Abstracts book
GOALS: Looking at experimental approaches in life sciences as if they were just developments of traditional tools would be misleading. Development of tools and techniques in chemistry and physics techniques have deeply changed the capacities and uses of experimental approaches.

New tools widen the traditional terms of reference of the “debate on 3Rs”: OMICs, organoids, bio-artificial organs, use of ex-vivo material, telemetry and not invasive measures, imaging in vivo and on cellular material...

Coupled with a better understanding about molecular, cellular and physiological mechanisms, these approaches enrich the studies in vivo, in vitro, in silico. They also make it possible to use both human and animal observational data, with clinical or epidemiological approaches, in pathological or healthy situations.

Some tools are so innovative that they do not Replace nor Reduce nor Refine any existing approaches.

The various fields which require the investigation of the living, such as basic research on physiology, pathologies, (eco) toxicity, drug development, production and quality control of animal and human health products, food control and even education can all benefit from these improvements. What is more, to these scientific breakthroughs allow more and more connections between those fields.

The European platform ECOPA is dedicated to the promotion of the 3R principle (refinement, reduction and replacement of animal experiments) and acts by:

- Dissemination of results obtained in this field
- Promotion of exchanges between the disciplines and especially between the four interested parties (associations, research, authorities, economic actors)

It has teamed up with its national french component FRANCOPA to organize this scientific symposium, which should illustrate the tremendous potential offered by the new tools for the investigation in life sciences to resolve ethical imperatives and concerns about understanding life.

This symposium will allow the stakeholders from the four pillars of ECOPA to exchange on those matters and to find new ways to structure the ethical debate on experiments, at a time where the new tools soften the distinction between experiment and observation, as well as they break some borders between disciplines.

ATTENDEES: Users of results in upstream and applied research, developers of methods, interested parties in product safety, in human and veterinary medicine, stakeholders or NGOs on ethical research...
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OPENING KEYNOTE

MODELING HUMAN PATHOGENESIS USING NOVEL IN VITRO AND IN SILICO METHODS

Animal experimentation has been considerably used to model human pathologies. Following the 3R concept, there has been a considerable effort to reduce and replace when relevant animal testing. Several in vitro systems have been established: cultured cells monolayers, 3D cell systems and organoids, microfluidic systems, co-culture using different tools, cellular stretching, shearing, etc. It is thought that through deep phenotyping of these systems it would be possible to compensate for the lack of a complete organism. Thus omics methodologies and systems biology were used in that respect. Computational methods were also used to model the different stages of disease development. A new approach in the field has been the use of Adverse Outcome Pathways (AOP) which represent a scheme describing the different key events at different scales that connect an initiating event with an adverse outcome. In complex conditions, AOP networks have been developed. The relevance of these approaches is quite large and covers different fields beyond toxicology. These developments represent a real opportunity to implement the 3Rs with relative confidence.

Keywords
Adverse outcome pathways, cell culture, omics, computational models

R. Barouki
- Inserm UMR-S 1124, University Paris Descartes, 45 rue des Saints Pères, 75006 Paris
- Hôpital Necker Enfants malades 75015 Paris
Contact e-mail: robert.barouki@univ-paris5.fr
Session 1
System biology and physiopathology
FROM ORGAN-ON-A-CHIP TOOLS TOWARDS PATIENTS ON CHIPS – ENFORCING A PARADIGM SHIFT IN DRUG DEVELOPMENT

Microfluidic microphysiological systems (MPS) have proven to be a powerful tool for recreating human tissue-and organ-like functions at research level. This provides the basis for the establishment of qualified preclinical assays with improved predictive power. Industrial adoption of microphysiological systems and respective assays is progressing slowly due to their complexity. In the first part of the presentation status quo of MPS development and examples of industrial adoption of single-organ chip and two-organ chip solutions are highlighted. The underlying universal microfluidic Multi-Organ-Chip (MOC) platform of a size of a microscopic slide integrating an on-chip micro-pump and capable to interconnect different organ equivalents will be presented. Sixteen different single organ equivalents have been established on that platform and nine organ combinations have been tested for stable long-term crosstalk yet. The second part of the presentation focusses on the challenges to translate a MOC-based combination of four human organ equivalents into a commercially useful tool for ADME profiling and toxicity testing of drug candidates. This four-organ tissue chip combines intestine, liver and kidney equivalents for adsorption, metabolism and excretion respectively. Furthermore, it provides an additional neuronal tissue culture compartment for extended toxicity testing. Issues to ensure long-term performance and industrial acceptance of such complex microphysiological systems, such as design criteria, tissue supply and on chip tissue homeostasis will be discussed. Finally, the presentation provides a roadmap towards on-chip patient models, which bear the potential of a paradigm shift in drug development.

Keywords
Organ-on-a-Chip, Multi-Organ-Chip, Microphysiological Systems, Safety Testing, Efficacy Testing, Therapeutic Window

Thanks
The work received funding from the German Federal Ministry for Education and Research, GO-Bio Grant No. 0315569 and from the European Union’s Horizon 2020 research and innovation program under grant agreement No 681002.

References


PRION DISEASES: TOWARD FURTHER REDUCTION OF ANIMAL EXPERIMENTATION

Prion diseases constitute a group of neurodegenerative disorders affecting humans and animals. Creutzfeldt-Jakob disease (CJD) in human, mad cow disease in bovine, scrapie in sheep and chronic wasting disease in cervids are the most common forms of this fatal disease. Prions are infectious proteic particles devoid of nucleic acid. The prion protein exists in two forms: the normal one (PrPC) and the abnormal and infectious one (PrPSc). Prion propagation occurs upon interaction of the abnormal form with the normal cellular form, which drives the normal protein to refold into the abnormal form, thus initiating the fatal infectious mechanism.

PrPC is also convertible into PrPSc in vitro after seeding of minute amounts of PrPSc into healthy mouse brain lysate: several cycles of sonication/incubation convert the normal form into PrPSc. The technique is known as Protein Misfolding Cyclic Amplification, PMCA. It allows the detection of subinfectious levels of PrPSc in several biological samples of infected human and animals.

We have developed a new PMCA procedure (miniaturized-beads PMCA) that is adapted to high throughput level. Using our technique, only one mouse brain is sufficient in experiments where classical bioassays using mice require over 400 animals. Furthermore, mb-PMCA is achieved in 2-4 days instead of 2 months to 2 years in bioassay.

This method for prion amplification has been further adapted by substituting brain lysates with lysates of cultured cells that express PrPC. Some prion strains could therefore be amplified without any animal material. In some instances, (i.e. variant-CJD prion), the cell lysate required the addition PrP0/0 mouse brain lysate (1/1 ratio) which brings brain factors required for amplification of this strain. In bioethical terms, our brain- and Cell-based PMCA constitute a significant step toward further reduction of animal experimentation in the prion field.

Keywords:
Prion - Creutzfeldt-Jacob - Mad cow - Scrapie - Animal experimentation - 3R principle

References:


Animal models are indispensable for identifying physiological mechanisms and developing new therapies for human diseases. Indeed, even if cellular studies can provide very important data on pathologies, they are generally insufficient to understand the complexity of events taking place in a multicellular organism (cell interactions, long-range signaling, metabolic regulation, ...). Despite the importance of rodent models in pre-clinical studies, the failure rate of treatments based on these studies remains far too important. Thus it is critical to define new strategies to 1) improve probabilities of success in clinical studies 2) limit the number of animals sacrificed in unsuccessful and expensive studies. Drosophila models emerged in the early 21st century after the discovery that 75% of the genes involved in human pathologies are conserved in Drosophila. Such models can be a tool of choice for these new approaches. In fact, sophisticated molecular genetic tools make it possible to control in vivo the expression of a gene of interest at 3 levels (spatial, temporal, intensity), to study the resulting phenotypes and to search for genetic or pharmacological modifiers in such induced pathologies, to validate them in a second time on rodent models.

I will illustrate the various areas of study that can be adapted to these approaches and the strategies involved. Finally, I will show how the new CRISPR / CAS9 technologies widen the scope of possibilities and reinforce the interest of interfacing Drosophila models with other strategies for studying human diseases.
GENOTOXIC AND CARCINOGENIC POTENTIAL OF 160 MYCOTOXINS IN HUMAN CELLS

Mycotoxins are metabolites produced by fungi causing diseases such as cancers in animals and humans. They commonly enter the food chain through contaminated food. More than 300 mycotoxins were described so far. Each mycotoxin is generally able to affect different organs and, probably due to differences in biotransformation capabilities, display pronounced interspecies toxicity dissimilarities. Only limited data exist on the genotoxicity of mycotoxins. Notably, some of these compounds are toxic to bacteria and could not be tested accurately with the Ames assay.

