Belgian Society for Toxicology and Ecotoxicology

Belgian Environmental Mutagen Society

Annual Meeting 2024

Novel Non-clinical Safety Testing Strategies for the Development of Human Medicinal Products

Ghent, December 5th, 2024

Abstract Book

Meeting Programme

8:30-9:00	Registration	
9:00-9:10	Welcome by the Presidents of BelTox and BEMS	*Steven Van Cruchten (UAntwerpen) and Birgit Mertens (Sciensano)*
	Morning Session, Chair: Vitalina Gryshkova (UCB)	
9:10-09:30	Introduction on Non-clinical Testing Strategies for Different Types of Drug Modalities	*Sonja Beken (FAMHP)*
09:30-10:00	Novel Non-clinical Safety Approaches for Small Molecule Drugs	*Jean-Pierre Valentin (UCB)*
10:00-10:30	Risk-based Approach for Non-clinical Evaluation of Advanced Therapy Medicinal Products	*Sofie Peirs (FAMHP)*
10:30-11:00	Coffee and Poster Session	
11:00-11:30	Preparing for a New ICH Guideline on Oligonucleotide Safety Testing: An Industry Perspective	*Joel Parry (GSK)*
11:30-11:45	BelTox General Assembly	*Steven Van Cruchten (BelTox)*
11:45-12:00	Short presentations on YSC posters	*Poster authors*
12:00-13:15	Lunch and Poster Session	

Afternoon Session, Chair: Ryan Wheeldon (J&J) and Sonja Beken (FAMHP)

13:15-13:45	Genetic Toxicology Testing Approaches for New Drug Modalities	*Caressa Van Dongen (J&J Innovative Medicine)*
13:45-14:15	Genomic Safety Considerations and Challenges in Genetically Modified Cell Therapies	*Parimal Pande (J&J Innovative Medicine)*
14:15-15:15	**Young Scientists Competition**	*Steven Van Cruchten (UAntwerpen)*
	Optimized 3D Explants for Ex Vivo Precision-Cut Tissue Slices: A Novel Platform for Drug Discovery and Personalized Medicine	*Ara Sargsian (KULeuven)*
	The Testicular Organoid as a Model to Study the Impact of Endocrine-disrupting Chemicals on Steroidogenesis	*Katerina Papageorgiou (VUB)*
	Advancing Nucleoside-based Therapeutics for Animal Trypanosomiasis: Balancing Efficacy with Environmental Safety	*Kayhan Ilbeigi (UAntwerpen)*
	Effect-based Monitoring of Emerging Organic Micropollutant Mixtures in Conventional Wastewater Treatment Plants Effluents in Flanders, Belgium	*Marie Pardon (KULeuven)*
15:15-15:45	Coffee and Poster Session	
15:45-16:15	Current State of and Alternatives to NHPs in Non-clinical Safety Studies	*Peter van Meer (MEB) Fred Brouta (UCB)*
16:15-16:45	Platform Approaches for mRNA Vaccines	*Claudia Lindemann (BioNTech)*
16:45-17:15	Proclamation of the Best YSC Presentations and Posters	*Steven Van Cruchten (UAntwerpen)*
17:15-17:30	Closure of Annual Meeting	*Steven Van Cruchten (Beltox), Birgit Mertens (BEMS)*

Organizing committee:
Chair: Steven Van Cruchten
Members: Joao Alves Barbosa, Sonja Beken, Vitalina Gryshkova, Mark Martens, Ryan Wheeldon, Viella Krieckemans

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Short CV Invited Speakers

Sonja Beken

Federal Agency for Medicines and Health Products, Belgium.

Sonja Beken holds a Master in Biological Sciences and PhD in Pharmaceutical Sciences from the Vrije Universiteit Brussel (VUB), Belgium and a Master in Applied Toxicology from the University of Surrey, UK. She is a European Registered Toxicologist. Sonja Beken is the Coordinator of the Unit of non-clinical evaluators at the Belgian Federal Agency for Medicines and Health Products. This Unit is responsible for the evaluation of non-clinical data submitted to support all phases of drug development (e.g. marketing authorizations, clinical trials, EU/national scientific advice, etc.). Sonja Beken is the Chair of the 3Rs Working Party and is also Member of the Non-Clinical Working Party at the European Medicines Agency. She was ICH Rapporteur for the revision of the S5(R2) Guideline and is current member of the Implementation Working Group for ICH S7B/E14. Over the years, Sonja Beken has contributed to the direct identification of opportunities for regulatory implementation of 3R testing paradigms through her active involvement in large-scale international initiatives. Her main areas of expertise relate to regulatory science, non-clinical drug development, (in vitro) toxicology and metabolism as well as alternative models to animal experiments (3Rs, NAMs).

