reviewed scientific journal, *Alternatives to Laboratory Animals (ATLA)*. Under his leadership for more than three decades, this journal has been publishing articles on cell toxicology and playing an important role in the implementation of *in vitro* testing methodologies and scientific achievements in this field. For several years, all the papers concerning the results of the Multicentre Evaluation of *In vitro* Cytotoxicity (MEIC) project (1989-1999), initiated and guided by Bjorn Ekwall, were been published in *ATLA*. Michael Balls collaborated with Björn Ekwall over many years, and became a close friend of Björn and his wife, Barbro.



In 1993, Michael Balls became the first Head of the European Centre for the Validation of Alternative Methods (ECVAM). He retired from this position in 2002. He is a member or an expert consultant of several national and international boards and advisory committees involved in the protection of animals and in the development and validation of *in vitro* tests. Michael Balls was elected as Honorary Member of the SSCT and of ESTIV. He has received many international awards for his work on laboratory animal welfare and alternatives to animal testing.

Professor Balls has published over 375 articles in peer-reviewed journals. In 2009, he published a book entitled *The Three Rs and the Humanity Criterion* which is an abridged version of *The Principles of Humane Experimental Technique*, by W.M.S. Russell and R.L. Burch (1959).

The BEM Award will be handed out at the 30th Workshop organized by the Scandinavian Society for Cell Toxicology (SSCT) together with the Swedish Toxicology Sciences Research Center (SweTox), in Bommersvik, Sweden, October 13-15, 2015.

On behalf of the board of Björn Ekwall Memorial Foundation

Ada Kolman President of BEMF	Hanna Tähti Secretary of BEMF

Annual INVITROM Symposium "Biology meets Technology: Organ-on-a-Chip & Biosensors" March 25, 2015 - Breda, the Netherlands

On March 25, 2015, INVITROM held the annual symposium entitled "Biology meets Technology: Organ-on-a-Chip & Biosensors" in Breda, the Netherlands. The presentation of the speakers resulted in interesting discussions. Included in the program was a 'pitch elevator presentations on posters' session. Young scientist awards were won by **Thomas Pauwelyn** (IMEC, Leuven, Belgium) for his poster on "Label-free cardiac contractility monitoring for drug screening applications based on compact high-speed lens-free imaging" and **Marinke van der Helm** (BIOS Lab-on-a-Chip group. University of Twente, The Netherlands) for her poster "A microfluidic platform to study and modulate the blood-brain barrier".

The program involved the following speakers:

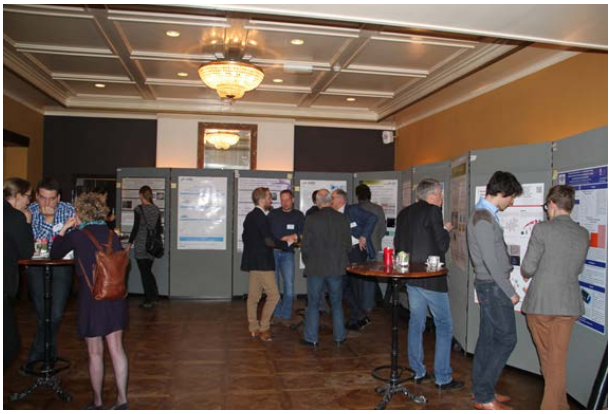
- **Andries van der Meer** (University of Twente / Wyss Institute, Harvard University): *Organs-on-chips: concept, technology and application*
- **Paul Vulto** (MIMETAS BV): *Organ-on-a-chip platform for high-throughput drug screening*
- **Dries Braeken** (Imec Life Science Technology, Leuven): *Think small for big changes: how nanoelectronics revolutionizes life sciences*
- **Jeroen Lammertijn** (KU Leuven, Department of Biosystems, MeBioS: Biosensor group): *Recent trends in biosensor development*
- **Menno Prins** (Eindhoven University of Technology, Molecular Biosensing for Medical Diagnostics): *Molecular biosensing for medical applications*
- **Jef Hooyberghs** (Vito Mol, Applied Bio & molecular Systems): *Detection of biomarkers: from physico chemical principles to clinical applications*
- **Heidi Ottevaere** ((Vrije Universiteit Brussel, Brussels Photonics Team, BPHOT): *Multifunctional biosensors: from lab to fab*

Even though the symposium was organized in parallel with the SOT in San Diego, the

symposium in Breda attracted a good number of attendants. The general reactions were very positive!

Next INVITROM symposium will take place in collaboration with BelTox, in December 2015 (Antwerp, Belgium).