The project aims to explore the genotoxic potential of 160 mycotoxins potentially found in food. We have recently developed a high throughput genotoxicity strategy based on the quantification of the histones H2AX and H3 in different human cell lines. This novel method permits to discriminate efficiently aneugens, clastogens and cytotoxic compounds. The used of human cell lines with different bioactivation capacities permit to differentiated proficiently direct genotoxins from bioactivated ones. We have screened 160 mycotoxins using the developed genotoxic assay in four human cell lines (HepG2, ACHN, LS-174T and SH-SY5Y) corresponding to mycotoxin target tissues (liver, kidney, colon and brain respectively). Over the 160 mycotoxins tested, 75 (47%) did not demonstrated any genotoxic potential. However, 66 (41%) demonstrated clastogenic properties, some of them with greater genotoxic potential than AFB1. 13 (8%) revealed aneugenic properties and six mycotoxins (4%) exhibited both aneugenic and clastogenic properties. For the clastogen mycotoxins, the use of different cell line allowed us to determine whether the tested compounds require a bioactivation reaction prior producing a genotoxic effect.

This is the first study that compare simultaneously the genotoxic potential of 160 mycotoxins in human cells. It will provide a critical tool to help risk assessors to reducing the uncertainties regarding the risk associated with mycotoxins contamination.

Keywords
Mycotoxins Genotoxicity H2AX Metabolism

Thanks
This research was funded by the PNREST Anses, Cancer TMOI AVIESAN, 2013/1/214 grant.

References
The rise in NAFLD (non-alcoholic fatty liver disease) prevalence constitutes an important public health concern worldwide. This disease, starting from hepatic steatosis (i.e. lipid accumulation) to one of its pathological complications, i.e. steatohepatitis, has been related to diverse etiologic factors, including alcohol, obesity and environmental pollutants\(^1\). However, only few studies have so far been realized in order to understand how these different factors might interplay regarding the progression of liver diseases.

Since NAFLD are pathologies that depend in part on intercellular interactions between liver cells but also on communications between the liver and the other organs, an \textit{in vivo} model is thus needed to integrate the complete physiology, which is not the case regarding \textit{in vitro} model. In this context, keeping concern of 3Rs issues, we decided to explore the possibility to use zebrafish larva to determine the impact on NAFLD of an environmental carcinogen, benzo[a]pyrene (B[a]P), in binary combination with ethanol, a well-known hepatotoxic lifestyle toxicant. Indeed, this model has two main advantages: (i) close similarities with human genetics and liver physiopathology; (ii) transparency of larva that allows to develop wide variety of imaging techniques adapted to high throughput studies\(^2\).

Concretely, we have generated a model of larva rapidly developing HFD-induced steatosis (1 day) before exposure to xenobiotics for 7 days. Using this model and diverse approaches including imaging, we have highlighted a role of co-exposure to B[a]P and ethanol in the progression of steatosis towards a steatohepatitis-like state, notably dependent on mechanisms linked to membrane remodeling\(^3\). In conclusion, zebrafish larva behaves as a promising model to more thoroughly study the mechanisms of liver disease progression, and to allow screening of environmental contaminants that are deleterious for human health as endocrine disrupters.

**Keywords**

\textit{In vivo} model, zebrafish larva, liver, steatosis, steatohepatitis, environmental contaminants, imaging.

**Thanks**

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**References**

Session 2

Organoids & organ on Chip
ORGANOIDs, ORGANS-ON-CHIP, 3D BIOPRINTERING IN A 3R CONTEXT

Organoids, organs-on-chip, 3D bioprinting may fill the existing gap between 2D culture of human cells and animals experimentation. By mimicking more accurately the molecular mechanisms at work in tissue development and diseases, the manufacturing of pluricellular 3D structures combined to genetic engineering will impact our current understanding of living tissues. These organ surrogates will also enable personalized pharmacological evaluation in physiologically relevant conditions, while reducing the number of tests on laboratory animals.

X. Gidrol

CEA, Université Grenoble Alpes, INSERM, BIG, BGE, Grenoble, France
CEA Grenoble, 17 rue des Martyrs, 38054 Grenoble.
Contact e-mail: xavier.gidrol@cea.fr
Liver plays a major role in the metabolism of drugs as well as the elimination of toxins compounds from the environment and food. Given the interspecies differences that exist between rodents and humans, development of relevant in vitro human models is crucial to investigate metabolism and toxicity in cells that exhibit the more in vivo-like response.

Our team recently developed a well-defined three-dimensional (3D) culture model of human hepatocytes in collagen gels. Both primary and transformed human cells were embedded in collagen matrix in which it is possible to vary matrix stiffness. Such approach allows a specific organization of the cells in spheroids which are polarized, expressing liver functions, including drug-metabolism enzymes at a significant level close to liver in vivo, after few days of culture and during at least four weeks. The human hepatocytes in 3D cultures allow both proliferation and differentiation. We demonstrated that our model is suitable many applications such as cell viability, apoptosis, xenobiotic biotransformation. Moreover, we analyzed DNA damages by comet assay, phosphorylation of the histone H2AX and DNA adducts identification following various genotoxic treatments.

All together, we set up a promising human hepatocytes in vitro 3D model able to predict drugs and contaminants toxicity and cell regulations.

**Keywords**

Human hepatocytes, primary cultures, three-dimensional culture, differentiation, proliferation, toxicity

**Thanks**

PNREST Anses, Cancer TMOI AVIESAN, 2013/1/166; Ligue contre le Cancer; Région Bretagne; Inserm; Université de Rennes 1.
HEPATOPEARLS: NEW GENERATION OF LIVER-MIMICKING SPHEROIDS

For several decades, cell-based assays have been used in drug discovery and development. The liver being the principal site of drug metabolism, primary human hepatocytes cultured as monolayer on two-dimensional substrates were traditionally used as the only practical option for cell-based screening. However, 2D cultured hepatocytes suffer disadvantages associated with the loss of tissue-specific architecture, mechanical and biochemical cues, and cell-to-cell and cell-to-matrix interactions affecting their metabolic function and lifespan, thus making them relatively poor models to predict drug responses for certain applications; prediction of hepatic clearance over long incubations with low-clearance compounds, toxicokinetics due to chronic drug exposure and drug-drug interaction predictability close to vivo.

To circumvent these drawbacks, we have developed a novel technology named "BioPearl", to fabricate miniaturized 3D spheroids from primary human hepatocytes with a rate of production adapted to high throughput screening. BioPearl is a millifluidic based technology allowing scaffold-free 3D liver spheroids to be grown in the liquid core of capsules under physiological conditions. Using this technology, miniaturized core-shell capsules composed of a thin layer of alginate and a liquid core of cells are generated with the elevated rate of 1500 capsules produced per second. "HepatoPearls" generated in this way display vivo-mimicking characteristics such as: a) Polarized epithelial morphology with the presence of bile canaliculi network, b) functional detoxification transporters, c) lifespan of up to 45 days d) high and stable metabolic activity of phase I and II metabolizing enzymes e) albumin secretion f) urea synthesis and g) CYP inducibility over 6 weeks.

HepatoPearls are considered a breakthrough in 3D liver models field thanks to their ease of handling, their capacity to integrate to any bioassay with different biomass needs, and their high metabolic functions stabilized up to 45 days. In this talk some applications of HepatoPearls in drug metabolism and pharmacokinetics, hepatotoxicity and drug-drug interaction screening will be presented.

Keywords
Spheroid, 3D cell culture, Physiologically relevant liver models, encapsulation, alginate, in vitro alternatives to animal models

Thanks
We would like to thank ESPCI Paris and Sanofi who granted the studies at the origin of development of the HepatoPearl model.

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DEVELOPMENT OF A NOVEL IN VITRO AEROSOL EXPOSURE SYSTEM: THE INDEPENDENT HOLISTIC AIR-LIQUID AEROSOL EXPOSURE SYSTEM (INHALES)

The human respiratory tract is functionally and structurally heterogeneous and the kinetics governing the transfer of gas or aerosol constituents from an inhaled volume of air to its epithelia are highly complex. Epithelia lining different regions of the respiratory tract are hence not uniformly exposed. The physical conditions epithelial cells experience as well as the amount and composition of an inhaled aerosol they perceive are highly different if, for instance, the upper airways are compared to the alveolar sacs.

Simulating this complexity for the purpose of in vitro aerosol exposure experiments poses a significant technical challenge. Relevant exposure parameters like flow velocities, flow patterns, the gradual changes in test atmosphere properties, residence times, and test atmosphere dilution as they occur in the living organism can generally not or only to a very limited extent be simulated 1-3.

In order to overcome these limitations, we are developing a novel aerosol exposure system, the Independent Holistic Air-Liquid Exposure System (InHALES)4, which structurally and functionally mimics the human respiratory tract. The key features of the system are that:
- It is able of breathing surrounding air, activating inhalers or taking puffs from cigarettes, i.e. it does not rely on any active aerosol supply (Independent).
- It represents the complete human respiratory tract from the oropharyngeal cavity down to the lung lumen and allows exposures of tissue cultures of all the according regions of the respiratory tract to be conducted in one single experiment (Holistic)

The system is able to simulate virtually any relevant breathing pattern. As structural and functional aspects of the human respiratory tract are combined, critical parameters such as flow velocities or aerosol dilution are, for each region of the respiratory tract, close to in vivo conditions by default.

We built a prototype of the system as a proof of concept and could demonstrate its functionality in cell-free exposure experiments using cigarette smoke and fluorescently labelled glycerol aerosols as test atmospheres. Currently, we are improving the geometry of the airway model. In particularly a 3D printed model of the bronchial tree reaching down to the transitional bronchioles is being developed.