Jean-Pierre Valentin

UCB Biopharma, Early Clinical Development & Translational Safety, Belgium.

Jean-Pierre holds a PhD in Physiology & Pharmacology from the University of Montpellier, France. Following a post-doc at UCSF, he joined the Pierre Fabre Research Centre where he contributed to the discovery and progression into development of 3 candidate drugs. Then Jean-Pierre joined AstraZeneca to build from inception, develop and lead the Department of Safety Pharmacology where he contributed to the safety evaluation of ~200 candidate drugs across a wide range of therapy areas, leading to the development and successful registration of several marketed products. In 2014 he joined UCB-Biopharma as Senior Director Head of Investigative Toxicology, supporting the entire portfolio across several therapy areas and drug modalities. He is an active member of several scientific societies; former President of the Safety Pharmacology Society; current co-chair of the HESI subcommittee on Pro-arrhythmia, and of the HESI Cardiac Steering Team. He is a member of the Board of Trustees and Executive Committee of HESI. He is also Chair of the IQ-DruSafe Secondary Pharmacology Working Group and is representing the EFPIA on the ICH E14-S7B committee. He is involved in training, education and mentoring programs associated with several scientific societies, universities, and trade associations. He is author/co-author of several patents and >200 peer review publications and book chapters.

Sofie Peirs

Federal Agency for Medicines and Health Products, Belgium.

In 2012, Sofie graduated from Ghent University with a Master of Science in Bioscience Engineering, specializing in cell and gene biotechnology. Then, she pursued and obtained a PhD in Health Sciences from Ghent University. Her doctoral and subsequent postdoctoral research focused on uncovering the molecular mechanisms driving T-cell acute lymphoblastic leukemia and identifying new therapeutic strategies for patients. In 2019, Sofie joined the Belgian Federal Agency for Medicines and Health Products (FAMHP) as a non-clinical assessor. Her main tasks include providing scientific advice to

applicants and evaluating non-clinical data in clinical trial and marketing authorisation applications. Her expertise lies particularly in oncology products and advanced therapy medicinal products (ATMPs).

Joel Parry

EFPIA Oligo Safety Working Group Chair and EFPIA ICH S13 co-lead/Preclinical Sciences, GSK R&D, UK.

Joel has worked in nonclinical safety within GSK R&D for 30+ years, conducting/directing mechanistic work for 20 of those. Over the last 15 years he has provided project toxicologist support, working on a range of modalities, although mainly oligonucleotides, both in discovery and development. In 2023 Joel joined the Nonclinical Safety Project Specialists department and lead a matrix team, accountable for implementation of a safety screening cascade for GSK's internal oligonucleotide discovery programs. Since being involved in oligonucleotide projects within GSK he participated in cross industry working groups (e.g., chairing the EFPIA Oligo Safety Group and member of DruSafe Oligo Safety group and various sub-committees of the OSWG). Joel has also recently been selected as one of the EFPIA leads for the ICH S13 Expert Working Group.

Caressa Van Dongen

Johnson & Johnson Innovative Medicine, Belgium.

Caressa has been a research scientist at Johnson & Johnson Innovative Medicine for the past 6 years based at the Beerse campus in Belgium. Caressa is a member of the Discovery Technology and Molecular Pharmacology group, specifically working on in vitro high throughput safety screening methodologies for the discovery portfolio. A recent accomplishment includes the development and implementation of a high content imaging assay for the detection and quantification of DNA damage. Caressa holds a master's in biomedical sciences from the University of Antwerp.

Parimal Pande

Johnson & Johnson Innovative Medicine, United States.