Jan van der Valk
INVITROM



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Series of Three Workshops Dedicated to Alternative Methods to Animal Testing for the Safety Assessment of Chemicals and Their Impact on Human Health
- Challenges and Perspectives (FAPESP)
- International and Brazilian views (unesp)
- Use for the Cosmetics Industry (Abihpec)

March 31 - April 2nd, 2015
São Paulo, Brazil

The first two workshops held on March 31st and April 1st addressed "Challenges and perspectives in research on alternatives to animal testing" and "Progress on alternative *in vitro* methods for the safety assessment of chemicals and their impact on human health: international and Brazilian views" respectively. The workshops were organized in São Paulo, Brazil, with the support of São Paulo Research Foundation, FAPESP. They presented initiatives and perspectives of the alternative methods to animal testing and exchanged ideas, concepts and innovations in the area.

The two meetings counted with 20 speakers who addressed their experiences on alternative methods to animal experimentation, three of which came abroad from Brazil. **Thomas Hartung (CAAT)** talked about the implementation of the Three Rs in the interests of good and humane Science and the limitations of animal models. **Raymond Tice (NIH)** presented the US multiagency collaborative effort on Tox21 project. **Chantra Eskes (SeCAM)** talked about the validation of alternative *in vitro* assays for toxicity evaluation and presented some integrated approaches on testing and safety assessment.

Among Brazilian speakers, the Brazilian Health Surveillance Agency (Anvisa) and National Council for Animal Experiments Control (CONCEA) as well as influential researchers of the State of São Paulo were present. The Brazilian network (RENAMA) for alternative methods, created by the Federal Government, as well as the Brazilian Society of Alternative Methods (SBMAlt) were also present at the meetings.

Due to the high demand, the registration had to be closed 3 days after the first announcement, with more than 300 attendees, which shows the interest of Brazilian scientific community in this topic.

Brazil has been making progress during the last few years and on July 2014, the Brazilian National Council for the Control of Animal Experimentation (CONCEA) started the process of reducing animal experimentation in Brazil. CONCEA established that Brazil has 5 years to implement 17 alternative methods validated internationally and recognized by the Organization for Economic Co-operation and Development (OECD). Among these methods, we can point out the skin sensitization, skin and eye irritation and corrosion potential, acute toxicity, skin absorption, phototoxicity potential and genotoxicity described by OECD Test Guidelines. Thus after this period testing on animals should not be carried out if only these 17 endpoints are addressed and there

will be mandatory replacement of the original method by the alternative one.

The links for the detailed program of these two workshops are listed below:
- http://www.fapesp.br/9310
- http://prope.unesp.br/eventos/fapesp/index.php

Organizing Committee:
Maria José S. M Giannini – PROPe-UNESP
Lorena R. Gaspar Cordeiro – FCFRP-USP
Silvya Stuchi Maria-Engler – FCF-USP
Suely Vilela – FCFRP-USP

In addition, on April 2nd, 2015, a third workshop was held at ABIHPEC, the Brazilian Association for the Cosmetics, Toiletry and Fragrances Industry, São Paulo, Brazil. The workshop entitled "Alternative methods to the use of animals for the cosmetics industry" gathered together scientists and Cosmetic companies and addressed the challenges and strategies for companies to adapt to the new legislation.

The detailed program of this workshop can be found at:
https://www.abihpec.org.br/2015/03/seminario-metodos-alternativos-ao-uso-de-animais-para-industria-de-higiene-pessoal-perfumaria-e-cosmeticos/

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II International Conference of Alternatives to Animal Experimentation
May 8-9, 2015
Lisbon, Portugal

The II International Conference of Alternatives to Animal Experimentation was held in Picoas Forum (Lisbon, Portugal) on the 8th and 9th of May 2015. This event was hosted by The Portuguese Society for Humane Education (www.spedh.com) and sponsored by ESTIV, the PT Foundation; Lumiar Town Council; MatTek IVLSL; New England Anti Vivisection Society (NEAVS); Xenometrix; PETA International Science Consorcium (PISC); SOS Animal; Liga Portuguesa para os Direitos do Animal (LPDA); Cultra; Paladar ZEN and Mercado do Site.

The Conference gathered approximately 80 participants coming from universities, animal facilities, research laboratories, animal welfare NGO's as well as general public willing to learn and committed to develop, validate and implement alternatives to animal experimentation. The Conference was divided in 5 sessions: 1. Animal experimentation: past and future; 2. The emergence of alternatives – *in vitro* technology; 3. The new paths of Europe, for citizenship and ethics; 4. The emergence of alternatives – *in silico* models; 5. Clinical research.

The Keynote Speaker for the first session was **Dr. Philip Low** (Neurovigil Inc.) who, amongst other scientific achievements, invented the iBrain™ device, a cutting-edge, single-channel EEG recording tool. This powerful tool has the potential to replace invasive animal studies in the field of neurosciences. Dr Philip Low also wrote the Declaration of Cambridge (2012) the Declaration of Curitiba, and the Declaration of Lisbon (available at www.spedh.com), written during this conference and ratified by its participants. Other speakers of this session included **Dr. Luís Vicente** (Faculty of Sciences, The University of Lisbon), who talked about the ethics surrounding the use of non-human animals in laboratory and field work; and **Dr. Robert Coleman** (Safer Medicines Trust) who presented an integrative approach for safety assessment and addressed the challenges regarding regulatory acceptance of alternative methods.

