SYMPOSIUM

Alternatives To Animal Experimentation

June 6-7, 2023 • Biocitech Paris-Romainville

Poster #1 proposé par : **CHERRY BIOTECH**

A New alternative method for skin sensitization and safety assessment based on an in vitro 3D fully differentiated skin model

OBJECTIVE: Before a new cosmetic ingredient is placed on the European market, the assessment of skin sensitization hazards and potency is mandatory. Currently, OECD 442 C, D and E provide guidelines for a tiered testing strategy to achieve a final classification based on the readout from several New Approach Methods (NAMs). To our knowledge none of them recapitulate and consider the interplay between epidermis/dermis cells and the resident immune cells. Therefore, the aim of our study was to create a new human full vascularized and immune-competent skin equivalent able to recapitulate the skin sensitization cascade.

METHODS: The model integrated human epidermal keratinocytes, normal human dermal fibroblasts (NHDF), Human Umbilical Vein Endothelial cells (HUVECS), and THP-1 (monocyte).

RESULTS: We obtained a multilayered, differentiated epidermis on top of the dermis. Presence of specific markers of human epidermis (Involucrin), dermis (CD26), endothelial (CD54) cells and resident macrophages (M1: TNF-alpha; M2: Dectin-1) were evaluated by immunofluorescence.

CONCLUSION: We developed an immunocompetent, differentiated, and vascularized full-skin in vitro model envisaging safety/sensitization assessments of cosmetic ingredients. Finally, our 3D skin model should allow in future investigations on strategies and drugs to prevent skin inflammation

Contact du poster : **Pierre GAUDRIAULT**

Poster #2 proposé par : **ALTERTOX**

Fun with NAMs

Education brings neither glory to the scientists nor a better h-index. Nevertheless, it is useful for multiple reasons such as knowledge sharing, capacity building and creation of an adequate ecosystem. Overall, one can admit that the education and training about 3Rs at university level has the merit to exist even it it could be possibly better advertised and communicated. he JRC launched a mapping exercise on this matter in 2018 but as far as the authors are concerned the results of the study were not published. A category of individuals that is rarely targeted properly is the general public as well as teaching at primary and secondary school. JRC took care of the latter by providing learning scenarios to empower the teachers. Moreover, organising open das as well as participating in science festivals are great venues for reaching out the general public. Still, there is space for creativity by providing other formats. At Altertox three new concepts and formats are expected to complement the current "arsenal" of tools available.

Contact du poster : **François BUSQUET**

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Poster #4 proposé par : **UNIVERSITÉ DE BORDEAUX / PRACTEEX**

**Development of a simulation platform using virtual animal testing
for pharmacy students**

During Pharmacy studies, practical lessons related to drug preclinical evaluation should include animal testing, but this raises questions regarding the 3R rules. In this context, we propose to develop a simulation platform dedicated to pharmacy students and using virtual animal testing. Thanks to virtual reality, students will be able to develop and test their own experimental protocols to assess therapeutic and/or adverse effects of various drugs affecting the central nervous system. In addition, this platform will help students to get familiar with ethical rules of animal testing and with the management and use of an animal facility, thus helping them to develop their professional skills. The use of virtual reality, avoiding the use of real animals, follows European directives on animal ethics and respects the recommendations of the 3R rules. Finally, since digital training of health professionals is currently a major challenge in France, the development of such a simulation platform will help reinforce Pharmacy students' digital culture.

Contact du poster : **Véronique MICHEL**

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Poster #5 proposé par : **FC3R**

French Center for 3Rs : reaching scientists

In 1959, the ethical 3Rs principle "Replace, Reduce, Refine" was developed by two scientists for scientists. However, 64 years later, it is still a challenge for 3R centers to make a direct impact on researchers with their daily work. The French Ministry of Higher Education and Research and the leading operators of public research created the French Center for 3R (FC3R), which priority is to encourage and implement the 3Rs in France through the promotion of responsible and innovative research, education, and transparent communication by targeting current and future generations of scientists specifically. To do so, the FC3R developed proactive strategies, engaging in concrete actions to federate a synergistic community around the researchers' needs and goals. The FC3R is working to: 1) disseminate and develop training offers adapted to the specific needs of students and researchers; 2) accompany researchers with their experimental design and the sharing of their unpublished/negative results through the creation of a dedicated platform; 3) communicate in regard to innovative non-animal methods, grants, the evolution of practices and regulations, but also recognize through interviews, conferences and awards stakeholders whose accomplishments lead forward promotion and implementation of the 3Rs in France; 4) fund research projects: two calls were completed in 2022/23 rewarding collaborative initiatives federating the French scientific community around the 3Rs principle and projects promoting Replacement of the use of animals or animal-derived products in science. FC3R will also facilitate scientific collaborations between the academic and industrial research communities, in biology and chemistry.

Contact du poster : **Susana GOMEZ**

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Poster #6 proposé par : **SABEU**

MEMBRANE TECHNOLOGY FOR TISSUE ENGINEERING

The incorporation of membranes into tissue engineering products has grown significantly. One example is the use of microporous membranes in systems like organ-on-a-chip and cell culture inserts. Tissue-culture treated membranes are excellent supports for cell growth [1]. To select the best membrane for tissue engineering it is important to consider the fabrication method, material, porosity and surface modifications [2]. Microporous membranes can be produced from ultra-thin PET films, that are bombarded with accelerated noble gas ions. The goal is to break the molecular chains of the polymer to create ion tracks that are clearly defined by their density and angles. The desired pore density is accurately determined by the ion beam intensity and the film velocity. The precise control of these factors allows the production of superior optical clear membranes. Afterwards, the ion tracks are chemically etched into pore channels. The diameter of these pores can be determined with sub-micrometer accuracy. Finally, membranes are treated with air plasma to promote optimal cell attachment. Microporous membranes are integrated in cell culture vessels [3] and in recent innovative organ-on-a-chip platforms supporting the 3D growth of human mature adipose tissue [4] and the creation of an oviduct-on-a-chip [5]. Cells grown on microporous membranes, have access to nutrients from the apical and basolateral side. This allows the separation into two compartments, simulating the physiological condition where the cell layer acts as a diffusion barrier. Due to the physiological relevance of this model, it is possible to mimic the complexity of human relevant systems, co-cultures and tissue barriers [6,7], such as the epithelium or endothelium [8].