Keywords
Aerosol exposure, in vitro, air-liquid interface system, 3R

Thanks
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References
NEUROSPHERES FOR SPECIES-SPECIFIC, MEDIUM-THROUGHPUT ANALYSES OF DEVELOPMENTAL NEUROTOXICITY (DNT) EVALUATION

Testing for developmental neurotoxicity (DNT) of compounds for regulatory purposes, e.g. for screening and prioritisation, by using an in vitro testing battery is currently under discussion at the OECD level. Within such a battery a variety of neurodevelopmental processes at different developmental stages needs consideration. We have been establishing a DNT high content assay based on time-matched fetal human and postnatal rodent as well as human induced pluripotent stem cell (hiPSC)-derived neural progenitor cells (NPCs), which proliferate in culture and - under differentiating conditions - migrate and differentiate into neurons and glia cells, i.e. radial glia, astrocytes and oligodendrocytes. In this so-called ‘Neurosphere Assay’ we analyzed multiple pathways for their functional implications in neurodevelopmental processes, amongst them oxidative stress, histone deacetylase inhibition or thyroid hormone disruption. For high content analyses of differentiated neurospheres, we developed the software ‘Omnisphero’ allowing simultaneous analyses of multiple neurodevelopmental endpoints like NPC migration, neuron and glia differentiation, neuronal morphology and neuronal migration. This set-up allows chemical testing in a medium throughput with the ‘Neurosphere Assay’ as part of a DNT testing battery, e.g. for industrial or regulatory usage.

Keywords
Neurosphere, neural progenitor cell, differentiation, development, brain
Session 3

Omnics, Biomaps, Exposome
INPUT OF PROTEOMIC ANALYSES FOR UNDERSTANDING CELLULAR RESPONSES TO NANOPARTICLES: TOWARD MECHANISTIC DATA AND EVIDENCE FOR CROSS-TOXIC EFFECTS

Nanoparticles are recently-introduced industrial products which impact on the environment and on human health is intensely debated. Most of the toxicological research carried to date on NPs follows very classical tracks, so that little is known yet on their mechanisms of toxicity, apart their propensity to induce oxidative stress and sometimes cytokine release. Because of their major scavenging role, macrophages are one of the major cell types to study when working on nanoparticles. In order to gain new insights on the interaction of nanoparticles with macrophages, we carried out a proteomic screen on cells treated with different nanoparticles (e.g. copper oxide, zinc oxide, metallic silver or silica). Very different responses have been observed: Copper oxide induces mainly a mitochondrial response and a strong response at the glutathione level. Zinc oxide rather induces a proteasomal and a metabolic response (gluolysis and pentose phosphate pathways). Proteomics was followed by targeted studies aiming at validating the proteomic results and at investigating major functions of macrophages (e.g. phagocytosis and cytokine production). In this way, we could demonstrate a role of DNA repair pathways for zinc oxide and silica nanoparticles, as well as a critical role of glutathione and heme oxygenase for survival to a copper oxide challenge. Mitochondrial dysfunction is also prominent following exposure to zinc oxide or copper oxide, and much less important following exposure to silver or silica. Different changes in the actin cytoskeleton were also observed in response to the nanoparticles tested. In conclusion, this combined approach has provided new ideas on how nanoparticles can exert their toxic effect.

Keywords
Nanoparticles; proteomics; zinc oxide; amorphous silica; copper oxide

Thanks
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HIGH-THROUGHPUT METHODS FOR AN INTEGRATIVE APPROACH OF THE NANOTOXICOCOLOGY

Regardless the positive impact of presence of nanoparticles in products on performance, stability, marketing and price, a big issue is emerging: do nanoparticles have a negative impact on human health and environment? To answer this question, industrial and legislator first considered that for a given compound, whatever their size (macro, micro or nano), the toxicity profile would be the same. But many studies suggested that new phys-chem properties come with new biological and environmental behavior. Thus, during last years, many studies have been published about toxicity evaluation of nanoparticles, leading to thousands of data (1 020 000 entries in Google Scholar and 18 481 in PubMed for “Nanoparticles AND Toxicology” asking). This huge amount of results is very difficult to understand and a clear message about toxicity of nanoparticles can’t be obtained.

This is mainly due to two factors: first a lack of experimental processes harmonization, and second a need of relevant and powerful methodology for data analysis. International consortia such as Nanogenotox\(^1\) and NanoReg\(^1-2\), proposed SOPs for studying nanoparticles toxicity \textit{in vitro} (from nanoparticles preparation to cell exposure through characterization).

A key point challenge regarding toxicology of nanoparticles is the development of high throughput screening (HTS) methods\(^3-4\). We developed an experimental HTS procedure based on cellular impedance measurement for the screening of nanoparticles’ toxicity\(^5-10\). We also coupled this approach with other HTS methods such as flow cytometry or fluorescence microplate readers. Possible interferences have, of course, been tested.

In order to analyse properly and efficiently the huge amount of data obtained, we also adapted transcriptomic analysis strategy, developed in our team, to toxicology.

This integrative approach represents an innovative strategy, in the line of global willingness of developing tools for a predictive nanotoxicology.

**Keywords**

Nanoparticles, Toxicology, High Throughput Screening (HTS)

**References**

1. Nanogenotox: The project. Available at: https://www.anses.fr/fr/node/120284.

\(^1\) Laboratoire de Cancérologie Expérimentale, CEA DRF/iRCM 18 route du Panorama, 92265 Fontenay-aux-Roses
\(^2\) Laboratoire de Toxicologie Génétique, Institut Pasteur de Lille, 59000 Lille
Contact e-mail: romain.grall@cea.fr
ROLE OF PERSISTANT ORGANICS
POLLUTANTS AND ADIPOCYTES IN THE
ACQUISITION OF A METASTATIC POTENTIAL
AND OF CHEMORESISTANCE IN BREAST
CANCER

Background:
In 2018, more than 250,000 new cases of invasive breast cancer are estimated to be diagnosed in the U.S., associated with more than 40,000 women who will die from breast cancer. Indeed, the 5-year relative survival rate of people with breast cancer is only 26% when diagnosed with distant metastasis. Recent in vivo and in vitro studies have suggested that environmental pollutants, mostly persistent organic pollutants or POPs, could influence tumor phenotype stimulating cellular processes relevant for metastasis such as migration and invasion. Adipose tissue, the main component of the peri-tumor stromal fraction in breast cancer, exerts a major endocrine and secretory role and it has been shown that these fat-storing cells and their precursors (pre-adiocytes) influence tumor behavior. POPs are a major public health concern because of their toxicity, persistence in the environment and we know that their accumulation is preferentially in adipose tissue.

Method:
To study the interactions between pre-adiocytes and breast cancer cells, we set up an original in vitro co-culture model using mainly human mammary tumor cells (MCF-7, MDA-MB-231) and human pre-adiocyte cells (hMADS, human multipotent adipose-derived stem cells), exposed or not to 2.3.7.8-TCDD, one of the most toxic POPs and the most active within the group of Aryl hydrocarbon Receptor (AHR) agonists and carried out experiments on several important cellular processes related to the acquisition of a metastatic phenotype. We hypothesized that TCDD modifies the phenotype of both adipose and MCF-7 cells responses compared to the co-culture alone.

Results:
Using the xCELLigence system to monitor MCF-7 properties (adhesion, spreading and proliferation of the cells), we showed that the cells properties were modified by the co-culture with hMADS cells and the co-exposure (co-culture + TCDD) with a decreased cell index value and increased cell invasion and/or migration. The expression of genes involved in tumor progression and metastasis were upregulated (Plasminogen activator inhibitor-1 (PAI), Human Enhancer of Filamentation 1 (HEF1, also known as NEDD9 or Cas-L) and Vascular endothelial growth factor (VEGF) and we found modifications of gene expression in favor to an epithelial-mesenchymal transition (EMT) (down-regulation of E-cadherin and upregulation of Slug (SNAI2) and Snail (SNAI1)), frequently associated with metastasis. In addition, we identified specifically in the co-exposure condition, a stem-cell biomarker, the Aldehyde dehydrogenase 1 family, member A3 (ALDH1A3). The stemness properties of treated cells were confirmed by tumorsphere formation assay and ALDH1A3 activity in FACS. In vivo, we examined the ability of cancer cells to metastasize in a Zebrafish larvae model and found that the co-exposure increased the frequency and the number of metastasis per fish. Considering the links between stem-like properties and chemo-resistance, we studied the expression of the estrogen receptor-alpha (ER alpha), a target of several endocrine agents, and showed a down-regulation, especially in the co-exposure condition. Finally, we observed important morphological changes in the co-exposure with the generation of giant polynuclear cells (PGCCs), a cancer sub-population strongly associated with aggressiveness and chemoresistance in cancer.

Conclusion:
These results suggest that the interaction between adipose tissue exposed to TCDD may affect the aggressiveness and progression of breast cancer. Further studies are required to confirm the acquisition of resistance to chemotherapy in cell lines and to clarify the underlying mechanisms.