The second session, focused on *in vitro* methods, was opened by **Dr. Elmer Heinzle** who presented the NOTOX European project on developing and validating predictive mathematical and bioinformatic models characterizing long term toxicity responses. Other speakers included **Dr. Teresa Rosete** who presented an *in vitro* approach which can potentially identify the chemicals responsible for triggering asthmatic reactions; **Dr. João Barroso** (EURL ECVAM) and **Dr. Helena Kandarova** (MatTek IVLSL) who described different *in vitro* methods being used for the study of chemical reactions in the eye and in the skin, respectively; and **Dr. Helen Wheadon** (Institute of Cancer Sciences, University of Glasgow) who presented on the development of *in vitro* stem cells to study leukaemia.

The second day of the Conference started with a main focus on legislation and regulatory acceptance. **Dr. Bas Blaauboer** (Utrecht University) presented a keynote lecture on regulatory acceptance and the use of 3R models in toxicology. **Dr. Ana Paula Martins**, member of Portuguese National Authority for Animal Health talked about the transposition of the Directive 2010/63/UE to the Portuguese law. **Dr. Gilly Stoddart** (PETA International Science Consortium) presented the joint efforts for reducing the number of animals required by REACH legislation, namely through the webinars promoted by PETA International Science Consortium, Ltd. and Chemical Watch and the need to increase the awareness on alternatives. **Dr. Manuel Rebelo** (Gulbenkian Institute for Science), presented the institute's ethics committee and its efforts in promoting the 3R policies.

The fourth session, focused on *in silico* methods, started with speech of **Dr. Mark Cronin** (Liverpool John Moores University) on the COSMOS project - an European initiative that facilitates the prediction of toxicity testing, whether *in vivo* or *in vitro*. **Dr. Vanessa Diaz** (Department of Mechanical Engineering, University College London) and **Dr. Miguel Rocha** (Engineering School, Minho University) presented two different projects with a similar goal: the creation of a virtual human model able to simulate the physiological reactions to different compounds, therefore helping the development of therapies for several diseases.

The last session of the Conference on clinical research counted with the presentation from **Dr. Diogo Ayres-de-Campos** (Faculty of Medicine, Oporto University) who used his expertise within the foetal monitoring field as an example of a biomedical area where large numbers of animals are used as models with no later applicability to human medicine; and from **Dr. Constança Carvalho** (Faculty of Sciences, Lisbon University) who demonstrated through citation analysis data the poor contribution of animal models to biomedical knowledge on Attention Deficit hyperactivity Disorder.

One of the main highlights of the Conference was the ratification of the Declaration of Lisbon, initiated and written by **Dr. Philip Low** and edited by **Dr. Andrew Knight, Dr. João Barroso** and Dr. Philip Low. This Declaration (available at www.spedh.com) gathered a prominent international group of animal researchers, veterinarians, ethicists and regulators to evaluate the costs and benefits of animal testing. Upon review of the available data on the subject, they decided to make and publicly sign the statement below.

"While recognizing that animal testing has long been a traditional component of biomedical research, it has become clearly apparent that the returns on investment in animal research are increasingly meager. To the extent that this type of research may continue, it is our recommendation that it be carried out after giving institutional committees, independent expert third party animal ethics committees, funding organizations and relevant regulatory authorities evaluating the proposed research (collectively 'The Parties') a more realistic and evidence-based estimate of the likely costs and benefits of the proposed protocols. Experimental animals should be filmed at all times and The Parties should have the option to view any footage they wish, to ensure that the proposed or approved research follows authorized and funded protocols, thus maximizing animal welfare and societal returns on investment in this research."

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MOON LIVER: Alternative models for liver hepatotoxicity
May 19, 2015 - Brescia, Italy

On May 19, 2015, a meeting on alternative methods for evaluating hepatotoxicity was organized by CELLTOX, Associazione Italiana Tossicologia *in Vitro*, in collaboration with the Italian Reference Center for Alternative Methods, Welfare and Care of Laboratory Animal and Mistral. The meeting was hosted by the Istituto Zooprofilattico Sperimentale della Lombardia e Emilia Romagna, Brescia.

After a brief introduction by **Maura Ferrari** on the activity of the Italian Reference Center for Alternative Methods, Welfare and Care of Laboratory Animal, **Dr. Emma Di Consiglio** from the Italian National Institute of Health in Rome presented the overall strategy of the European Project Predict-IV and the final conclusions reached for the improvement of the prediction capacity of *in vitro* tests using biokinetics data obtained on hepatic cellular models. **Dr. Barbara de Servi** from VitroScreen presented 3D Co-cultures: The 'Microliver' model - characterization and applications. **Prof. Giovanna Mazzoleni** from University of Brescia focused on model systems that use fluid-dynamic microenvironments while **Dr. Annalisa Manenti** showed the application of bioengineering in biomedical research.
Alfonso Lostia from JRC presented an update on relevant Adverse Outcome Pathways (AOPs), and the rationale and scientific strategy employed to design a case study for predicting repeated dose liver toxicity in the SEURAT-1 EU project.