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Contact du poster : **Karina CUANALO-CONTRERAS**

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Poster #7 proposé par : **HCS PHARMA**

Bridging the gap between in vitro & in vivo:
3D cell culture considering the extracellular matrix

The extracellular matrix (ECM) is present in all tissues and is a master regulator of cellular behaviour and phenotype. In each tissue/organ, it is characterized by a specific composition, and by biochemical and biophysical properties. Importantly, ECM features are modified in different types of disease, like cancers and fibrotic conditions. We have developed 3D cellular models using BIOMIMESYS®, a patented hydroscaffold™ for 3D cell culture with unique dual properties: hydrogel and solid scaffold features in a single matrix. Suitable for long-term 3D cell culture, BIOMIMESYS® is based on Hyaluronic Acid (HA), a major component of the ECM, biofunctionalized with other ECM components depending on the organ/tissue of interest. Moreover, the stiffness is also modulated to fit with the healthy or pathological ECM to reproduce, in an organ-specific manner. We will show how we can model the tumoral matrix with a representative range of stiffnesses (1, 8 and 16 kPa) in vitro, and how this can better mimic pathological tissues and their responses. Our 3D in vitro models, by taking into account the ECM, aim at bridging the gap between 2D in vitro models and in vivo situation for a better prediction of human outcomes. As such, human 3D in vitro models based on BIOMIMESYS® would provide an interesting alternative to animal testing in New Approach Methods (NAM) batteries.

Contact du poster : **Elodie VANDENHAUTE**

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Poster #8 proposé par : **ANIMAL FREE RESEARCH UK**

Fostering Awareness, Accessibility, and Acceptance of Animal-Free Antibodies

Most antibodies used in research are derived from animals, with about 1 million animals used in their production each year in Europe [1]. The emerging animal-free production techniques, like phage display, can allow improved reproducibility for research, diagnostic, and therapeutic purposes, as well as having potential for greater functionality and upscale [2]. These techniques are gaining worldwide recognition as the way forward in biomedical research.

However, there is a general lack of awareness around this topic. As animal-free antibodies are relatively 'new', it raises questions around their validity, affordability, and quality, even when researchers are aware of them. Many suppliers and researchers who are aware are hesitant to change materials or techniques that are well established without good cause.

The European Centre for the Validation of Alternative Methods (ECVAM) published recommendations that " animals should no longer be used for the development and production of antibodies for research, regulatory, diagnostic and therapeutic application" [1]. As part of this initiative, Animal Free Research UK will launch an online animal-free antibody database in April 2023. This platform aims to encourage researchers to prioritize the use of animal-free antibodies while facilitating their access. We hope this will be a strong basis for creating more impetus towards acceptance of animal-free biomaterials, and for building more informative and collaborative resources as a support for scientists embracing human-relevant research.

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Contact du poster : **Lilas COURTOT**

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Poster #10 proposé par : **ICARE LABORATOIRE**

Innovative in vitro 3D skin model for safety assessment: a case report for the skin sensitization

Prevalence of allergic contact dermatitis is increasing in worldwide [1], justifying the need to assess skin sensitization potential for any ingredient intended for topical applications. Three technical test guidelines (OECD 442 C, D and E) should be considered, including a battery of in vitro tests. Despite the significant advances of skin model equivalents (SME) validated by the governmental organizations, none of them consider the interplay between epidermis/dermis cells and the resident immune cells. The aim of our study was to create a new SME that includes an endothelial barrier and some resident immune cells involved in the skin sensitization cascade.

METHODS: The model integrated human epidermal keratinocytes, normal human dermal fibroblasts (NHDF), Human Umbilical Vein Endothelial cells (HUVECS), and THP-1 (a human leukemia monocytic cell line). Structure and cell-specific markers were thoroughly characterized. To compare the developed model with the IL-8 Luc assay, assay which has been validated as an in vitro alternative providing information on key event 3 (KE3) in the adverse outcome pathway for skin sensitization, the model was exposed to molecules with known in vivo and in vitro properties (sensitizing substances and negative controls, cf OECD 442) such as ethylene glycol dimethacrylate; phenylenediamine and formaldehyde.

Contact du poster : **Edith FILAIRE**

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Poster #11 proposé par : **MOLECULAR DEVICES**

Automation of 3D bioprinting assays for high-content imaging and assessment of compound effects

The automation of the 3D cell models results in a significant reduction in the time and effort involved, as well as an increase in assay precision and throughput. Here we describe methods for an automated generation of organoids and 3D models using automated 3D
bioprinting. Cells mixed with hydrogel-based inks or matrices were dispensed or printed into a 96-well plate using the multi-tool robotic platform, BioAssemblyBot®400 (BAB400). The BAB400 platform enabled efficient dispensing/printing of cells into domes, lines, or
other patterns, plate handling, and media addition and exchange. This assay was used for compound testing and evaluation of the anti-cancer effects of various drugs. The results showed the workflow for automated bioprinting/dispensing 3D cellular models with ECM matrices for anti-cancer drug screening workflows. An increase in throughput and ease of operation was achieved through automation. Also, imaging and data analysis methods provided valuable information about complex compound effects in 3D printed
and cell-tissue-engineered models.

Contact du poster : **Simon TENDIL**

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ADEBIOTECH
THINK TANK ONE HEALTH

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Tissue Engineering: Applications & Scopes

June 6-7, 2023 • Biocitech Paris-Romainville

Poster #12 proposé par : **LABORATOIRE EUROFINS BIO-EC**

From ex vivo to perfex vivo: human skin explant as a powerful tool to replace animal models in dermo-cosmetic

Ex vivo human skin models are robust and powerful tools to evaluate the efficacy of dermo-cosmetics and medical devices. They are well known to be close to the normal in vivo skin conditions, particularly the human skin explants (HSE). Any product that is applied to the real skin can be applied to HSE. However, it is sometimes necessary to apply the product or a treatment on the excess human skin tissue after plastic surgery, before making the explants. For instance, a needle roller can be applied over a large skin surface before applying the product which will favor better penetration, afterward the explants will be realized. Using ex vivo tracer staining studies, we have shown that hyaluronic acid-based microneedles applicator is capable of penetrating the skin epidermis and delivering substances embedded in the needle polymer matrix.

In parallel we have developed a model, perfex vivo human skin, where the skin is kept in the open air with a regulated temperature and a fluidic system ensures the circulation of the culture medium from a CO_2 incubator to the explant. Thus, separated from the disadvantageous culture environment, the explant gets closer to reality.

The Perfex vivo support and cellular environment present optimal conditions of tension, hydration and temperature allowing to test reliably dermo-cosmetics and medical devices.