Keywords
AhR, TCDD, breast cancer, chemoresistance, metastasis, stem cells, polyploid giant cells

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M. Koual (1), (2) C. Tomkiewicz (1), X. Coumoul (1), R. Barouki (1)
(1) INSERM UMR-S1124, Toxicologie Pharmacologie et Signalisation cellulaire
(2) Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges-Pompidou, Service de Chirurgie Cancérologique Gynécologique et du Sein, Paris, France
Contact e-mail :
meriemkoual@hotmail.com
celine.tomkiewicz@inserm.fr
MODERN SCIENCE FOR 3RS ALTERNATIVE APPROACHES AND BETTER QUALITY CONTROL OF HUMAN VACCINES

The 3Rs principles – Replacement, Reduction, Refinement – have been established in 1959 and since then have been adopted widely and particularly in Europe with the European Directive 2010/63/EU. The demand for implementing 3Rs is not only coming from legislators but also the general public who is more and more sensitive on the ethics of animal use in research, as in other fields. The vaccine industry has been committed to the 3Rs principles for several years. Whereas animal testing has been successfully removed from lot release testing of well-characterized human vaccines, in vivo safety and potency quality control testing are still used for established inactivated vaccines such as rabies, pertussis, diphtheria, and tetanus vaccines. Moreover, regulatory requirements often differ for various parts of the world, resulting in either duplication of animal testing or partial implementation of 3Rs for some vaccines when distributed worldwide. This reinforces the need for enhancing international harmonization and cooperation efforts in order to support more rapid progress towards 3Rs for human vaccines testing. The presentation will briefly review the use of laboratory animals in human vaccines research and testing and will describe the vaccine manufacturing industry’s commitment and its concrete programs for implementing 3Rs principles in R&D and industrial operations processes using modern science and consistency approach. It will highlight the successes as well as the barriers that are encountered when implementing 3Rs principles, as well as the ongoing efforts that include external collaborations with other industries, public organizations and Health Authorities for the acceptance of alternative methods.

S. Uhlrich (1), E. Coppens (1), F. Moysan (1), S. Nelson (2), N. Nougarede (1)

(1) SANOFI PASTEUR, France
(2) SANOFI PASTEUR, Canada
Contact e-mail: sylvie.uhlrich@sanofi.com
DEVELOPMENT OF SENS-IS, AN IN VITRO ASSAY TO MEASURE SKIN SENSITIZATION POTENCY OF CHEMICALS

In vitro approaches for the identification of skin sensitizing chemicals are highly desired as a way to replace the animal tests that are not accepted anymore for testing cosmetic ingredients due to public and political concerns and European cosmetics regulations (EC) No 1223/2009. Analysis of genes modulated during the sensitization process either on mice (LLNA) or human (blisters) combined with data mining has allowed the definition of a comprehensive panel of sensitization biomarkers. The expression of this set of genes has been measured on reconstituted human epidermis models (Episkin) exposed to various sensitizers and non-sensitizers. Fine analysis of their expression pattern indicates that it is the number of modulated genes rather than the intensity of up-regulation that correlates best with the sensitization potential of a chemical. By combining the expression data, it is now possible to identify a wide variety of sensitizers on a test system (in vitro reconstructed human epidermis) that is very similar to the in vivo situation and compatible with a large variety of test substance characteristics. The SENS-IS assay has been shown to be robust and easily transferable. It’s capacity to predict hazard is excellent as demonstrated by Cooper statistics values over 95% on a large panel of chemicals. The SENS-IS assay being based on a reconstructed human skin model as the test system, is providing a wide applicability domain very similar to the expected “typical use” of the tested products. The SENS-IS assay thus represents a serious alternative to the available in vivo sensitization tests.

Keywords
Skin sensitization, Alternatives to animal tests, Toxicogenomics, Reconstituted epidermis, SENS-IS

References
Session 4

Education and training in medicine
A. TESNIERE (ILUMENS)
C. VOGT (CLAUDE BERNARD UNIVERSITY – LYON 1)
OR
F. STORCK (VETERINARY SCHOOL OF ALFORT)
A FRAMEWORK PROGRAM FOR THE TEACHING OF ALTERNATIVE METHODS (REPLACEMENT, REDUCTION, REFINEMENT) TO ANIMAL EXPERIMENTATION

Development of improved communication and education strategies is important to make alternatives to the use of animals, and the broad range of applications of the 3Rs concept better known and understood by different audiences. The final goal is beyond just replacing, reducing and refining the use of animals for scientific purposes rather to present a new approach that should allow a better prediction of the effect on humans. The concept of 3Rs is getting outdated in favour of the modern NAMs (New Approach Methodologies) to indicate a novel technique that is disruptive with the past, including any techniques that may contribute in the understanding of the real mechanism in the human organism. It is a new topic and general experience is still little. That's why a specific teaching framework program may help in the education of young scientists who are just facing this fascinating topic, as well as senior scientists who need to enlarge their expertise.

This type of new training should start from the correct definition of the audience and the message that is to be communicated. The program should include the description of the specific tools joined by all other relevant aspects such as the concept of validation, quality procedures, regulatory acceptance and so on. Unfortunately, more and more in the EU the funding dedicated for training is limited both in terms of timing that operators can dedicate to that and resources that are necessary for the organisation of hands on training in the lab. Next step is therefore the proper management of these limits with suitable maximisation of the available length of the training and the tools that are available.

In spite of the challenges, teaching such innovative and inspiring approach is exciting and the experts of the field are strongly invited to make any effort in this sense. Specific focus should be dedicated also to pupils from high schools, that represent the scientists of the future. The goal is not the simple dissemination of notions of 3Rs and NAMs, rather to transmit the curiosity and the willingness to never stop in front of traditional approach and being open minded towards any new possibility that science may offer now and in the future.

Keywords
3Rs, NAMs, education

References
THE 3RS IMPLEMENTATION IN FELASA ACCREDITED COURSES

The Directive on the Protection of Animals Used for Scientific Purposes 2010/63/EU recognises the importance of education and training of all persons involved with the use of laboratory animals. FELASA accredits modular initial training courses (Gyger et al., 2018) addressing both the Directive 2010/63/EU and the related European Commission guidance document (European Commission guidance document, 2014).

The FELASA accreditation scheme needs to give a concrete and convincing argumentation showing the application of the 3Rs, and to address the ethical approval of the procedures done on living animals on practical classes. Course audits are key-elements of the FELASA accreditation scheme, going towards best practice in LAS education and training. A special focus will be made on the 3Rs in the course programme.

The presentation will elaborate on the FELASA accredited function courses applications and on audits reports, the main features characterising these courses will be summarized and analysed, focusing on the implementation of the 3Rs in practical works. The improvements of practical sessions include

Reducing the number of animals per student
Replacing animals by using mannekins/phantoms, dead animals, materials from slaughter houses. Refining animals use, mainly by a continuous and direct supervision and by the use of anesthetized animals in the training of procedures. The staff student ratio during these classes is a criterium needing major attention, because this represents the main parameter for refining the practical training, especially with awake animals.
Re-using animals for other pedagogic or scientific purposes instead of using another set of animals

Concrete examples and illustrations for 3Rs application in teaching will be given.

References
Session 5
Computational Toxicology & Drug efficiency
MARRYING IN VITRO AND COMPUTATIONAL APPROACHES TO IMPROVE DRUG SAFETY AND EFFICACY

Human in vitro methodologies are becoming invaluable tools for uncovering mechanisms of chemical-induced perturbation. This coupled with techniques such as transcriptomics, metabolomics, proteomics and high content imaging can produce large amounts of data, capturing large chunks of biology. For such systems to be applicable to safety assessment, the kinetics of the chemical in the systems needs to be determined, and reverse dosimetry to the whole body should be applied. Computational approaches, including docking and dynamic models can also be used to supplement cheminformatics to predict chemical interaction with enzymes and receptors. The integration of biological data streams, with cheminformatics, kinetics and in vitro to in vivo extrapolation requires the marriage of in vitro and computational approaches. The improvement of efficacy and safety prediction is dependent on the evolution of these individual aspects in an integrated synergistic way.

Keywords
Stress response, transcriptomics, safety, efficacy

References

P. Jennings
Division of Molecular and Computational Toxicology, Amsterdam Institute for Molecules, Medicines and Systems, Vrije Universiteit Amsterdam, Contact e-mail: p.jennings@vu.nl
**SPATIAL-TEMPORAL MULTISCALE-MULTILEVEL MODELING OF APAP DAMAGE AND ITS CONSEQUENCE ON AMMONIA DETOXIFICATION: STEPS TOWARDS A VIRTUAL LIVER**

In vivo experiments are expensive, time consuming, and underlie strict ethic rules. Their results only partially apply to human. Modern experimental methods composed of imaging at high resolution, in 3D, or in living tissues provide information that increasingly permit development and parameterization of multi-level computational models, that allow for virtual experiments. In such models, hypotheses on molecular, cell-level or tissue-level mechanisms can be implemented and their consequence been tested in silico. The results can be used to guide experimental decisions and designs. Prospectively, this can permit feeding computational models with patient-specific information at each of the above levels and studying the prospective impact of therapeutic interventions.