During the conference, different methodological approaches were illustrated as well as *in vitro/ex vivo* experimental models currently available for the study of liver function. For each of these, the main features, the specificity of answers obtained, the relevance and significance of use (including financial considerations) were presented. Each model/system was presented, considering the advancement of knowledge of experimental methods used to generate it, the current scope of use (advantages and limits, regulatory issues) and possible prospects for implementation/dissemination.

Particular attention was paid to the culture techniques used to keep the differentiated hepatocyte functions *in vitro*, to the importance of the liver microenvironment (three-dimensional histo/cyto architecture) and to the specific cell-cell/extracellular cell matrix interactions. An overview of the contribution of tissue engineering, emerging micro/nano technologies and the use of bioengineering in the design and optimization of new models of liver function for application in toxicology, were also presented.

Francesca Caloni and Yula Sambuy
CELLTOX

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In Vitro Testing Industrial Platform (IVTIP) meeting, 28 – 29th May, 2015, Copenhagen, Denmark

Cyrille Krul (president of IVTIP) opened the non-members section of the meeting with a brief introduction to IVTIP and its goals of promoting dissemination of information and new developments within *in vitro* methods by informing members and regulatory agencies of the impact and needs for *in vitro* test methods. The group has 42 member companies and is active through research programs and publishing meeting reports.

The first session focused on regulation with talks from the Danish 3Rs centre emphasising the work taking place to unite national 3Rs principles efforts across Europe with EURL-ECVAM taking on a coordinator role. The European Commission has invested much effort to bring available *in vitro* methods into use on a larger scale through Integrated Testing Strategies (ITS), Weight of Evidence (WoE) and Integrated Approaches to Testing and Assessment (IATA) initiatives. The REACh and Cosmetics Directives have promoted significant efforts into developing alternative methods and establishment of the European partnership for alternative approaches to animals (EPAAA), the DB-ALM and TSAR databases for alternative methods has made the existing knowledge more accessible. Once validated, process of implementation in member states is slow however, often held up by translation into the many languages. The final talk of the session then posed the (long) list of questions developers must ask themselves and address

when preparing a method for submission (e.g.: usability, implementation ease, cost, relevance to human, capture *in vivo* MoA, purpose etc.). It was commented that uptake of new methods appears to be a vicious circle as regulators are reluctant to accept data without proof of applicability domain therefore companies continue to use proven (older) methods. The necessity to involve all stakeholders from the beginning of new method development was seen as key to swift (as possible) progression and adoption at the EU level.

There then followed four shorter invited talks about new developments and relevance to *in vitro* lung toxicity. Presentations were received from Kirkstall on their Quasi-vivo system, an *in vitro* lung inhalation model from TNO, the lung GARD assay from Senzagen and a lung surfactant model from the National Research Centre, Denmark. Each gave an overview of their systems, applications and the work they have performed to date demonstrating the need for integrated models (Kirkstall), appropriate biology (National Research Centre, Denmark), selection of appropriate model (TNO) and analysis at the genetic level (SenzaGen) in order to replicate the complex biology of lung toxicity in and *in vitro* arena.

The second day began with a comprehensive overview of Adverse Outcome Pathways (AOPs) and how this paradigm drives an understanding of toxicity at the mechanistic level. Over 50 AOPs have been entered into the AOPWiki (www.aopwiki.org) – an OECD driven resource. AOPs require a Molecular Initiating Event (MIE) and an Adverse Outcome (AO) linked by Key Events (KE) which have a definable relationship to each other. These KEs can be used to design relevant assays for *in vitro* testing and can be combined in a testing strategy relevant to the human situation. AOPs are living documents to be added to and refined as knowledge becomes available however it was emphasised that using them to select test methods still needs some expertise. The following three talks in the session covered developmental/reproductive toxicity, developmental neurotoxicity using neurospheres and ChemScreen - an FP7 funded project to generate mechanism based reporter bio-assays. It is accepted that complete data only exists on approximately 1,000 chemicals but there are >40,000 used in Europe and >83,000 in the USA. Reproductive toxicity, be it development or neurological or another organ, is a challenging area for *in vitro* approaches but developments in assays such as the EST and advances in neurospheres has enabled some known developmental toxins to be successfully ranked following *in vitro* testing to the known *in vivo* outcomes. It was mentioned that it could be possible to use developmental neurotoxicity testing in the AOP framework as certain KE apply to multiple AOPs therefore one data set could be applicable to multiple pathways as AOPs are vertically as well as horizontally arranged. ChemScreen has developed a panel of luciferase reporters for reprotox applications and can also be applied for Read-Across purposes. Of the 50 reporters available, it was proposed that as few as 30 could yield suitable data depending on depth of information required and that pharmacokinetic data was helpful in improving overall predictions, however these predictions were very much types of toxicity.