Contact du poster : **Giuseppe PERCOCO**

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Poster #13 proposé par : **UMRT BIOECOAGRO 1158 UNIV. LILLE**

In vitro and in vivo comparison of the DPP-IV inhibitory activity of dietary proteins from different origins after gastrointestinal digestion

Dipeptidyl-peptidase IV (DPP-IV) plays an essential role in glucose metabolism by inactivating incretins. In this context, food-protein-derived DPP-IV inhibitors are promising glycemic regulators which may act by preventing the onset of type 2 diabetes in personalized nutrition. In this study, the DPP-IV-inhibitory potential of proteins from diverse origins was compared for the first time in vitro and in vivo in rat plasma after the intestinal barrier (IB) passage of the indigested proteins. In parallel, these proteins were digested in vitro using the harmonized INFOGEST protocol. The DPP-IV-inhibitory activity was measured after IB passage using a Caco2/HT29-MTX mixed-cell model. The peptide profiles were analyzed using RP-HPLC-MS/MS with MS data bioinformatics management, and the IC50 of the identified peptides was predicted in silico. The in vitro and in vivo DPP-IV-inhibitory activity of the proteins differed according to their origin. The correlation existing between the in vivo and in vitro results is discussed in this work

Contact du poster : **Benoit CUDENNEC**

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Poster #14 proposé par : ITERG

Nutrition – alternative to in vivo mode

The use of alternative methods in animal experimentation is now a necessity with regards to societal concerns about ethical approach to animal experimentation. In this context, the EFSA Scientific Committee highlights the importance of minimizing tests on animals

(in vivo tests) and of promoting alternative approaches, wherever possible. These alternatives include laboratory tests (in vitro) or tests performed by computer simulation (in silico). For example, it is recommended to replace or, when appropriate, to reduce the number of animals used in the experiments and to improve their well-being. The EU legislation on the protection of animals used for scientific purposes establishes guidelines for the ethical use of animals in experimental procedures (Directive 2010/63/EU). These are the "3Rs": "Replacement (substitution and replacing animal models whenever possible), Reduction (reducing the number of animals in experiments) and refinement (optimizing the methodology applied to animals). Studying the intestinal absorption of a compound and its metabolic fate in the organism is the result of different chemical, enzymatic and mechanical processes that occur simultaneously in the organism under the effect of complex regulatory pathways. In the case of studying the digestion of a compound, different approaches can be considered : in vivo, in vitro or in silico methods. However, in vitro or in silico models do not reproduce the biological complexity of the digestive tract and its metabolism. In a regulatory and societal context, it is important to know the advantages and limitations of the different models/approaches, in order to set up the best model that complete the study objectives,

especially in projects dedicated to the evaluation of the absorption and metabolic fate of target molecules. Through our research program, we defined different alternative methods to animal studies to follow the digestion and absorption of specific compounds. Also, a comparative study between in vitro and in vivo methods has been implemented to evaluate the intestinal absorption of nutrional molecules. The aim is to potentially identify an in vitro method that allows to all or a part of an approach on in vivo model.

Contact du poster : **Leslie COUEDELO**

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Poster #15 proposé par : **UNIVERSITÉ PARIS CITÉ**

Toxicological evaluation of a co-exposure to microplastics and benzo(a)pyrene in vitro using human bronchial epithelial 3D model

Airborne microplastics (MPs) have been recently detected in human lung tissue, but their fate and impact on airways is still unrevealed. Hence, MPs have become a major public health concern, as they might act as carriers of toxic pollutants like benzo(a)pyrene (BaP), a polycyclic aromatic hydrocarbon with inflammatory and tumorigenic effects. Our study focuses on the impacts of polyethylene terephthalate (PET, 69nm) or polystyrene (PS, 307nm) alone or combined with BaP on an in vitro model of human bronchial epithelium that we recently developed to study short and long-term effects, using air-liquid interface grown Calu-3 cell line. Biological effects of the pollutants are being assessed by evaluating the integrity of the barrier, cytotoxicity, inflammation, antioxidant defense and Aryl Hydrocarbon Receptor pathway biomarkers and mucus production. Uptake of MPs is being tracked using cell imaging. No major effect on the barrier integrity and cell viability was observed after 24h of exposure to PS or PET MPs alone. However, cells internalized PET MPs, and these were found in intracellular vesicles. We are currently clarifying the impact of MPs on the bioavailability of BaP. This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 965367 (PlasticsFatE).

Contact du poster : **Safaa MAWAS**

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Poster #16 proposé par : **CYPRIO SAS**

Encapsulated animal and stem cell-derived hepatocytes in liquid core alginate capsules as new models for in vitro in vivo correlations and DMPK

Hepatocytes cultured as a monolayer are widely used in the pharmaceutical industries for multiple in vitro applications, such as drug metabolism and pharmacokinetics (DMPK), hepatotoxicity or drug-induced liver injury (DILI). However, this model is not optimal to predict toxicity and/or efficacy of a new drug due to the rapid loss of functionality and viability of these cells. While the living animal models could provide an alternative, there is, additionally to some interspecies differences, a crucial and ethical need to reduce their use to follow the 3Rs recommendations. At Cyprio, we have developed human HepatoPearls, a liquid-core capsule containing primary human hepatocytes that self-assemble into a three-dimensional spheroid. The alginate shell serves as a physical barrier protecting the spheroid from the mechanical stress thus facilitating manipulations while simultaneously increasing its long-term viability and maintaining its metabolic activities. Here, we present our other Pearl models currently under development, such as rat and mouse HepatoPearls aimed for in vitro in vivo correlation assays as well as encapsulated stem cell-derived hepatocytes (iPearls) and pooled human HepatoPearls designed to reduce the inter-donor variability of the primary human hepatocytes. These models also highlight all the hallmarks of hepatocyte-specific functions, such as CYP activity and inducibility, albumin synthesis and the presence of bile canaliculi.

Contact du poster : **Jérôme CARON**

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Poster #17 proposé par : **INSIGHT BIOSOLUTIONS**

Anti-aging efficacy prediction of cosmetic ingredients in a complex mixture by In-silico and In-vitro approaches

The skin aging process, which is activated by longer exposure to sunlight, pollution, stress, and age leads to overexpression of several protein biomarkers and Matrix Metalloproteinase (MMP) is one of them. MMP-1 is the major protease that initiates the fragmentation of collagen fibers, which are predominantly type I and III in human skin and are further degraded by MMP-3 and MMP-9 [1,2]. Many commercially available chemical MMP inhibitors are added to the cosmetic formulation, but the effective detection of the anti-aging potential of cosmetic ingredients is still a challenging task.

Here we focused on finding new anti-aging cosmetic ingredients from natural sources for developing sustainable and green cosmetics using integrated in silico/in vitro approaches. Our molecular modeling study found that two active ingredients CPD2 (-9.0 kcal/mol) & CPD3 (-10.9 kcal/mol) gave stronger & comparable affinities against MMP enzymes as compared to known chemical MMP's inhibitors (-9.0 ~ -10.5 kcal/mol).