Parimal Pande has obtained a PhD from the University of Connecticut USA and a master's degree from the University of Houston Clear-Lake. He is currently working as Sr. Principal Scientist at Johnson & Johnson USA in the global toxicology and safety pharmacology department. At J&J, he is actively involved in developing a strategy to assess the off-target effects and insertional mutagenesis of the lentivirus and CRISPR therapeutics. Previously, he had worked at Boehringer Ingelheim Pharmaceuticals for 10 years in the investigative toxicology department, where he supported biomarkers and toxicogenomic assessments of preclinical phase I enabling studies. He has also supported the safety strategy of AAV based therapeutics. Parimal has over 16 years of experience in the field of toxicology and developed expertise in the field of molecular genetics. He is actively involved in the external consortiums like HESI CT-TRACs where he is currently leading a workstream to answer questions related to genomic stability in the allogeneic iPSC derived CAR-T therapeutics.

Peter van Meer

Medicines Evaluation Board, The Netherlands

Peter van Meer is a senior non-clinical assessor at the Medicines Evaluation Board and the Dutch representative in the European Medicines Agency Non-clinical Working Party. Peter studied molecular pharmacology and toxicology at the Free University Amsterdam and holds a PhD in biopharmaceutics from Utrecht University. He is also a steering group member of the Regulatory Science Network Netherlands and is member of the scientific board of the Dutch 'More Knowledge with Fewer Animals' program. Peter's scientific interests are in 3Rs applications in support of regulatory efficiency and regulatory science in general, where he collaborates with several national and international research groups and is in the scientific advisory board of European research programs. He is affiliated to the Universities of Nijmegen and Utrecht University, where he supervises two PhD candidates who are involved in 3Rs research.

Fred Brouta

Non-Clinical Safety Evaluation at UCB Biopharma, Belgium

A veterinarian by training, Fred Brouta earned a PhD in Veterinary Sciences from the Faculty of Veterinary Medicine at the University of Liège. He began his career at UCB in 2002 and has since held multiple leadership and scientific roles in the domain of non-clinical safety for various Immunology and Neurology projects. Currently, Fred is the head of non-clinical safety evaluation at UCB Biopharma and board-certified by the American Board of Toxicology (DABT). He also represents UCB at the Preclinical Development Expert Group (PDEG) within EFPIA.

Claudia Lindemann

BioNTech UK Limited, UK

Claudia Lindemann has been working at BioNTech since 2018 and currently holds the position of Director of Non-clinical Safety, Portfolio Lead for Infectious Diseases since September 2022. In this role, she leads a team of non-clinical safety project representatives in the field of infectious diseases and oncology. She is responsible for developing and executing integrated non-clinical safety strategies for RNA-based vaccines and therapeutics across the Infectious Disease Portfolio. Prior to this, Claudia held positions as Associate Director Non-clinical Safety and Scientist Non-clinical Development in the Department of Non-clinical Safety. Claudia's academic background includes a DPhil in Clinical Medicine from the University of Oxford, an M.Sc. in Pharmaceutical Science from the WWU Münster, and a B.Sc. in Chemical Biology from the TU Dortmund. Claudia has authored several scientific publications, including works on the preclinical characterization of an mRNA-encoded anti-Claudin 18.2 antibody, an anti-Claudin 6 bispecific antibody and a vaccine against M-Pox (BNT166) as well as on the toxicological assessments of BNT162b2, a COVID-19 vaccine

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Introduction on Non-clinical Testing Strategies for Different Types of Drug Modalities

Sonja Beken

Federal Agency for Medicines and Health Products, Brussels, Belgium.