Four shorter talks then were given covering three lung models and a workshop report on tobacco and COPD (IIVS). The tobacco and COPD report was from an event set up by the IIVS in December 2014 for tobacco related stakeholders and regulators to meet and discuss regulation of that industry and will soon be published. 63 people attended and a second workshop was requested and will take place in April 2016 following successful funding being granted. The three lung model talks consisted of an isolated lung perfusion model (Fraunhofer ITEM), precision-cut lung slices (Fraunhofer ITEM) and nanoparticles in the pulmonary epithelium (TNO).

The perfused whole lung model had a range of parameters that could be measured including tidal volume, blood gases, weight and revealed pathological effects that were reversible by the addition of lung surfactant back into the tissue. So far, the maximum time anyone has maintained this model is 20 days. The precision-cut lung slices allow for direct application and also gaseous exposure and because the tissues contain all relevant cell types in situ, cytokine profiles can be obtained. Currently only diseased tissue is readily available. MucilAir was the model of choice for analysing nanoparticles on the pulmonary

epithelium and readouts included trans-epithelial electrical resistance (TEER), lactate dehydrogenase release and cytokine release as well as viability measurements. Data revealed that sub-lethal effects were observed using apoptosis and ROS readouts, but not observed in the colourmetric readouts and that acute exposure did not reveal any effect but chronic exposures did.

The final four presentations talked about the air/blood barrier, use of crowdsourcing for improving pathway data in respiratory computer models, lung-slices and the necessity of using the optimal model for lung toxicity *in vitro*. If particles can cross the air/blood barrier, then systemic exposure is possible therefore it is imperative to successfully model this exposure route. Using a new, purpose derived cell model with functioning tight junctions (unlike A549 cells) in co-culture with macrophages, induced inflammation responses were reversible with coated nanoparticles. The use of crowdsourcing to verify data predictions was used by Marja and colleagues when they produced intricate BEL-language maps of processes with common points (NODES) and effector routes mapped out. Releasing the map into the public domain allowed external users to verify or request amendments to the map thus validating the connections and improving the predictivity of the model.

The last presentation considered the optimal requirements for an *in vitro* lung model. With diverse readouts such as sensitisation, inflammation, disease induction, carcinogenicity, barrier function, local tolerance, cilia beat frequency, TEER, cytokine release and viability to mention a few, it is clear that the more complex and human-based models are most suitable. The reconstructed tissue models (RTMs) such as EpiAirway and MucilAir, were high on this list as even though the precision-cut lung slices were still considered a better overall model, the availability of the RTMs makes them a very useful tool for *in vitro* lung toxicology.

Darren Kidd
Covance

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CELLTOX Poster Prize for Young Investigator at "Advances in Cell & Tissue Culture"
June 15-17, 2015 - Pisa, Italy

During the 7th Annual Kirkstall Conference on "Advances in Cell & Tissue Culture" organized by Kirkstall in collaboration with the University of Pisa and Centro Piaggio from 15th to 17th June 2015, CELLTOX, the Italian Association for *In Vitro* Toxicology (www.celltox.it) offered a prize of € 150 for the best poster presented by a young investigator.

During the Conference several new models of 3D cell cultures and co-culture systems were presented, aimed at improving the physiology and differentiation of *in vitro* models for the development of alternative methods for *in vitro* toxicology. Novel chemical and bioengineering approaches were presented for the development of hydrogels and scaffolds allowing better *in vitro* reconstitution of tissue cytoarchitecture.

The CELLTOX prize was awarded to the poster presented by **Dr Daniele Cei** of "Centro Piaggio" and the University of Pisa, who recently obtained his PhD on this research topic, describing the development of an *in vitro* model of the intestinal barrier based on an electroactive polymer (EAP) membrane capable of applying stretching impulses to the intestinal cell culture, thus reproducing the peristaltic movements of the mucosa.

Dr Daniele Cei and two other young investigators **Dr Emanuele Bronzini**

(University of Pisa) and **Dr Steven Webb** (University of Liverpool) also received prizes sponsored by F1000 (free subscription and free article submission to F1000 Research (http://f1000research.com).

Francesca Caloni
CELLTOX

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2nd COLAMA: The Latin-American Congress on Alternative Methods for Research, Testing, Industrial and Education Purposes
July 5-7, Varadero, Cuba

For the second time, Latin America has organized its largest congress dedicated to the use of Alternative Methods for investigative, testing, industrial and educational purposes. Organized by the Cuban Society of Pharmacology and the Latin-Ibero-American Network on Alternative Methods, the Congress counted with 30 speakers, 50 posters and a broad participation by international and national scientists.