The ingredients mixture with CPD2 & CPD3 were evaluated for their potential to reduce Radical Oxygen Species (ROS) production and MMP1 release in culture of human keratinocytes and human fibloblasts illuminated with visible light or infrared. The addition of the ingredients, significantly reduces the production of ROS in keratinocytes cultures and decreases concentration of MMP1 in fibroblasts during illumination with visible or infrared light ($p<0.05$). These active ingredients act on keratinocytes and fibroblasts, two of the major cell types of the skin, to protect it from the harmful effects of light.

The results obtained from the two different approaches show that the ingredient mixture with CPD2 & CPD3 has beneficial anti-aging effect, owing to their both anti-oxidant, down-regulation of MMP-1. and their inhibitory binding affinity against MMP-3 and MMP-9 proteins. Ours integrated in silico and in vitro methods offer a framework for testing and validating the mechanism of action of anti-aging cosmetic products.

Contact du poster : **Ashwani SHARMA**

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Poster #18 proposé par : **INERIS**

Alternative methods to investigate the toxicological effects of airborne pollutants

The development of alternative methods to animal testing has become a priority in the framework of regulatory toxicology. Up to now, few approaches other than in vivo experiments have been developed to investigate hazards of airborne pollutants. This methodological paucity is due to the difficulties for alternative tests to reproduce repeated exposure and to evaluate chronic and integrative endpoints (e.g., reprotoxicity, clinical and cognitive effects, etc.). The objective of our work is to combine in vitro exposure models and in silico analysis to leverage regulatory expertise and replace animal assays to assess the toxicological effects of airborne pollutants. For this purpose, the exposure of different cell types (pulmonary, nasal, coupled lung and liver cells, skin, and ocular models) to various air pollutants (particulate matter, complex aerosols etc.) are being tested. A detailed characterization is performed to explore the links between the physicochemical properties of the tested atmospheres and the observed biological responses. Cellular and integrative in vitro and in vivo data are compared, correlated, and integrated with in silico tools. The overall aim is to identify pertinent biomarkers and implement predictive alternative exposure/effects models. In the context of regulatory hazard assessment, these alternative methods coupled to physicochemical properties characterization could be part of an integrated testing strategy for assessing inhalation health hazards of substances to avoid animal testing.

Contact du poster : **Julie PEIFFER**

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Poster #19 proposé par : **OXIPROTEOMICS**

Targeted Proteomics Approaches for Assessing Efficacy Testing in in vitro Human Skin Models

The design of rational dermo-cosmetics interventions targeting the root causes of skin dysfunctionmay help slow down skin structure & function decline there by keeping a "healthyskin". To achieve this purpose, identification of reliable bio-markers of skin longevity is needed. Increased oxidative stress and inflammaging are pivotal drivers of accelerated skin aging. Both indissociable processes are transversal to different "unhealthy" skin phenotypes: such as atopic skin, sensible skin, oily skin or photo-aged skin. Extrinsic factors (photodamage, urban pollution, microbiome dysbiosis), as well as internal factors (neurogenic inflammation, mood disorders) disrupt innate functions and delay skin repair and renewal. Thus, to track the rate of aging and develop a comprehensive set of skin

aging biomarkers, we set-up a novel targeted proteomics approach for the assessment,

quantification and identification of oxidatively damaged (carbonylated) proteins at the proteome level. Distinct signatures of biomarkers (i.e specific proteins from different anatomical compartments or organelles) have been observed upon skin exposition (in vitro and ex vivo) to different types of stress. The use of precise and specific aging biomarkers is essential to support transition to rational cosmetics approaches and more generally to P4 Cosmetics (predictive, preventative, personalized), and finally to precision cosmetics.

Contact du poster : **Martin BARAIBAR**

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Poster #21 proposé par : **OROXCELL**

Skin sensitization and photosensitization evaluation through LC-MS/MS, HRMS and 3D reconstructed tissue approaches : An integrative evaluation strategy addressing the mixtures challenge

Sensitization evaluation is an essential part of the safety assessment of substances in the dermo cosmetic industry. In order to comply with the current ban of in vivo assays for the evaluation of cosmetics raw materials in Europe, several in vitro assays for the detection of sensitization were developed to permit the toxicological evaluation of these substances without the use of animals.

In parallel, the skin sensitization process was conceptualized as an Adverse Outcome Pathway (AOP) based on four key events (KE), and the existing, validated, assays can be linked to the evaluation of specific KE of the AOP through the defined Integrated Approaches to Testing and Assessment (IATAs).

However, the validated tests were developed for the evaluation of simple substances and not for substances difficult to test such as lipophilic substances requiring the use of organic solvents, or complex mixtures such as botanical extracts. Although possible, the testing was shown to be difficult mainly due to solubilization issues, to interference related to cytotoxic effects from the organic solvent or from a component of the mixture, or to the unavailability of the composition of the mixture.

We discuss here the limits of the tests described in the standard guidelines, together with the options developed at Oroxcell to address these issues, both by setting up innovative mixture characterization methods, by the development of new assays and by the adaptation of existing tests to substances difficult to test. Our integrative approach considerably extends the field of application of the assays towards complex substances and mixtures for toxicological assessment and efficacy against sensitization effect, and for the evaluation of photosensitization effect of these substances.

Contact du poster : **Éric ANDRES**

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Poster #22 proposé par : OROXCELL

Identification of soluble biomarkers for skin sensitization in a co-culture model of reconstructed human epidermis and monocyte-derived dendritic cells

Over the last years, the better understanding of the mechanisms leading to skin sensitization has permitted the development of alternative in vitro methods for replacing the classical animal models for studies in toxicology. Nonetheless, the gold standard methods for the evaluation of the sensitizing potential of new chemicals still relies on animal testing, since no method so far has been able to recapitulate the complexity of molecular and cellular pathways leading to skin sensitization. Therefore, the demand for developing accurate non-animal alternative methods for safety assessment has been a high priority for cosmetic industry since animal models were banned for testing cosmetic ingredients. Currently, the in vitro methods testing skin sensitization are based on the detection of few protein biomarkers (e.g., IL-18), a large set of genes (e.g., Genomic Allergen Rapid Detection – GARDTM), and monoculture systems, relying then in single endpoints to study a very complex and multi-faceted process. Herein, we propose the development of a co-culture model using 3D reconstructed human epidermis (RHE) and monocyte-derived dendritic cells (THP-1) for integration of key event 2 (the generation of danger signals by keratinocytes) and 3 (activation of dendritic cells) of skin sensitization. A previous study [1] has shown that the expression of key markers of dendritic activation (CD54 and CD86) at basal conditions (no treatment) was elevated in THP-1 cells co-cultured with RHE in comparison to THP-1 monocultures. Therefore, although THP-1 is considered a good candidate tool for sensitization screening [2], such findings suggest that new activation markers should be investigated for better sensitization prediction. In this context, our study is designed to identify soluble biomarkers in the supernatant of RHE/THP-1 co-cultures. Following the exposure to skin sensitizers and irritants, the expression of CD54 and CD86 will be determined in THP-1 cells by flow cytometry. In parallel, the expression of a set of candidate genes will be evaluated in RHE and THP-1 cells by quantitative PCR for screening of potential upregulated targets. Afterwards, the correlation between dendritic activation and genic expression will determine the most relevant targets leading to skin sensitization. Among them, a few targets will be selected for creating a panel (5-10 targets) for performing a bead-based immunoassay (BioLegend's LEGENDplexTM) to detect overexpressed soluble factors in the supernatant of RHE/THP-1 co-cultures by using flow cytometry. We foresee that such experiments might allow the identification of soluble biomarkers of skin sensitization which will be useful for rapid and reliable safety assessment. References [1] Schellenberger et al. Toxicology in Vitro. 2019; 57:62-66. [2] Clouet et al. Arch Toxicol. 2019; 93:941-951.