As a step towards a virtual liver lobule, we in this presentation will report the stepwise development of a multilevel model of drug-induced damage, regeneration and the detoxification of ammonia during regeneration. Hyperammonemia (too high ammonia blood concentration) is a severe complication after drug induced liver damage, for example resulting from overdosing acetaminophen (paracetamol), and can lead to encephalopathy and dead of the patient. We will first present an integrated model, integrating a compartment model of ammonia detoxification and a spatial-temporal micro-architectural agent-based model of liver regeneration after drug induced liver damage, that was able to identify lack of a critical ammonia sink mechanism in the 40-years old consensus reaction scheme (Schliess et al., Hepatology, 2014). The finding has led to identification of a so far unrecognized ammonia sink mechanism that could be experimentally demonstrated to represent a potential therapy approach in hyperammonemia. In a further step we redo the analysis in a full spatial temporal micro-architecture model of the smallest virtual functional micro-anatomical unit (called lobule) obtained from image analysis, whereby the detoxification reactions are executed in each individual hepatocyte.

Unlike the integrative model, the spatial-temporal multiscale model is able to predict the consequences of architectural distortions as they occur in liver fibrosis on liver metabolism.

Finally, we extend the multiscale model by integrating a model of toxic damage by acetaminophen in each hepatocyte, and discuss the relation of in vivo to in vitro experiments in the light of the model, as well as HGF - induced cell progression during the regeneration of tissue damage caused by acetaminophen if times allows.

**Keywords**
Multilevel spatial-temporal modeling, acetaminophen, ammonia detoxification

**Thanks**
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**References**
The Organisation for Economic Co-operation and Development (OECD) has been making effort with member countries and other stakeholders to expand use of alternative methods in assessing hazard of chemicals. This activity has included development of guidance documents and tools such as (Q)SAR, chemical categories and Adverse Outcome Pathways (AOPs) as a part of Integrated Approaches to Testing and Assessment (IATA). OECD countries have established principles and criteria for validating QSARs for use in a regulatory context and have developed the QSAR Toolbox which allows users to draw upon collective information and knowledge in a variety of areas: grouping of chemicals, read-across, using structural or physical-chemical properties to profile chemicals, harnessing mechanistic information to inform the prediction of hazard. The Toolbox has evolved by continuous development and linking with other tools and approaches, such as AOPs, and will help the future of IATA-based approaches where hazard prediction is informed by integrating results from one or many methodological approaches.

In 2015, the IATA Case Studies Project was launched to increase experience with the use of IATA and novel hazard methodologies by developing case studies, which constitute examples of predictions that are fit-for-regulatory use. This activity has identified the importance area to develop future guidance documents. The lesson and learning from the case studies will help for harmonizing and improving chemical safety assessment methods.

Highlights of these initiatives will be presented.

**Keywords**
AOP; Chemical category; Data Gap filling; Environmental health; IATA; in silico; QSAR; read-across; Toxicity

**Thanks**
The Authors would like to thank OECD member countries for contribution to and support of the projects.

**References**
MERLIN-EXPO: A LIBRARY OF MULTIMEDIA CHEMICAL FATE MODELS AND PBPK MODELS FOR ASSESSING ENVIRONMENTAL AND HUMAN EXPOSURE TO CHEMICALS UNDER UNCERTAIN CONDITIONS

The objective of the presentation is to show the principles and capability of a new software called MERLIN-Expo dedicated to exposure assessment of chemicals. MERLIN-Expo incorporates advanced models simulating the fate of chemicals in the environment and in human body (PBPK models) into an easy-to-use tool. To avoid the ‘black box’ approach, models available in the MERLIN-Expo library are implemented on a common ‘easy-to-use’ and ‘difficult-to-abuse’ platform to facilitate integrated full-chain assessments for combined exposures. Complex scenarios can thus be built by combining independent modules that are available in the library. Internal exposures for different human populations (e.g. children) can thus be estimated for a wide range of scenarios. Considering the recently published WHO guideline related to uncertainty in exposure assessment, MERLIN-Expo also contains functionality for uncertainty/sensitivity analysis (from screening methods to variance-based approaches). Finally, MERLIN-Expo follows a Quality Assurance and Standardisation process for documentation in collaboration with CEN (European Committee for Standardisation).

These issues will be illustrated through specific case studies related to transfer of contaminants in water, air, soil and biota systems, as well as in human body.

Keywords
Exposure assessment - Fate of chemicals in the environment - PBPK - Uncertainty analysis - Sensitivity analysis

Thanks
This work was financially supported by the project ‘The FUture of FUllly integrated human exposure assessment of chemicals: Ensuring the long-term viability and technology transfer of the EU-FUNded 2-FUN tools as standardised solution’ (4FUN), Grant agreement n. 308440, funded by the European Commission under the 7th Framework Programme.

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P. Ciffroy
EDF R&D, Laboratoire National d’Hydraulique de Environnement
6, quai Watier - 78400 Chatou (F)
Contact e-mail : philippe.ciffroy@edf.fr
MECHOAS (MECHANISM OF ACTION) SAR MODEL AND SKIN SENSITIZATION SCREENING: 3-METHOXYPHENOL, 4-METHOXYPHENOL AND 1,4-DIMETHOXYBENZENE CASE STUDY

What is MechoA?
MechoAs use the molecular structure of a substance (SMILES code) to determine the toxicity mechanisms of the parent substance and its major metabolites. The model classifies the substances into 6 major classes (membrane destabilization, enzymatic hydrolysis, reactivity, pro-activity, indirect enzyme disruption, direct docking disruption) and a total of 23 subclasses. The model has been trained with fish, daphnids, algae and rodent in vivo toxicity data, therefore, the MechoA model can discern the differences found in toxicity among these species uniting toxicology and eco-toxicology under one and only classification method: MechoAs. Here-in, we have chosen to illustrate the application of MechoAs for skin sensitization (SS), a human health endpoint required for all chemical Regulations involving Human health assessment.

Where is MechoA located in the AOP? & How does it relate to skin sensitization?
MechoAs predict the Molecular Initiating Event (MIE) phase by scanning for electrophilic reactivity and the major metabolites of the parent substance. Therefore, identifying if these substances are capable of binding covalently to proteins, which is the MIE of the skin sensitization Adverse Outcome Pathway (AOP).

Case study methoxyphenol analogs: how do MechoAs help to predict skin sensitization (SS)?
4-methoxyphenol and 3-methoxyphenol are positional isomers, while 1,4-dimethoxybenzene is the methylated form of 4-methoxyphenol. Even though these substances are structurally very similar they show different sensitization profiles:

4-methoxyphenol and 3-methoxyphenol (SS): The MechoA model assists in predicting these substances as skin sensitizing, by showing that these compounds can form 2 major metabolites of the type: catechol and a o-quinone, which are capable of covalently binding to proteins, the MIE of the skin sensitization AOP. This prediction is backed up by an LLNA study for 4-methoxyphenol which classifies this substance as being sensitizing to skin. And, an in vitro study showing that 3-methoxyphenol can be metabolized to catechol by CYP2E1 and subsequently to o-quinone by superoxide dismutase (SOD).

1,4-dimethoxybenzene (non-SS): The MechoA model assists in predicting these substances as non-skin sensitizing, by showing that the substance in a non-reactive substance and not significantly. This prediction is backed up by an in vivo guinea pig study showing that this substance is not sensitizing to skin.

Why MechoA and not MOA (Mode of action)? & Comparison to other predictive tools for SS
These conclusions reached using the MechoA model were compared to other models, such as, the classical Verhaar MOA model, which classifies all 3 substances as class 1: non-polar narcotics; therefore, non-reactive. Toxtree’s skin sensitization reactivity domains: which un-correctly predicts 4-methoxyphenol, and Toxtree’s protein binding alerts module: which un-correctly predicts 1,4-dimethoxybenzene.

Keywords
skin sensitization, mechanism of action, MechoA, mode of action, MOA

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M. Delannoy (1), F. Bauer (1), C. Charmeau-Genevois (1,2), P. Thomas (1,2)
(1) KREATIS, 23 rue du Creuzat, 38080 L’Isle d’Abeau, France.
(2) CEHTRA, 23 rue du Creuzat, 38080 L’Isle d’Abeau, France
Contact e-mail : melanie.delannoy@kreatis.eu
Session 6
Telemetry & Imaging
THE FUTURE OF HIGH CONTENT SCREENING FOR ANIMAL-FREE CHEMICAL SAFETY TESTING

Adverse outcome pathway (AOP) has been proposed to support in the future chemical risk assessment based on mechanistic reasoning. However, for most adverse effects there exists limited information about the underlying molecular mechanisms. Therefore, one central element of the AOP concept is to understand toxicological activity on the molecular and cell biological level. Finally, a molecular initiating event (MIE) and essential, predictive biological key events (KEs) should be identified, who are responsible for an adverse outcome (AO). Generally speaking, high-content screening (HCS) is a technology which supports biological research to understand the workings of normal and diseased cells. Thus, on the one side HCS is able to support identification of MIEs and single KEs and KE relationships to fill data gaps in toxicity knowledge generation. On the other side, toxicological chemical risk assessment demands more and more the development of HCS-compatible animal-free tests. The benefit especially of image-based HCS toxicity tests is that (1) a higher amount of biological data can be extracted from each experiment, and (2) testing occurs in the living cellular environment (3) a high number of experiments can be performed in a short period of time (4) automatisation of data acquisition and analysis limits bias. However, there are still some challenges to overcome before an image-based HCS toxicity test can be approved for toxicity assessment, e.g. existing difficulties in standardization, as well as limited reproducibility, and transferability.