The scientific sessions covered the following themes:

- *In vitro* methods for the toxicological and pharmacological evaluation of products chaired by **Pilar Vinardell (Spain) and Idania Rodeiro Guerra (Cuba)**
- Use of bioinformatics and other tools for the development of new products chaired by **Ulises Jáuregui and Danys Siveiro (Cuba)**
- Validation and regulatory acceptance of alternative methods chaired by **Chantra Eskes (Switzerland) and Lázara Martínez (Cuba)**
- Alternative methods in Education chaired by **Manuel Cedeño (Cuba)**
- Alternative methods and for potency and toxicity testing for vaccines and other pharmaceutical products chaired by **Mario Landys Chovel (Cuba)**

In addition the event counted with the unexpected visit from Miriam Palacios, a Cuban scientist in Pharmacology and Toxicology who made a career in the UK and presented on 'Mobility, migration and networking of the Cuban scientific community'.

The organizers did a great job in gathering together a very interesting program and social activities, notwithstanding the strong difficulties encountered in obtaining appropriate funding for the congress. Most important, they were able to guarantee the presence of local scientists that strive in advancing research, testing and use of alternative methods despite the limited resources.

The next COLAMA meeting is foreseen to take place in Argentina in 2018.

Chantra Eskes
SeCAM, Switzerland

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Highlights

ESTIV has been shortlisted for the LUSH prize in the category Training and Education.

The prize in this category recognises the importance of dissemination of methods among commercial scientists, researchers and students. The criteria for training is broad, and includes training existing scientists in new techniques, open-source databases, and the education of school children.

More information available at:
www.lushprize.org

THE 2015 LUSH PRIZE SUPPORTING ANIMAL-FREE TESTING

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Meetings and Workshops calendar

CELL AND DEVELOPMENTAL SYSTEMS (EMBO WORKSHOP)
August 18-22, 2015
Arolla, Switzerland

EUROPEAN ENVIRONMENTAL MUTAGEN SOCIETY 44TH ANNUAL MEETING
August 23-27, 2015
Prague, Czech Republic

SCIENTIFIC ADVISORY COMMITTEE ON ALTERNATIVE TOXICOLOGICAL METHODS
September 2-3, 2015
Research Triangle Park, North Carolina, US

6TH EMBO MEETING, ADVANCING THE LIFE SCIENCES
September 5-8, 2015
Birmingham, UK

10TH INTERNATIONAL CONFERENCE ON ENVIRONMENTAL EFFECTS OF NANOPARTICLES AND NANOMATERIALS
September 6-10, 2015
Vienna, Austria

SETAC LATIN AMERICA 2015 MEETING: THE ROLE OF SCIENCE IN ENVIRONMENTAL DECISION-MAKING
September 7–10, 2015
Buenos Aires, Argentina

THE 2015 TISSUE ENGINEERING CONGRESS
Sept 8-10, 2015
London, UK
Buenos Aires, Argentina

51ST CONGRESS OF THE EUROPEAN SOCIETIES OF TOXICOLOGY (EUROTOX 2015)
September 13-16, 2015
Porto, Portugal
www.eurotox2015.com

CURRENT METHODS IN CELL BIOLOGY, EMBO PRACTICAL COURSE
September 14-22, 2015
Heidelberg, Germany

CELL DEATH
September 15–19, 2015
Cold Spring Harbor, New York, US

ARGENTINE CONGRESS OF TOXICOLOGY AND MEETING OF THE LATIN AMERICAN ASSOCIATION OF MUTAGENESIS, CARCINOGENESIS, AND ENVIRONMENTAL TERATOGENICITY (ALAMCTA)
September 16–18, 2015
Buenos Aires, Argentina

3RS ALTERNATIVES AND CONSISTENCY TESTING IN VACCINE LOT RELEASE TESTING, INTERNATIONAL ALLIANCE FOR BIOLOGICAL STANDARDIZATION CONFERENCE
September 16-18, 2015
Egmond aan Zee, The Netherlands

EUSAAT/LINZ 2015: 19TH EUROPEAN CONGRESS ON ALTERNATIVES TO ANIMAL TESTING
Sept 20-23, 2015
Linz, Austria

ALTERNATIVE APPROACHES TO IDENTIFYING ACUTE SYSTEMIC TOXICITY: MOVING FROM RESEARCH TO REGULATORY TESTING
September 24-25, 2015
Bethesda, Maryland, US

12TH CHE-PON6; 12TH INTERNATIONAL MEETING ON CHOLINESTERASES AND SIXTH INTERNATIONAL CONFERENCE ON PARAOXONASES
27 Sept-2 Oct 2015
Elche-Alicante, Spain,
www.12thChE.org