While traditional small-molecules and biotechnology derived pharmaceuticals, such as monoclonal antibodies, are well established, numerous new modalities have been added to the therapeutic toolbox over the past 20 years. These include new chemical modalities (e.g. RNA therapeutics, protein degraders, cyclopeptides) as well as complex biotechnology-derived medicinal products, antibody-drug conjugates, gene and cell therapies, and live biotherapeutic products, to name but a few.
In compliance with Directive 2001/83/EC and its associated guidelines, regulatory non-clinical testing of medicinal products is carried out to support first administration of a new medicinal product to humans; before carrying out clinical trials in larger populations and before marketing authorisation. The ICH guidelines, ICH M3(R2), ICH S6(R1) and ICH S9 provide specific modality- and indication-based non-clinical testing frameworks. Novel types of drug modalities, however, clearly require adapted non-clinical testing strategies and methods and ongoing initiatives will be introduced.
Standard pre- and non-clinical models are becoming increasingly limited in their utility for effective prediction of safety and efficacy of novel drug modalities. This can for example be related to the absence or insufficient pharmacological activity. The EMA guideline on strategies to identify and mitigate risks for first-in-human and early clinical trials (EMEA/CHMP/SWP/28367/07 Rev. 1) encourages the use of in vitro studies with human-derived material, either as additional support or as an alternative to in vivo testing. The EMA 3Rs Working Party and the European regulatory network have a long-standing commitment towards the application of the principles of Replacement, Reduction and Refinement (3Rs). This is driven both by the requirements of Directive 2010/63/EU, as well as by the crucial need for better tools to predict quality, safety and efficacy of new medicinal products. In this context, the leverage and regulatory acceptance of non-animal approaches, including human-based complex in vitro models, in silico tools, etc, in the regulatory non-clinical testing of human medicinal products is fostered. Experience gained from past related regulatory discussions (e.g. ICH E14:S7B Q&A, ICH S1B(R1), ICH S5(R3)) will be discussed, both with respect to acceptance of (human based) in vitro systems for a pre-defined context of use as well as with regards to the regulatory acceptance of weight of evidence approaches.

Novel Non-clinical Safety Approaches for Small Molecule Drugs

Jean-Pierre Valentin

UCB Biopharma, Early Clinical Development & Translational Safety, Braine l'Alleud, Belgium.

Safety is a significant contributor to drug candidate's discontinuation and market withdrawals of pharmaceuticals. This presentation will provide a perspective on the evolution of non-clinical safety testing for small molecule pharmaceutical drugs, considering scientific and technological advancements in drug safety science, the paradigm shift in the drug discovery and development process, and the ongoing evolution of the regulatory landscape. Gaps in safety sciences arise from the inability to identify safety hazards across the cardiovascular system and interconnected organs/functions, to predict and assess risks with certainty, and to optimally manage and mitigate the CV safety liabilities of drugs. Difficult aspects of non-clinical safety evaluation have included understanding the mechanisms of toxicity to develop, validate, and deploy new approach methodologies (NAMs), such as human-relevant or human-based in silico, in vitro, and in vivo testing systems to address specific or selective forms of human toxicities. Examples of hazard identification and de-risking strategies will be presented, focusing on cardiovascular toxicity (e.g., QT prolongation and torsades de pointes arrhythmias), and off-target secondary pharmacology profiling. Optimal management and mitigation of non-clinical safety risks have resulted from better estimation of safety margins understanding, the provision and use of human-relevant safety biomarkers, and an understanding of the translation of in silico, in vitro, and in vivo studies to humans.

Risk-based Approach for Non-clinical Evaluation of Advanced Therapy Medicinal Products

Sofie Peirs

Federal Agency for Medicines and Health Products, Brussels, Belgium.

Advanced therapy medicinal products (ATMPs) comprise gene therapy medicinal products, somatic cell therapy medicinal products and tissue engineered products. Due to the specific nature of ATMPs, a risk-based approach may be applied to determine the extent of quality, non-clinical and clinical data to be included in the marketing authorisation application. This presentation will focus on the non-clinical evaluation of ATMPs. An overview of the relevant regulations and scientific guidelines will be provided. The risk factors that may be considered in the risk analysis will be discussed as well as how the risk profile determines which non-clinical data is needed for a marketing authorisation application. To illustrate the risk-based approach, examples from real marketing authorisation dossiers will be given.

Preparing for a New ICH Guideline on Oligonucleotide Safety Testing: An Industry Perspective

Joel Parry

EFPIA Oligo Safety Working Group Chair and EFPIA ICH S13 co-lead/Preclinical Sciences, GSK R&D, Stevenage, United Kingdom.

Oligonucleotide Therapeutics (ONTs) have now matured as a modality, with more than 20 medicines approved world-wide to treat a variety of diseases. They comprise diverse modes of action, including mRNA degradation, modulation of splicing or non-coding RNA, RNA editing, translational interference, targeting proteins or immunostimulation. ONTs are chemically synthesised and very often contain a variety of modifications to improve their potency, disposition, and safety properties. They differ from small molecules (e.g., in size, chemical composition) and have several specific safety considerations (e.g., extended tissue half-life, durability of pharmacodynamic effect, hybridisation-mediated off target effects (OTEs)). Although there are a few regional regulatory guidance documents for the modality, these contain some divergent perspectives. In the absence of harmonised guidance, sponsors and regulators have tended to default to following International Council for Harmonization (ICH) M3(R2). However, this small molecule guidance does not adequately address the more unique aspects of assessing ONT safety, often leading to case-by-case decisions and inefficiencies in development strategies.