Contact du poster : **Rodrigo AZEVEDO LOIOLA**

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Poster #23 proposé par : **NESTLÉ**

Deciphering the origin of total estrogenic activity of complex mixtures

Identifying compounds with endocrine properties in food is getting increasingly important. This is complex since foods are mixtures containing thousands of chemicals, with many of them being unknown. Recently, the application of bioassays has been promoted for their potential to detect unknown bioactive substances and to provide information on possible interactions between molecules. The present study addresses a promising approach using High Performance Thin-Layer Chromatography (HPTLC) coupled to bioassays to link analytical and biological activity data. Two soy protein isolates anticipated to contain estrogenic chemicals served as case studies. Seven isoflavones in the isolates were identified using Liquid Chromatography Mass-Spectrometry (LC-MS) analysis. The use of chemical derivatization for natural products using HPTLC allowed the depiction of the correlation between the soy isolates extracts and the identified isoflavones. Moreover, coupling HPTLC with the Estrogen Screen Yeast assay (p-YES) revealed the presence of an estrogenic bioactive zone. Analysis of the bioactive zone through Liquid Chromatography coupled to High Resolution Mass Spectrometry (LC-HRMS) highlighted signals corresponding to several isoflavones already detected in the isolates as well as two additional ones.

Contact du poster : **Bastien GENTILI**

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Poster #24 proposé par : **GENEVOLUTION**

Could CTA be a reliable alternative method to identify non-genotoxic substances in Food Contact Material extract ?

It has been estimated than 10–20% of recognized human cancer carcinogens classified as class I by the IARC act by non-genotoxic mechanisms (Hernandez et al., 2009). The only way to detect compounds with non-genotoxic action mechanisms will be to perform long term in vivo study but animal testing can be waived for most of regulations (for ex Food Contact Materials (FCM)) when results from genotoxicity are clearly negative and when no structural alerts are identified. Furthermore, in vivo assays have some limitations; large number of animals, prolonged duration (2 years), large quantity of substance to test (very difficult with Non-Intentionally Added Substances (NIAS)). They are very expensive with scarce mechanistic information making difficult to completely understand the human relevance (Mascolo et al., 2018, Madia et al., 2019). Carcinogenesis is a field where the request for alternative methods to animal testing In accordance with the 3R rules is particularly high. In regulatory toxicology, in vitro evaluation methods need to be robust, reliable and standardized (Dusinska et al., 2017). The Cell Transformation Assay (CTA) using Bhas 42 cells is a test to evaluate the genotoxic and non-genotoxic compound using the two phases of carcinogenesis, initiation and promotion mode. Bhas42 is a mouse fibroblast 3T3 cell line transfected with many Ras gene copies allowing a process of cellular transformation. This bioassay method has many advantages, e.g., high sensitivity, short experimental period, use of smaller amounts of materials, and simplicity of the procedure (Asada et al., 2005). In this work, we miniaturized 6-well protocol in 96-well protocol with a high throughput and automatically analysis in order to use less chemical agent, media.... The advantage of this miniaturization is also the classification and quantification of foci. The aim of this work is to demonstrate the molecular mechanism leading to in vitro malignant transformation.

This bioassay will be performed on FCM extracts in order to identify any non-predictable non-genotoxic NIAS in mixture.

Contact du poster : **Théo LACOUR**

SYMPOSIUM

Alternatives To Animal Experimentation

June 6-7, 2023 • Biocitech Paris-Romainville

Poster #25 proposé par : **ADAMAS UNIVERSITY**

Choose to be kind, choose to be cruelty -free

Trying to mirror human diseases or toxicity by artificially creating symptoms in mice, dogs or monkeys has major scientific limitations that cannot be overcome. Very often the symptoms and responses to potential treatments seen in other species are dissimilar to those of human patients.

The wider field of human health research could benefit from a similar shift in paradigm. Many disease areas have seen little or no progress despite decades of animal research.

Contact du poster : **Asika GHOSH**

Poster #26 proposé par : **EPITHELIX**

Novel fully primary human airway epithelium-alveolar macrophages in vitro co-cultures models to study host pathogen interactions

Being the first line of defense of the organism against airborne pathogens, the respiratory epithelium acts as a physical barrier and is a potent immune-regulator which orchestrates both innate and adaptive immune responses upon bacterial or viral infections.

Here we established new co-culture models using well characterized, standardized human airway epithelium (MucilAirTM, SmallAirTM) and primary human lung macrophages (CD45+,HLA-DR+, CD206+, CD11b+ and CD14-) for studying bacterial and viral infections. The co-culture models are functional (phagocytosis) and respond to pro-inflammatory stimuli such as LPS, TNF-□ and Poly(I:C) with an increased IL-8 secretion.

MucilAir™-macrophages showed stronger immune responses upon Streptococcus pneumonia (Sp19F) bacterial infection than with methicillin-susceptible Staphylococcus aureus strain (MSSA) compared to MucilAir™ monocultures. Upon MSSA infection, the presence of macrophages led to a decrease of 1.5Log10 CFU with IL-8 and β-defensin-2 secretions decreased after 24 hours while a reduction of Sp19F growth of 3.5Log10 CFU was observed.

These novel in vitro models might find applications in understanding the role of immune-epithelial cell interactions in infection diseases and inhalation toxicity assessment.