Keywords
high content screening, toxicology, adverse outcome pathway, animal testing alternatives

References

FRANCE BIOIMAGING, A COORDINATED INFRASTRUCTURE FOR QUANTITATIVE BIOLOGICAL IMAGING AT MULTIPLE SCALES

France-BioImaging (FBI) is a National Infrastructure in Biology and Health (INBS) in the field of biological imaging. It represents the Unique French Node of the European Landmark ESFRI EuroBioImaging. FBI is at the crossroads between molecular and cell biology, biophysics and engineering, mathematics and informatics. This unique coordinated infrastructure gathers several large biological imaging facilities and laboratories specializing in R&D for imaging in 5 local and one transversal Node (Image Processing and Data Management; IPDM). France-BioImaging aims at creating the most efficient adoption of the latest advances in all technologies and methods related to microscopy, by the users of the imaging facilities. These technologies and methods, reinforced by a strong support in computational analysis, provide quantitative measures and integrative understanding of a wide range of cell and tissue activities in biological models, from the simplest, to small animals in normal and pathological situations.

- As a multi-disciplinary task force to investigate new avenues and encourage their application in Biological Sciences, we open a large scale research tool for many areas, from research on stem cells to cancer studies.
- As a coordinated Infrastructure speeding up the technological transfer of bio-imaging innovations on its Core Facilities, we provide fast access to advanced techniques and methods.
- As a portal for collaborative projects between public and private sectors in the field of advanced microscopy, we participate in socio-economic development through industrial partnerships and innovations.

Beyond the overall presentation of our Research Infrastructure, case studies will be presented that covers the most recent technological approaches and their applications on a broad range of biological topics.

Keywords
Microscopy, Live cell and animal Imaging, Bioimage Informatics, CLEM, Super-resolution and Light Sheet Microscopy, High Content Screening

Thanks
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https://france-bioimaging.org/fr/
NOVEL APPROACH FOR LABEL-FREE LIVE CELL IMAGING BY RAMAN MICROSCOPY AND APPLICATION TO NANOTOXICOLOGY

Nanotoxicology investigates the effect of nanomaterials of various size, shape, chemical composition and functionalization on cells and organisms. Nanomaterials can therefore have very different physical chemical properties and biological activities, depending on their nature, the organism and the route of exposure. In vitro models can be used to investigate their toxicity, in particular on human cells, and the development of 3D cell culture models is particularly promising for assessing their effect on tissues as well as their long-term toxicity.

However, localizing nanomaterials within the cells and following their translocation through biological barriers remain challenging and it has so far been limited to fluorescent or labelled nanoparticles. Fluorescence microscopy is a very powerful tool to determine the subcellular localization of fluorescent nanoparticles, but it excludes a large set of environmental, industrial or clinical non-fluorescent materials. Radioactive labelling can prevent labelling artifacts but requires specific facilities.

Raman microscopy is a label-free, non-destructive vibrational spectroscopy technique which can be used for imaging biological samples. This technique combines Raman scattering effect with confocal microscopy. Using this setup, the Raman spectrum of the sample can be acquired at a cellular or subcellular level, within a few minutes, without any labelling and with limited sample preparation. This analysis unveils the “molecular fingerprint” of the sample and gives access to a broad information content on its chemical and biochemical composition. Raman microscopy has become an easy-to-use, high-speed imaging technique with numerous applications in the medical field. I will present here this novel technique for label-free live cell imaging and its applications to in vitro studies in nanotoxicology.

Keywords
Raman microscopy; nanotoxicology; live-cell imaging

Thanks
Dr. Grégory Lefèvre (RM2D, Chimie Paris Tech).

References
A. LE PAPE (CNRS – CIPA)
Conclusion

Innovation for new tools and new tools for advances in life sciences
THE NEED TO IMPROVE HUMAN RELEVANCE IN BIOMEDICAL RESEARCH

Non-communicable chronic diseases are becoming increasingly prevalent in Western countries, accounting for more than 86% of total premature deaths. Such diseases are generally the result of a combination of genetic, physiological, and environmental factors (e.g., diet, exercise and smoking). Animal models have been extensively utilized to elucidate the pathophysiological mechanisms of human diseases and for drug development. However, basic/fundamental and pre-clinical research successes have not, in most cases, translated into effective therapeutic treatments for humans. This failure has been particularly prominent in the field of Alzheimer’s disease research, for which no human effective drugs have been developed so far. On the other hand, the use and the implementation of human-based investigational methods, both in vitro and in silico, together with non-invasive imaging technologies, and large-scale epidemiological data set repositories, may contribute to the development of new preventive and treatment strategies. Here we present the challenges and opportunities in biomedical research, presenting Alzheimer’s disease research as a possible case study, and propose how we can mitigate this translational barrier by employing human-based methods to elucidate disease processes occurring at multiple levels of biological complexity. A paradigm shift towards human relevant research, accounting for a multi-dimensional and multi-disciplinary approach, is highly needed to tackle the ever-increasing prevalence of non-communicable diseases.

Keywords
Alternative methods, human relevance, biomedical research, chronic diseases, Alzheimer’s disease

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F. Pistollato, PhD
Joint Research Centre, European Commission, Ispra, Italy
Contact e-mail: francesca.pistollato@ec.europa.eu
POSTERS
THE EUROPEAN CONSENSUS PLATFORM FOR 3R ALTERNATIVES TO ANIMAL EXPERIMENTATION (ECOPA)

The ecopa is a non-profit organization which explores all possible means to improve the exchange of information in the area of alternative methods. The primary mission of ecopa is to raise public, governmental and scientific awareness for a better use of alternative methods for scientific purposes with the final goal of fully replacing all in vivo tests while improving the quality of risk assessment in humans. Presently, ecopa is composed by 7 National Consensus Platforms (French, Finland, Norway, Spain, Italy, Germany and Switzerland) and has other members as individuals to include academic institutions, professional associations, companies, and any other organisation (or one of its divisions) which support ecopa’s aims. National Consensus Platforms are usually structured with representatives from ecopa’s four stakeholders: Government and regulatory authorities, academia, industry, and animal protection and welfare organisations.

The aims of ecopa are to facilitate the exchange of scientific information, expertise and experience between national consensus platforms to enhance implementation of refinement, reduction and replacement in the EU and worldwide. ecopa recommends the use of alternative strategies that may elucidate the real mechanism of actions of the different substances in humans, in particular when the animal models fail in predicting an effect. This is particularly true in the area of drug development where so many differences exist between humans and animals.

Adhering to the 3Rs, ecopa strives to raise public, governmental and scientific awareness for a better acceptance of alternatives both at member state and European levels. Members of ecopa are part of ESAC at EURL-ECVAM and it is constantly in contact with DG Environment and DG Health & Consumers. Additionally, many members are participating in European Integrated Projects. The ecopa is also an accredited stakeholder at ECHA. Some national consensus platforms also have strong interest in the food and feed sectors even though the main focus remains in the methodology for risk assessment.

New Approach Methodologies (NAMs) integrating advanced in vitro, in silico and in chemico tools should be developed and included in testing strategies to increase our understanding of toxicity mechanisms in humans and the risks to the environment. The same goal applies to biomedical research. ecopa’s strategic goals apply to all areas where animals are used for scientific or regulatory purposes.

Keywords
3Rs, Consensus Platforms
FRANCOPA: THE FRENCH PLATFORM FOR THE DEVELOPMENT OF ALTERNATIVE METHODS IN ANIMAL TESTING

FRANCOPA is the French platform dedicated to development, validation, and dissemination of alternative methods in animal testing and is a member of the European platform Ecopa (European Consensus-Platform for Alternatives). Like the other european platform, Francopa is structured with representatives from government and regulatory authorities, academia, industry and animal protection and welfare organizations. The platform gathers 15 French institutional partners to promote the development of alternative methods for the reducing or the replacement of animal testing, extending to the other fields the experience gained in the fields in the development, the evaluation, and control of health products and chemicals. Francopa is a consensus platform where solutions are developed together, rather than a place to fuel controversies about use of animals.

Expert groups are developed within the platform and are in charge to elaborate proposals and reflection to promote 3R methods in specific domain area (education and trainings, limits of methods, basic and applied research...). The exchanges in these different expert groups result in a report, a symposium or a workshop of Francopa.

To promote the 3R methods and in order to facilitate the exchange of information and tools, the platform and its partners organise workshops and seminars (waiving animal testing for regulatory purposes, read-across, use of organoids and ex vivo approaches in basic and applied research).

Francopa published a state of the art on 3R methods and practices in France, together with recommendations to the French government in 2010, last updates in 2016.