Genetic Toxicology Testing Approaches for New Drug Modalities

Caressa Van Dongen[1,2], Natalie Mesens[1]

1. Johnson & Johnson Innovative Medicine, Turnhoutseweg 30, 2340 Beerse, Belgium; 2. Corresponding author.

Genotoxicity testing is a regulatory requirement for many drug modalities, and drug candidates need to be proven to be free of DNA damage potential before starting clinical trials. Currently, regulated in vitro assays addressing genotoxicity such as the in vitro micronucleus test (MNT) run late in discovery; at high costs in low throughput with a high hit rate requiring intensive de-risking and in vivo follow up.

The in vitro MNT is a genotoxicity test for the detection of micronuclei in the cytoplasm of interphase cells. Micronuclei may originate from acentric chromosome fragments (i.e. lacking a centromere), or whole chromosomes that are unable to migrate to the poles during the anaphase stage of cell division. Therefore, the in vitro MNT is a method that provides a comprehensive basis for investigating DNA damage potential because both aneugens and clastogens can be detected in cells that have undergone cell division during or after exposure to the drug. In addition to using the in vitro MNT to identify substances that induce micronuclei, the use of immunochemical labelling kinetochores, or hybridization with centromeric/telomeric probes (fluorescence in situ hybridization (FISH)), can provide additional information on the Mode of Action (MoA) of the drug. The genotoxic MoA classification is important as it requires a different risk management strategy to address potential safety concerns for humans.

Within J&J, the Multiplexed Endpoint Genotoxicity Assay (MEGAScreen) was developed. The MEGAScreen is a High Content Imaging (HCI) In vitro Micronucleus Assay which simultaneously assesses micronuclei and two additional cellular markers associated with genotoxicity; the γH2AX and kinetochore biomarker – allowing MoA classification. In this way, J&J transformed their current genotoxicity strategy: the high throughput obtained by automated HCI analysis shifts micronucleus assessment towards early stages of drug discovery and integrates MoA information enabling immediate understanding of the potential issue at series level for early navigation or deselection.

Genomic Safety Considerations and Challenges in Genetically Modified Cell Therapies

Parimal Pande

Johnson & Johnson Innovative Medicine, 1400 McKean Rd, Lower Gwynedd Township, PA 19002, United States.

Autologous cell therapy has proven to be effective for the patients with relapsed and refractory cancers, where standard of care failed to show efficacy. The autologous cell therapy although beneficial, suffers manufacturing challenges. The vein-to-vein time are long and the cost to manufacture the personalized therapy is very high. Allogeneic “off-the-shelf” approach is being developed to make the CAR-T therapies available quickly and since these drug products are manufactured in bulk the cost is considerably low compared with the autologous cell therapy. Manufacturing of the allogeneic cell therapy products involve complex engineering process, where cells undergo extended culturing and multiple passaging. The cells accumulate small and large structural variations that are donor specific, associated with extended culturing or could be because of editing performed using CRISPR/ other nucleases. These structural variations may offer genetic fitness to the cells and the cells may proliferate uncontrollably leading to development of secondary malignancy in patients. There are four critical stage gates, at which genetic safety of the allogeneic product should be assessed before proceeding to the next stage. The presentation is focused on providing details around these stages and the assays that can be used to address genomic safety related questions.

Highlights from the 2023 EFPIA Survey on Non-Human Primate (NHP) Use in Toxicology Studies

Fred Brouta

Non-Clinical Safety Evaluation at UCB Biopharma, Belgium.

This presentation will discuss the findings of the European Federation of Pharmaceutical Industries and Associations' (EFPIA) 2023 survey on the use of non-human primates (NHPs) in toxicology studies, exploring potential pathways to reduce and replace NHP use, and highlighting industry perspectives. This recent survey gathered insights from over 25 pharmaceutical companies to evaluate trends, challenges, and advancements in the replacement, reduction, and refinement (3Rs) of NHP use in preclinical testing. Designed with input from the Non-Clinical Working Party (NcWP) of the European Medicines Agency (EMA), the survey covers industry practices related to species selection, study design, and the adoption of New Approach Methodologies (NAMs).