Contact du poster : **Mendy BOUVERET**

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Poster #27 proposé par : **WATCHFROG**

New Transgenic Medaka Model to Detect Disruption of Thyroid Signalling

Endocrine disruption caused by chemicals has been considered as a major issue over the last few decades for both human and wildlife health. Identifying endocrine disrupting chemicals in order to limit their usage is therefore a priority and is required according to EU regulations (REACH, pesticides, biocides). To date no OECD test guideline based on fish are available for the detection of Thyroid Disrupting Chemicals. This study aimed to fill this gap by developing a new test for the detection of Thyroid Active Chemicals (TACs) at eleutheroembryonic life stages. It's based on the development of a new medaka transgenic fish line (Oryzias latipes), expressing Green Fluorescent Protein (GFP) in thyroid follicles. We quantified variations of total fluorescence after 72 hours exposure to TACs only, which is proportional to thyroid axis activity, reflecting the negative feedback loop of Hypothalamic–Pituitary–Thyroid axis. This promising assay on eleuthero-embryos will provide an alternative to animal experiments as this early life-stages are not defined as protected and, therefore, research involving them do not come within the regulatory frameworks dealing with animal experimentation.

Contact du poster : **Elise PESCE**

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Poster #28 proposé par : **UMR1011**

Microfluidic pump systems: a way to replace animal models to study aortic endothelial cells response and viability to high shear stress

Severe heart failure leads to the implantation of cardiac assist devices in patients waiting for a heart transplant. These pumps induce a change in the blood flow regime: from a physiological and pulsatile flow to a pathological and continuous flow with higher shear stress, provoking bleedings in half of the patients. Animal models to study the response to different types of flow require surgical procedures and thus, additional training in animal experimentation making those procedures inaccessible to some biologists. In addition, these practices require more animals used because of the development of the surgery protocol and the survival rate of these operations. Besides, large surgical animal models used to study cardiac assist devices provide macroscopic information while only few explorations are feasible at the cellular and molecular level. Now, microfluidic pump system can be used to study the flow in vitro and we tested the IBIDI® pump system to study the response of aortic endothelial cells to high shear stress. The cells were subjected to different flow types with a high pathological shear stress. Immunofluorescence and qPCR analyses were also performed to study the inflammatory response and cell viability. Plasma (with and without platelets) were perfused into this model to check the response of blood elements to high shear stress and see the relevance of the model.

Contact du poster : **Christina LE TANNO**

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Poster #29 proposé par : **GENOSKIN**

The Hyposkin® platform: a unique framework to decipher the early steps of human immune response to vaccines at the site of injection.

Traditional ex vivo vaccine readouts often fail to recapitulate the complexity of the human immune system found at the site of injection. We have developed a proprietary technology that maintain natural human skin biopsies (Hyposkin®) alive and immunocompetent. We then have designed a general framework to study the early steps of human immune responses to vaccines and adjuvants at the site of injection.

Combining 3-D imaging, multiplexed cytokines dosage and single cell RNA sequencing, we show that 1) immune and structural compartments in HypoSkin® stay alive and functional for 10 days and that 2) the injection of the COVID19 Moderna vaccine triggers the release of a specific signature of chemokine well known to be involved in T cells and monocytes recruitment. Finally, 3) the longitudinal single cell analysis of skin-resident immune cells transcriptome showed a time-dependent modulation of myeloid and lymphoid cells activation status, strongly suggesting the generation of a coordinated immune response at the site of injection.

The Hyposkin® platform provides a unique framework for the study of the early steps of immune response to drugs at the injection site that might help to maximize specific immune targeting or evaluate adjuvant potency ahead of clinical trials.

Contact du poster : **Nicolas GAUDENZIO**

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Poster #30 proposé par : **GALAPAGOS**

Assessing compounds using an animal-free testing strategy

Currently in vivo studies remain essential in the discovery, development and approval of new medicines. Regulatory authorities worldwide require that new medicines are evaluated in both animals and humans to ensure the quality, safety, and efficacy of these products. However, there is increasing societal pressure to reduce/eliminate to use of laboratory animals. Galapagos is therefore keen to implement and demonstrate the company's commitment and responsibility to refine, reduce and replace testing involving the use of animals to the greatest possible extent.

Since 2021, Galapagos is part of The Virtual Human Platform for Safety Assessment (VHP4Safety) platform, which aims to accelerate an animal-free testing strategy. VHP4Safety intends to assess the safety of compounds solely based on human physiology and biology by integrating innovations in data sciences, human tissue culture models, and transition management. To build the "virtual human" and feed the platform human, relevant scenarios have been assessed, integrating key variables such as disease state, life course exposure, and gender and age. Subsequently the platform will be implemented to ensure stakeholder acceptance, governance and sustainability.

The VHP4Safety project is funded by the Netherlands Research Council (NWO) 'Netherlands Research Agenda: Research on Routes by Consortia' (NWA-ORC 1292.19.272).

Contact du poster : **Astrid CAPELLO**

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Poster #31 proposé par : **GALAPAGOS**

3R initiatives at Galapagos

Over the last few decades, in vivo animal experiments have been the gold standard for the assessment of safety of pharmaceuticals for human health. Over the last year the societal pressure, and knowledge gain regarding the animal testing limitations, supported the development of alternative methods to animal testing. Consequently, an increasing number of new 3R approaches and techniques are currently available to refine, reduce and replace existing animal experiments. However, the new techniques also come with their own challenges in translatability and acceptance.

Starting from 2019, the year the animal welfare committee was initiated, Galapagos has used less animals year by year. Part of this reduction was attributed to the initiatives taken in research as well as in development of new drug compounds.

Several 3R examples included the increased usage of PK/PD modeling to rank compounds and thus limit unnecessary in vivo testing, the use of micro-sampling in main treatment groups of toxicity studies to make satellite animals redundant. We also embraced the 'organ on a chip' approaches to develop miniaturized 3D cell culture models that mimic specific aspects of human vascular diseases and we increased the development of humanized in vitro models using human biopsies.

For in-house in vivo studies, implementation of imaging technologies (bioluminescence, X-Ray,..) allowed us to evaluate the progression of the pathology with fewer animals per study. Efforts were also engaged at the level of animal housing with adaptation of bedding and adapted enrichment. Health monitoring was adapted with alternative method using a non-terminal sterile flocked swab method allowing reuse of the sentinel animals.