An important diffusion tool is the info-center. Francopa Info-center aims at facilitating the sharing and methods in animal testing. It is an interactive website with different sections:
- Database with a searching engine (data from FRANCOPA and associated websites).
- Discussion forum.
- Newsletter, four per year, related to methodological advances and FRANCOPA actuality.
- Frequently Asked Questions.
- Links to related websites.

Keywords
3R, national platform, regulatory authorities, academia, industry, NGO

Francopa members

Thanks
The French Ministry of the Ecological and Solidary Transition.
USE OF LUCS (LIGHT-UP CELL SYSTEM) AS AN ALTERNATIVE LIVE CELL METHOD FOR ACUTE ORAL TOXICITY TESTING

LUCS (Light-Up Cell System) is a new live cell assay based on the light-induced property of a cyanine dye leading to oxidative stress production\(^1\). The assay allows to assessing cell homeostasis and its alteration by toxic agents. The VALITOX research program (coordinated by PROANIMA foundation, France) aims at demonstrating the use of LUCS as an alternative test for regulatory applications including acute oral toxicity. In this study, we collected the LUCS EC\(_{50}\)s (50% Efficacy Concentrations) obtained in human HepG2 cells pre-treated for 24 hours with 53 chemicals selected from the ACuteTox EU database. These in vitro LUCS EC\(_{50}\) were compared with available in vivo data, i.e. human blood LC\(_{50}\) (50% Lethal Concentration) values derived from human acute poisoning cases\(^2\) and rodent oral acute toxicity LD\(_{50}\)s (50% Lethal Dose)\(^3\)\(^,\)\(^4\). Linear regression analyses between LUCS EC\(_{50}\) vs human LC\(_{50}\) or rodent LD\(_{50}\) showed a higher correlation with human (r\(^2\) = 0.69, n= 53) than with rodents (r\(^2\) = 0.34, n=35 and r\(^2\) = 0.43, n=36 for two sets of rat data and r\(^2\) = 0.45, n=23 for mouse data). As LUCS assay original protocol necessitates LED flashes with a dedicated device, a chemical-based protocol was developed to avoid the illumination step, leading to similar results (r\(^2\) = 0.96, n = 20 chemicals). Together with the previously published demonstration (1) of LUCS’ robustness and high throughput applications, the data presented here clearly position LUCS procedure as a Weight of Evidence (WoE) approach for testing acute systemic toxicity for the EU REACH and other regulations where pertinent alternative methods are still lacking.

References
DEVELOPMENT OF EXPOSURE STRATEGIES TO PERFORM REPEATED TREATMENTS OF BRONCHIAL EPITHELIAL CELLS IN AIR-LIQUID INTERFACE CULTURES TO STUDY THE LONG-TERM EFFECTS OF PARTICLES

Culturing normal human bronchial epithelial (NHBE) primary cells at the Air-Liquid Interface (ALI) allows their differentiation to establish a mucociliary epithelium. These cultures could be maintained for several weeks allowing the study of long-term effects after repeated exposures. We studied the long-term effects of atmospheric particulate matter (PM), diesel exhaust particles (DEP) or CeO$_2$ nanoparticles (NP) after repeated treatments.

Firstly, we studied the effect of particles on the differentiation of NHBE cells. Undifferentiated NHBE cultures were treated four times (every 48 hours) with 100 µL/cm$^2$ of DEP or PM suspensions for 4 hours allowing particle sedimentation. Then the culture media were removed and cells cultured at ALI for up to one month. This exposure of undifferentiated cells to DEP or PM (coarse, fine or ultrafine fractions) induced a sustained pro-inflammatory response, induction of airway remodeling markers, and a shift towards a mucosal phenotype. Secondly, we studied the effects of NPs on differentiated NHBE cultures. After 3 weeks of culture at ALI to obtain mucociliary differentiation, we performed repeated treatments (every 48 or 72 hours) with 30 µL/cm$^2$ of CeO$_2$ NP suspensions for up to one month. We evaluated the effects of CeO$_2$ NPs on cytokine secretion and mucus production. For both exposure scenarios the control cultures did not show any signs of toxicity.

In conclusion, both treatment strategies allowed performing repeated treatments without altering epithelial integrity, even after long-term exposures. Treatments with particles promoted the differentiation into a hyper-secretory epithelium and induced a sustained pro-inflammatory response.

Keywords
Nanoparticles, atmospheric particulate matter, Diesel exhaust particles, long-term exposure, bronchial epithelial cells

Thanks
IMPROVING LIMITATIONS OF *IN VITRO* MODELS IN DRUG TOXICITY TESTING

The regulatory environment for non-clinical drug development promotes ethical considerations to reduce the number of tests on animals, such as the development of *in vitro* models. Many drugs are currently being withdrawn from the market due to hepatic toxicity. The aim of this study is to present an original approach improving the limitations of *in vitro* hepatic model for drug toxicity testing.

HepaRG is a human hepatoma cell line able to differentiate *in vitro* into mature hepatocyte cells. HepaRG is cultured in a William’s E medium supplemented with 10% of fetal calf serum (FCS) that provides factors of attachment and stimulator of hepatoma cell proliferation. According to the manufacturer’s protocol, the addition of 2% dimethyl sulfoxide (DMSO) to the culture medium induces differentiation of these cells into hepatocyte-like phenotypes.

In this study, we used a statistical method called the design of experiments in order to minimize the number of experiments necessary to optimize phase I metabolism enzyme (CYP1A2, 2B6, 2D6, 2E1, 3A4) and phase II enzyme (sulfotransferase, SULT and UDP-glucose-glycoprotein glucosyltransferase, UGT) expressions, and the specific marker of adult hepatocytes, albumin production. Fetal markers such as alpha-fetoprotein (AFP) and CYP3A7 were tested. Continuous variables were examined at low, medium, and high levels, between 0% and 2% for DMSO and between 2% and 18% for FCS and with a centrally repeated condition. Using this approach, we observed that culture conditions can impact the relevance of a study model in toxicology. AFP expression was not observed in this study. Moreover, we observed that a decrease in concentrations of FCS (2%) compared to the manufacturer’s protocol (10%) could allow optimal expression of genes involved in the metabolism of drugs (CYP 2D6 and CYP 3A4), and in the production of albumin. For phase II enzymes, SULT and UGT, maximal expression was observed with concentrations of 18% FCS and 2% DMSO. High concentrations of DMSO (2%) corresponding to the upper limit of the field of this study were associated with maximum expression of the genes involved in the metabolism and in albumin production. In contrast, 2% of DMSO increase CYP3A7 expression too. Manufacturer’s recommendations do not seem to be optimal and can raise questions on the results obtained with this model.

This study focuses attention on the special needs of new approaches to develop translational research.

**Keywords**

HepaRG, toxicity testing, translational research, metabolism

**Thanks**

CHABANOL Estelle, BACQUET Solenn, BRUYER Anne-Sophie.

**References**


USE OF IN SILICO METHODS FOR SKIN SENSITISATION IN THE CONTEXT OF REACH REGULATION

REACH regulation requirements for all substance to be registered include assessment of skin sensitisation potential. According to the European Chemical Agency, around 30000 registration dossiers have been submitted for REACH registration deadline 2018 (substances in the 1-100 tons per year band). Previously based only on in vivo animal testing methods, the annex VII has been revised to introduce non-animal testing. The local lymph node assay (LLNA) was considered as the preferred option as it gives information on potency and dose-responses relationship.

The main challenge for the replacement of animal model under REACH, such as LLNA, is thus to keep the same level of protection of human health for workers and consumers. Therefore, the new methods shall be able to identify skin sensitisation hazard but also to determine potency (e.g. strong or extreme sensitisers), and be suitable for risk assessment. In vitro approaches have been developed for the identification of skin sensitisation and some tests have been validated at OECD level. However, they are not yet validated for determining skin sensitisation potential. No single non-animal test method is thus able to provide data that would fully substitute the in vivo approach currently use. Combination of test methods used with integrated approach on testing and assessment (IATA) is thus necessary. Integrated approach and define approaches are currently being developed by OECD.

The main limitations and remaining uncertainties of in vitro test methods developed to identify skin sensitisation hazard are on the ability of these methods to identify strong sensitisers but also to identify sensitisers that need metabolisation or autoxidation to express their skin sensitisation potential. Moreover, some challenges are also to well-defined the application domain of each method prior to their uses.

The poster will present the current challenges for replacing in vivo test methods and how in silico tools could be used in a weight-of-evidence approach in combination of in vitro test method to predict skin sensitisation and overcome the above issues.

Keywords
REACH, skin sensitisation, in silico tools
PBPK MODELING OF ZEBRAFISH EMBRYO FOR REPROTOXICITY ASSESSMENT OF VALPROIC ACID AND SOME OF ITS ANALOGS

Introduction: Understanding and predicting chemical effects on development and reproduction is a complex challenge. The zebrafish embryo is an in vitro test system increasingly used for its transparency, short development time, easy husbandry and gene homologies with humans.

Objectives: To better explain and predict developmental toxic effects observed in zebrafish embryo, we are developing a generic physiologically-based pharmacokinetic (PBPK) model to predict target organ concentrations of neutral or ionizable chemicals. We present an application of the model in the assessment of the teratogenicity of valproic acid (VPA) and nine analogs.