SAFETY PHARMACOLOGY SOCIETY
September 28-October 1, 2015
Prague, Czech Republic

OPENTOX EURO 2015
September 30-October 2, 2015
Dublin, Ireland

SSCT-Swetox Workshop Järna, Sweden
13-15 October 2015
http://www.ssct.se

EFSA'S 2ND SCIENTIFIC CONFERENCE – SHAPING THE FUTURE OF FOOD SAFETY, TOGETHER
October 14-16, 2015
Milan, Italy

9TH CONGRESS OF TOXICOLOGY IN DEVELOPING COUNTRIES
Nov 7-10, 2015
Natal, Brazil

SEURAT-1 SYMPOSIUM, PAINTING THE FUTURE ANIMAL-FREE SAFETY ASSESSMENT OF CHEMICAL SUBSTANCES: ACHIEVEMENTS OF SEURAT-1
December 4, 2015
Brussels, Belgium

OPENTOX ASIA 2015
December 7-9, 2015
Seoul, Korea

JSAAE 28TH ANNUAL MEETING, DEPARTURE FROM PAST PRACTICE AND KICKOFF TOWARDS TO THE FUTURE
Dec 10-12, 2015
Yokohama, Kanagawa, Japan

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Recent Publications of ESTIV members

Cazelle E., Eskes C., Hermann M., Jones P., McNamee P., Prinsen M., Taylor H., Wijnands M.V.W. (2015). Suitability of the Isolated Chicken Eye Test for Classification of Extreme pH Detergents and Cleaning Products. Toxicology In Vitro 29, 609-616.

De Wever B., Goldberg A., Eskes C., Roggen E., Vanparys P., Schroeder K., Le Varlet B., Maibach H., Beken S., De Wilde B., Turchina C., Bogaert G., Bogaert J.-P. (2015). Applied In vitro Toxicology,1. DOI: 10.1089/aivt.2014.0011.

Estevan C, Fuster E, Del Río E, Pamies D, Vilanova E, Sogorb MA. Organophosphorus pesticide chlorpyrifos and its metabolites alter the expression of biomarker genes of differentiation in D3 mouse embryonic stem cells in a comparable way to other model neurodevelopmental toxicants. Chem Res Toxicol. 2014 Sep 15;27(9):1487-95.

Hayden PJ., Bachelor M, Ayehunie S, Letasiova S, Kaluzhny Y, Klausner M and Kandárová H. Application of MatTek *In Vitro* Reconstructed Human Skin Models for Safety, Efficacy Screening, and Basic Preclinical Research. Applied *In Vitro* Toxicology, 2015

Kaluzhny Y, Kandárová H, Handa Y, J DeLuca, T Truong, A Hunter, ... The EpiOcular Eye Irritation Test (EIT) for hazard identification and labelling of eye irritating chemicals: protocol optimisation for solid materials and the results after extended shipment. Alternatives to laboratory animals: ATLA 43 (2), 101-127

Kaluzhny Y, Kandárová H, d'Argembeau-Thornton L, Kearney P, Klausner M. Eye Irritation Test (EIT) for Hazard Identification of Eye Irritating Chemicals using Reconstructed Human Cornea-like Epithelial (RhCE) Tissue Model. *J. Vis. Exp., 2015* (in press), e52979, doi:10.3791/52979 (2015).

Leist M, Hasiwa N, Rovida C, Daneshian M, Basketter D, Kimber I, Clewell H, Gocht T, Goldberg A, Busquet F, Rossi AM, Schwarz M, Stephens M, Taalman R, Knudsen TB, McKim J, Harris G, Pamies D, Hartung T. Consensus report on the future of animal-free systemic toxicity testing. ALTEX. 2014;31(3):341-56.

Pamies D, Bal-Price A, Fabbri M, Gribaldo L, Scelfo B, Harris G, Collotta A, Vilanova E, Sogorb MA. Silencing of PNPLA6, the neuropathy target esterase (NTE) codifying gene, alters neurodifferentiation of human embryonal carcinoma stem cells (NT2). Neuroscience. 2014 Sep 26;281C:54-67.
Pamies D, Hartung T, Hogberg HT. Biological and medical applications of a brain-on-a-chip. Exp Biol Med (Maywood). 2014 Sep;239(9):1096-107.

Rovida C, Alépée N, Api AM, Basketter DA, Bois FY, Caloni F, Corsini E, Daneshian M, Eskes C, Ezendam J, Fuchs H, Hayden P, Hegele-Hartung C, Hoffmann S, Hubesch B, Jacobs MN, Jaworska J, Kleensang A, Kleinstreuer N, Lalko J, Landsiedel R, Lebreux F, Luechtefeld T, Locatelli M, Mehling A, Natsch A, Pitchford JW, Prater D, Prieto P, Schepky A, Schuurmann G, Smirnova L, Toole C, van Vliet E, Weisensee D, Hartung T. (2015). Integrated Testing Strategies (ITS) for safety assessment. ALTEX. 32, 25-40.

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Toxicology *in vitro*

Official Journal of the European Society of Toxicology in vitro

Editors: Daniel Acosta,

TOP 5 Hottest TIV Articles

January to March 2015
(from http://top25.sciencedirect.com/)

Comparison of alamar blue and MTT assays for high through-put screening
TIV, Volume 18, Issue 5, Pages 703-710
Hamid, R.; Rotshteyn, Y.; Rabadi, L.; Parikh, R.; Bullock, P.