Contact du poster : **Ludovic WAECKEL**

Poster #32 proposé par : **MATTEK IN VITRO LIFE SCIENCE LABORATORIES**

Development and Validation of in vitro Human Inhalation Toxicity Tests

for Volatile Liquids, Mists, and Sprays

Testing of acute respiratory toxicity currently (ART) largely relies on the use of animal models. Besides the ethical issues associated with such testing, the animal models have been discredited many times as reliable predictors of human physiological response. In this study we utilized EpiAirway reconstructed human tracheobronchial epithelium model to develop physiologically relevant ART test, assess its correlation with GHS categorization and investigate interlaboratory reproducibility. The experiments with 53 test articles were performed in parallel on the tissues produced by 2 MatTek laboratories in USA and EU and utilized two ART protocols , the Direct Application Protocol (DAP), and the Vapor Cap Protocol (VCP). The effects on tissue viability (MTT assay) and barrier properties (TEER) were determined. Using the MTT assay, the DAP discriminated between GHS Cat.1&2/3&4/5&NC with a Sensitivity/Specificity/Accuracy (S/S/A) of 63.5/76.1/69.8% (USA) and 63.8/76.1/70.0% (EU). Utilizing the changes in TEER, the DAP showed S/S/A of 65.9/76.7/71.3% (USA) and 64.1/76.6/70.3% (EU). The correlation coefficient between the laboratories was R2 =0.91 for MTT and 0.76 for TEER. Using the MTT assay, VCP discriminated between GHS categories with S/S/A of 70.8/83.2/77.0 (USA) and 71.9/83.2/77.5% (EU). TEER showed S/S/A of 64.4/78.5/71.5 (USA) and 67.1/80.1/73.6 (EU). The correlation coefficient between the laboratories was R2 =0.96 for MTT and 0.93 for TEER.

Contact du poster : **Jan MARKUS**

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June 6-7, 2023 • Biocitech Paris-Romainville

Poster #33 proposé par : **GENEVOLUTION**

Participate in the RENEB inter-laboratory comparison exercise 2021

Cytogenetic assays for biological dosimetry: dicentric chromosome assay and cytokinesis-block micronucleus assay following telomere and centromere hybridization

RENEB 2021 inter-laboratory comparison of three blinded samples exposed to x-rays had the purpose of simulating an emergency scenario in which early dose estimates are required for immediate medical management support. The 40 participating labs were asked to provide fast analysis in a triage mode and report results in a quickly manner. The three blinded samples provided included one unexposed, one lower exposed and one higher exposed sample. The cytogenetic assay teams receive 2.6 ml of whole blood per sample.

Dicentric chromosome assay [DCA] and cytokinesis-block micronucleus assay [CBMN] were performed using telomere and centromere staining for MN and dicentric scoring allowing the improvement of the techniques compared to uniform staining generally used by other participating laboratories (Giemsa or DAPI). The introduction TC staining to the evaluation of chromosomal aberrations renders the analysis easy, without requiring a high level of expertise.

Both The CBMN assay and the DCA assay allowed classification of samples in the correct triage categories, with a slight increase in both irradiated doses, a fact unexpectedly found systematically also by other participating labs.

Contact du poster : **Corina CUCEU PETRENCI**

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Poster #34 proposé par : **MATTEK IN VITRO LIFE SCIENCE LABORATORIES**

EpiOcular time-to-toxicity test method for eye hazard subcategorization

In vivo Draize eye irritation test (OECD TG 405) was used to assess a serious eye damage/eye irritation. In 2015, OECD TG 492, based on reconstructed human cornea-like epithelium (RhCE), was accepted as an in vitro alternative. However, this method could not distinguish between chemicals causing serious eye damage (GHS Category 1 or Cat 1) and less-severe eye irritation (GHS Category 2 or Cat 2). Recently, OECD TG 492B has been accepted which describes in vitro procedure allowing the identification of chemicals that: a) do not require labeling (No Category or No Cat), b) Cat 1, and c) Cat 2. The EpiOcular™ time-to-toxicity test method was developed for eye hazard identification of liquid and solid chemicals according to the three UN GHS categories. The proposed testing strategy developed in CON4EI project and confirmed in ALT4EI project and additional testing resulted in a robust final set of 144 reference chemicals – 78 liquids and 66 solids. Using proposed testing strategy for liquids, we were able to correctly identify 78.7% of Cat 1 (N=27), 63.5% of Cat 2 (N=26) and 82.0% of No Cat (N=25). Using proposed testing strategy for solids, we correctly predicted 75.0% of Cat 1 (N=28), 59.4% of Cat 2 (N=16) and 80.3% of No Cat (N=22). Overall, 76.8% of Cat 1 (N=55), 61.9% of Cat 2 (N=42), and 81.2% of No Cat (N=47) were correctly predicted. The EpiOcular™ time-to-toxicity test method is a novel approach for subcategorizing both liquid and solid compounds into 3 UN GHS ocular hazard categories: No Cat, Cat 2, and Cat 1.

Contact du poster : **Silvia LETASIOVA**

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Poster #35 proposé par : **TEBUBIO**

Organ-On-Chip: physiologically relevant 3D modeling for pre-clinical screenings.

Human in vitro cellular models have been developed to answer biomarker discovery for in numerous pathologies. Despite the extensive work done so far in cellular models, emulating all functions and responses of an organ remains a challenging task, as the experimental microenvironment impacts on function and assay readout ability. Tebubio has developed 3D in vitro systems that help predict biomarkers toxicity in physiological environment, biotransformation, drug-drug interaction, drugs' adverse effects, and long-term toxicity in preclinical stages. Our methodology takes advantage of Synvivo 3D Tissue and Organ-On-Chip models to recreate and study physiological events such as the invasion of tumor cells into a vascular compartment in response to drug treatment or the capacity of new drugs to pass through the blood brain barrier. At Tebubio, we demonstrated that a combination of controllable assays in 3D cultures can successfully be used for compound screening in preclinical studies. We are currently offering to our clients fully customizable 3D models capable of reliably recapitulating physiological responses that are complementary to animal model assays.

Contact du poster : **Célia BOSSO-LEFÈVRE**

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Poster #36 proposé par : **GIS FC3R**

Mapping of Replacement in France based on the projects submitted for the second call for projects of the FC3R

The French Center for the 3Rs (FC3R) promotes and supports the application of the 3Rs principles (Replacement of animals if possible, Reduce the number of animals used and Refine, enhancing wellbeing and reducing pain and stress) in scientific projects in favor of responsible and innovative research.

In November 2022, 159 projects from the academic research field were submitted during the second call for projects owed to replace animals and products from animal origin in research and the results released in March 2023 after an analysis from the Scientific Committee of the FC3R. From these data, we analyzed several items that characterize non-animal models : the species replaced in the project, the body system studied, the non-animal approach used, the area of research (oncology, pathophysiology, etc...) and the location of the laboratories. We thus observed that organoids and organs-on-chips were the most commonly used in vitro methods. These data highlights how non-animal models are implanted in French academic research and will help us to understand the role of academic research as a factor of innovative non-animal approaches in science.