Material and Methods: Quasi steady-state distribution of chemicals is modeled in ten embryo organs, and in two sub-cellular organelles: lysosomes and mitochondria. The partition coefficients between the organs or organelles and the culture medium depend on physico-chemical properties of the substances. Organ volumes grow over the first 5 days of life. Liver metabolism can be linear or saturable. For VPA and analogs, metabolic parameters were estimated by Bayesian fitting of the model to data on culture medium and embryo concentrations during repeated dosing.

Results: The kinetic data were reasonably well fitted by the model (Figure 1), even though residual uncertainty was substantial. Linear clearance estimates for VPA and analogs were around 10-10 to 10-11 L/min, which for an embryo volume of 3x10^-7 L, correspond to half-lives of about 11.5h. Embryo organ concentrations were used to calculate dose-response for general lethality and heart defect caused deaths. Naive dose-responses were also calculated using nominal dose. Using target organ concentrations shifts substantially the magnitude of dose-response parameters and the relative toxicity ranking of the VPA analogs studied (Figure 2).

Conclusion: The model can be used to relate zebrafish effects observed in vitro to cellular exposures. It should improve the translation of in vitro zebrafish data to humans for safety assessment.

“This project has received funding from the European Union’s Horizon 2020 research and innovation program under grant agreement No 681002”.

Figure 1:
Predicted (line) and observed (points) embryo concentrations as a function of time for VPA.

Figure 2:
Illustration of the differences between concentration inducing 10% of effects from nominal dose or corrected by the pharmacokinetic factor, for VPA and its nine analogs.
SIMULTANEOUS MEASUREMENT OF CONTRACTILITY, ELECTROPHYSIOLOGY AND BIOMARKER SECRETION TO DETERMINE CARDIOTOXICITY IN HIPSC DERIVED CARDIOMYOCYTES

Many novel oncology therapeutics may induce cardiotoxicity by inhibiting survival pathways which are shared by both tumors and cardiac cells. Traditional methods to assess cardiotoxicity have relied upon in vitro overexpressing human cell lines or use in vivo animal models. These models often lack the complexity of human cardiomyocytes; whereas animal models may lack predictivity due to inherent species differences. Therefore, there is a need for the development of more predictive and specific assays that allow for multiparametric assessment of potential cardiotoxic side effects of new drugs in humans.

Using proprietary hiPSC-derived ventricular cardiomyocytes (Pluricyte® Cardiomyocytes) that recapitulate a human myocyte’s contractile and electrophysiological profile, as well as mature sarcomere organization, we developed a multiparametric assay to measure potential cardiotoxic effects in vitro. Here, both acute and long-term drug effects on contraction, electrophysiology and cardiac Troponin I release are determined simultaneously from each well of a 48 well plate. To assess the effects of anticancer drugs on the physiology of Pluricyte® Cardiomyocytes, the cells were incubated with concentration ranges of Nilotinib, Lapatinib, Doxorubicin and Ponatinib for up to 64 hours, and analyzed simultaneously using microelectrode array (MEA), impedance and cardiac Troponin I (cTnI) release assays. This resulted in defined cardiotoxicity profiles for each compound. Whereas treatment with Nilotinib and Lapatinib caused a transient, short term (functional) contractile/electrophysiological deficit, Doxorubicin exhibited a continuous/long-term toxic effect in both MEA and impedance measurements. While Lapatinib and Nilotinib did not cause structural toxicity as measured by cTnI release; however, Ponatinib and Doxorubicin induced a dose-dependent increase in cTnI release. The dose-dependent increases in cTnI release correlated with reduced cell index values obtained in the impedance assay.

These data suggest that a multiplexed analysis is crucial to investigate short and long-term cardiac liabilities as it provides a more comprehensive readout that generates mechanism-specific cardiotoxicity profiles, leading to better prediction of drug-induced cardiotoxicity.


Ncardia, Galileiweg 8, 2333 BD Leiden, The Netherlands
Contact e-mail: alexandre.fouassier@ncardia.com
IN-SILICO MODELIZATION OF COMPOUNDS INTERACTION WITH BILE SALT EXPORT PUMP (BSEP): AN ALTERNATIVE APPROACH TO PREDICT HEPATOTOXICITY

Background:
BSEP is an efflux transporter protein present in the hepatocytes membrane that plays important role in flow of bile acid from hepatocyte cell into the bile canaliculi\(^1,2\). Impaired BSEP activity due to drug interaction leads to accumulation of bile acid within the hepatocyte cells and results in cholestasis liver injury (DILI)\(^3\). However, due to the lack of a X-ray structure of BSEP, there is no detail information about interaction of compounds with BSEP.

Objective:
To develop 3D model for BSEP transporter protein and analyzing its affinity with compounds using docking-based prediction in order to classify the compounds as inhibitor or substrate and understand predictive toxicity.

Methods:
The 3D model of BSEP (Uniprot code: Q9QY30) was determined by homology modeling, and further validated by ramachandran method. FDA approved compounds and cosmetic ingredients from European Commission COSING database were selected and docked against the BSEP structure. The post docking complex was analyzed in order to evaluate the behavior of compounds with BSEP and to determine their role in hepatotoxicity.

Results:
Homology modeling produced 3D model of BSEP using mouse p-glycoprotein as template with more than 70 % homology. Ramachandran plot confirms that 3D model has more than 90% of amino acid residues in the most favored regions and that all torsion angles Φ and Ψ are in good order. Molecular docking between 3D model of BSEP and FDA approved compounds shows that bosentan (positive control) produced higher affinity for BSEP as compare to the caffeine with docking energy of - 15.21 kcal/mole (IC\(_{50}\) = 38.1 μM for bosentan) than - 4.60 kcal/mole (IC\(_{50}\) > 135 μM for caffeine). Similarly, most of the cosmetic ingredients studied are found to have less affinity for BSEP than bosentan.

Conclusion:
Our in silico approach confirmed that bosentan has very high affinity than caffeine and act as inhibitor of BSEP which plays major role in formation of cholestasis. Comparison of cosmetics ingredients affinities with bosentan predicted that most of ingredients are non-inhibitor of BSEP and play no role in hepatotoxicity. Therefore, our in silico approach may be helpful to design in-vitro experiments using BSEP in order to predict precisely the role of compounds in hepatotoxicity.

Reference:
List of authors

AUDEBERT Marc – INRA - marc.audebert@inra.fr
BAROUKI Robert – INSERM - robert.barouki@univ-paris5.fr
CIFFROY Philippe – EDF - philippe.ciffroy@edf.fr
DELANNOY Stéphanie – KREATIS - melanie.delannoy@kreatis.eu
DEVINEAU Stéphanie – Paris Diderot University - stephanie.devineau@univ-paris-diderot.fr
DIANAT Noushin – CYPRIOS - noushin.dianat@cyprios.fr
DRASDO Dirk – INRIA - dirk.drasdo@inria.fr
FOUASSIER Alexandre – Ncardia - alexandre.fouassier@ncardia.com
FRITSCH Ellen – IU, Leibniz Research Institute for Environmental Medicine - Ellen.Fritsche@uni-duesseldorf.de
GIDROL Xavier – CEA - Xavier.Gidrol@cea.fr
GRALL Romain – CEA - romain.grall@cea.fr
GROUX Hervé – IMMUNOSEARCH - hgroux@immunosearch.fr
HORIE Masashi – OECD - Masashi.HORIE@oecd.org
IMRAN Muhammad – INSERM - muhammad.imran@univ-rennes1.fr
JENNINGS Paul – Vrije University of Amsterdam - p.jennings@vu.nl
KOLF-CLAUW Martine – Veterinary School of Toulouse - m.kolf-clauw@envt.fr
KOUL Meriem – INSERM – meriemkoul@hotmail.com
KUCZAJ Arkadiusz – Philip Morris International, University of Twente - Arkadiusz.Kuczaj@pmi.com
LANGOUET-PRIGENT Sophie – INSERM - sophie.langouet-prigent@univ-rennes1.fr
LE PAPE Alain – CNRS, CIPA - lepape@cnrs-orleans.fr
MARX Uwe – TissUse, GmbH - uwe.marx@tissuse.com
MAXIMILIEN Rémi – CEA - remi.maximilien@cea.fr
MOUDJOU Mohammed – INRA - mohammed.moudjou@inra.fr
MOYSAN Frederic – SANOFI Pasteur - Frederic.Moysan@sanofi.com
PISTOLLATO Francesca – ECVAM - Francesca.PISTOLLATO@ec.europa.eu
RABILLOUD Thierry – CEA - thierry.rabilloud@cea.fr
ROVIDA Costanza – CAAT - costanza.rovida@chimici.it
SALAMERO Jean – INBS France Biolmaging - jean.salamero@curie.fr
SCHOENFELDER Gilbert – BFR - Gilbert.Schoenfelder@bfr.bund.de
SHARMA Ashwani – EUROSATH - ashwani.sharma@eurosafe.fr
STORCK Fanny – Veterinary School of Alfort - fstorck@vet-alfort.fr
TESNIERE Antoine – ILUMENS - Antoine.tesniere@ilumens.org
TRICOIRE Hervé – Paris Diderot University - herve.tricoire@univ-paris-diderot.fr