Good Caco-2 cell culture practices
TIV, Volume 26, Issue 8, Pages 1243-1246
Natoli, M.; Leoni, B.D.; D'Agnano, I.; Zucco, F.; Felsani, A.

Dichloro-dihydro-fluorescein diacetate (DCFH-DA) assay: A quantitative method for oxidative stress assessment of nanoparticle-treated cells
TIV, Volume 27, Issue 2, Pages 954-963
Aranda, A.; Sequedo, L.; Tolosa, L.; Quintas, G.; Burello, E.; Castell, J.V.; Gombau, L.

Simultaneous evaluation of cell viability by neutral red, MTT and crystal violet staining assays of the same cells
TIV, Volume 12, Issue 3, Pages 251-258
Chiba, K.; Kawakami, K.; Tohyama, K.

In vitro toxicity of nanoparticles in BRL 3A rat liver cells
TIV, Volume 19, Issue 7, Pages 975-983
Hussain, S.M.; Hess, K.L.; Gearhart, J.M.; Geiss, K.T.; Schlager, J.J.

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ESTIV Corporate members

MatTek In Vitro Life Science Laboratories, an European subsidiary of MatTek Corporation (USA) produces since 2011 reconstructed 3D human tissue models for the EU and Asia market in Bratislava, Slovakia. MatTek is at the forefront of tissue engineering and is a world leader in the production of innovative 3D reconstructed human tissue models.
MatTek's skin and ocular tissue models are used in regulatory toxicology (OECD, EU guidelines) and address toxicology and efficacy concerns throughout the cosmetics, chemical, pharmaceutical and household product industries. MatTek Corporation celebrates 30 years anniversary in 2015.
www.mattek.com

Epithelix proposes innovative *in vitro* solutions for respiratory diseases and chemical testing. It provides reconstituted human *in vitro* tissues with long shelf-life and associated services for research laboratory, personal care, chemical and pharmaceutical industry.
www.epithelix.com

Novozymes is a world leader in bio-innovations. Together with customers across a broad array of industries, Novozymes creates industrial bio-solutions, which both improves our customers' business and the use of the planet's resources.
www.novozymes.com

As independent and customer-oriented research organisation, VITO provides innovative technological solutions as well as scientifically based advice and support in order to stimulate sustainable development and reinforce the economic and social fabric of Flanders.
https://www.vito.be

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ESTIV Affiliated Societies

Associazione Italiana Tossicologia *In vitro* - CellTox
Dutch-Belgium Society for *In vitro* Methods–INVITROM
UK *In vitro* Toxicology Society – IVTS
Brazilian Society on Alternative Methods to animal testing (SMAlt)
Scandinavian Society for Cell Toxicology – SCCT

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ESTIV membership fee

Membership fee
The membership for an individual member for 2015 is € 30.00. If you are also a member of one of the affiliated societies (CellTOX, SSCT, INVITROM, IVTS), the membership amount to € 18.00.

Method of Payment
Bank Transfer
RABOBANK, IBAN #: NL20 RABO 0101 4857 51
BIC: RABONL2U

Attention of: ESTIV
Polderkade 1, NL-5345 RR Oss, The Netherlands
Due to the high costs of applying for and cashing EuroCheques, please do not use this means of payment.

It is also possible to pay the membership fees by our convenient and secure online credit card payment services (PayPal), To use these services, please visit the ESTIV website at:
http://www.estiv.org/member.html

Laura Suter-Dick

ESTIV e-mail list

ESTIV has an e-mail list, which has the potential to be a very valuable resource. There are many types of questions that you could pose to the list, whether you are a junior or a senior scientist. To send a message to all ESTIV members on the list (presently more than 200 colleagues), simply address your e-mail to estiv@freelists.org
This is a “closed” list, which means the “list-owner” (Elsa Casimiro) is able to select who is allowed to join. Only ESTIV members will receive the message. However, please note that this list should not be used to send confidential messages or attachments as these are uploaded to the 'freelists' archive that can be accessed by the general public. If you have never received a message from the ESTIV list, it is because you have not informed us of your e-mail address. Please correct this by sending a message to me at secretariat@estiv.org and your name will be added.

Elsa Casimiro

"ESTIV also owns a group on LinkedIn, to communicate and to allow ESTIV members to update each other on career moves, etc. The group is only open to ESTIV members. Search for the group "ESTIV" and register".

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ESTIV Executive Board Members 2015

EXECUTIVE BOARD	*POSITION*	*E-Mail*
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ESTIV Honorary Members

Monique Adolphem, Michael Balls, Diane Benford, Bas Blaauboer, Bob Combes, Sjeng Horbach, Horst Spielmann, Jan Van der Valk, Flavia Zucco

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For more information on ESTIV and membership application contact

Elsa Casimiro (ESTIV Secretary)
E-mail: secretariat@estiv.org

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