Contact du poster : **Alan DUBOIS**

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Poster #37 proposé par : **HYBRIGENICS SERVICES**

Chemical Proteomics and Nanobody Eelection Platforms as Alternative Methods to Reduce Animal Testing

Hybrigenics has developed a chemical proteomic method based on a unique Chemical Yeast Two-Hybrid screening system. We identify on- and off-targets of small bioactive molecules to elucidate the mechanism of action and anticipate potential side-effects. The yeast is the screening tool. The process allows to screen in an exhaustive and unbiased manner proprietary highly complex cDNA libraries (+130 libraries, +45 species) constructed from any tissue or cell type (human, rodents, plants, …).

Antibodies are essential for research diagnostic and therapeutic. After many decades of use of rabbits, mouse and lamas, synthetic biology takes the lead. We present a platform of Nanobody selection based on synthetic library using phage and yeast. We have already selected a large set of Nanobodies for our customers. The published targets are form GPCR to peptide or even DNA. Direct access to the nanobody sequence allows a maximum of flexibility in the applications from research to diagnosis and therapeutics.

Contact du poster : **Jean-Christophe RAIN**

Poster #38 proposé par : **UNIVERSITÉ PARIS CITÉ**

Hormonal impact of bisphenols A, F and S in human placental cells

Bisphenol A, used in the production of plastics, is classified as an endocrine disruptor. It is therefore now restricted by several European regulations and directives and replaced by other bisphenols such as bisphenol F or S. Bisphenol A has estrogenic properties and alters hormone levels. Hormone levels are critical to the pregnancy process and to the growth of the fetus. During pregnancy, the production, metabolization and regulation of hormone levels are dependent on the endocrine function of the placenta. Any alteration in the hormone level is associated with adverse pregnancy outcomes such as preeclampsia, preterm birth, intrauterine growth restriction. The purpose of this study is to evaluate the hormonal alteration induced by bisphenol A and if its substitutes, bisphenol F or S, also induce hormonal alteration. We incubated human placental JEG-Tox cells with this three bisphenols for 72 hours as we previously demonstrated that JEG-Tox cells can be of great value in placental toxicology studies and are able to secrete many hormones. Hormonal secretion (estradiol, progesterone, hPL, hCG and leptin) was evaluated. All the tested bisphenols alter hormonal secretion and bisphenol A substitution by bisphenols F or S doesn't appear to be a safe alternative for pregnant women.

Contact du poster : **Elodie OLIVIER**

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Poster #39 proposé par : **INERIS**

Francopa's point of view: where are we with the use of NAMs for regulatory purpose?

FRANCOPA, the French platform on alternative methods, is particularly devoted to the replacement of animal toxicity tests for regulatory purposes. On 22 November 2023, Francopa brought together national and European researchers and regulators in a webinar to exchange views on recent and future developments of New Approach Methodologies (NAMs). On this basis of discussion, Francopa proposes to analyse the opportunities and challenges of NAM development from a regulatory perspective. The context seems more favorable to the integration of these methods due to the increasing number of actions pros the replacement of animal testing : in September 2021, the European Parliament adopted a resolution calling on the European Commission to draw up an EU-wide action plan to actively abolish animal testing; the European Citizens' Initiative ("Save cruelty free cosmetics - commit to a Europe without animal testing") has collected 1.3 million signatures. In parallel, the Commission adopted the Chemicals Strategy for Sustainability and the revisions of the REACh and CLP regulations were launched. Even though, skin corrosion and irritation, skin sensitization, eye damage and irritation, genotoxicity and mutagenicity can already be addressed using NAMs, systemic effects (repeated dose toxicity, reproductive and developmental toxicity) and more complex effects as developmental neurotoxicity or immunotoxicity seem more challenging. Building confidence in NAMs is linked to data integrity and transparency, technical characterization and validation, identify the uncertainty and the biological relevance of these new methods.

Contact du poster : **Laure GEOFFROY**

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Poster #40 proposé par : **SOCOTEC ENVIRONNEMENT**

Use of new approach methodologies in the toxicological field: how it can help to step away from animal testing requirements

Directive 2010/63/EU on the use of animals for scientific use and REACH (EC 1272/2008) regulation set animal experimentation as only bearable in last resort, for environment or health protection purpose. Yet, the number of animals used under REACH regulation is still significant due to standard data requirements. For instance for each high tonnage substance, no less than 2500 animals are required in reproductive and developmental toxicity studies (i.e. OECD TG 443 and OECD TG 414 in two species) constituting a minimal data requirement for the high tonnage band. Adaptations of the data requirements are possible by applying a weight-of-evidence analysis or a using read-across (grouping) from analogue substances. However, the regulatory acceptance of complying with data requirements using these approaches is not as straightforward as by generating data in animal experiments. Interestingly, an in vitro assays battery has been developed and one is under development respectively for Developmental Neurotoxicity and for Developmental ImmunoToxicity, which are aspects investigated in OECD TG443 study. It opens the way to use new approach methodologies (NAMs) for regulation purposes. However, acceptability of approaches different from standard data requirement is not warranted, especially when no guidance is available. In particular, guidance is needed to better characterize acceptable Weight of Evidence analysis implying in vitro data generation.

Contact du poster : **Constantin DALLOT**

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Poster #41 proposé par : **PIERRE FABRE DERMO-COSMÉTIQUE**

**PhotoSENSIL-18 assay development: enhancing the safety testing
of cosmetic raw materials and finished products to support
the in vitro photosensitization assessment ?**

Although photosensitization remains a major toxicological endpoint for the safety assessment of cosmetic products, there is no validated in vitro method available so far. Given that previous studies have proposed that the Interleukine-18 (IL-18) play a key role in keratinocyte-drivens pro-inflammatory responses, we hypothesize that IL-18 might be used as a specific biomarker for in vitro photosensitization assessment.

The aim of the present study was the set-up of a new in vitro assay using IL-18 production as a biomarker for the identification of photosensitizers in a reconstructed human epidermis (RHE) model.

EpiCSTM were incubated with a set of 16 known sensitising / Phototoxic / Photosensitizing substances and exposed to ultra-violet (UV) irradiation.

For each tested substance, the IL-18 production level was expressed as UV+/UV- ratio. Our data shows that the cut-off of 1.5 (UV+/UV- ratio) is the most predictive model among the tested conditions, being capable of identifying true positive photosensitizers (8 of 9) with a good prediction in comparison with in vivo data.

As preliminary results, our data suggests that PhotoSENSIL-18 is a promising in vitro method for identification of photosensitizing substances. Although further studies are necessary to optimize the model, we foresee that PhotoSENSIL-18 can be an alternative method to safety assessment of cosmetic products within a global Integrative Approach to Testing and Assessment (IATA).

Contact du poster : **Richard NGUYEN**

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