NHPs are generally not the default choice for non-rodent toxicology studies. Although alternative species, such as dogs and minipigs, are increasingly utilized, NHPs remain essential in specific cases—particularly for New Biological Entities (NBEs) and gene therapies—due to pharmacological, physiological, and anatomical requirements that other species cannot fully meet.

Companies are actively working to reduce animal use by minimizing control groups and incorporating historical control data, especially in non-GLP (Good Laboratory Practice) studies. The survey highlighted a need for more robust virtual control data and clearer regulatory guidance to facilitate the ongoing reduction of concurrent control animals. Recovery group management varies across companies, with some opting for high-dose recovery groups, especially in chronic studies. Early alignment with regulatory authorities was identified as a valuable strategy to limit NHP use in a scientifically justified manner.

The adoption of NAMs is emerging as a promising means to reduce in vivo NHP testing. However, limitations in regulatory acceptance, method validation, and the inability of NAMs to fully replicate complex biological systems currently restrict their widespread implementation as alternatives to animal studies. Respondents stressed the importance of validated NAMs that can reliably translate to human models, which would help bridge the gap between exploratory studies and regulatory submissions.

The survey findings reflect the industry's ongoing efforts to ethically balance scientific rigor with regulatory requirements by employing alternative species, optimizing study designs, and piloting NAMs. Continued collaboration with regulatory bodies will be critical to advancing the 3Rs approach in toxicology studies, ultimately reducing reliance on NHPs and fostering innovation in non-clinical safety assessment.

New approaches to identify the need for developmental toxicity studies in NHP

Peter van Meer

Medicines Evaluation Board, The Netherlands.

The need for NHP to evaluate safety of biologics, and particularly monoclonal antibodies (mAbs), is driven by the high affinity to their target and target species specificity. This has led to specific guidance, ICH S6(R1), for this class of products tailored to ensure relevant safety assessment. In this guidance, the limited placental transfer of mAbs in the first trimester of pregnancy in NHP is also recognized, which has resulted in merging embryofetal toxicity and pre- and postnatal toxicity studies, the enhanced PPND study. However, the safety profile of mAbs is generally driven by its pharmacology and immune responses. Thus, adverse developmental effects are likely also driven by these mechanisms. A weight of evidence approach (WoE) for developmental risk of monoclonal antibodies (mAbs) may be able to evaluate the need for an ePPND study. We reviewed developmental and reproductive toxicity programs of 65 mAbs and compared these to our WoE. Our review suggests that a WoE is able to identify developmental toxicants and despite being conservative, this WoE approach could characterize presence and absence of developmental risk without animal studies. The current WoE could have reduced the need for developmental toxicity studies by 42% without loss of important patient information in the label.

Platform approaches for mRNA vaccines

Claudia Lindemann

BioNTech UK Limited, United Kingdom.

The development of mRNA-based pharmaceuticals presents an unparalleled opportunity for the utilization of platform technology. By altering the encoding sequence of the mRNA, drugs can be adapted for individualized treatments, strain adaptations, and expanded clinical indications while maintaining technical specifications. The use of platform technology not only offers a manufacturing advantage but can also streamline the non-clinical package. This presentation will delve into the applicability of platform preclinical studies, specifically toxicology approaches, for mRNA-based vaccines and therapeutics.

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Abstracts Young Scientist Competition Oral Presentations

Optimized 3D Explants for Ex Vivo Precision-Cut Tissue Slices: A Novel Platform for Drug Discovery and Personalized Medicine

Ara Sargsian[1,2], Hermon Germatsion[1,2], Bart Ghesquière[3], Stefaan J. Soenen[1,2], Bella B. Manshian[1,2*]

1. Translational Cell and Tissue Research Unit, Department of Imaging and Pathology, KU Leuven, Belgium; 2. NanoHealth and Optical Imaging Group, Department of Imaging and Pathology, KU Leuven, Belgium; 3. Metabolomics Expertise Center, Center for Cancer Biology, VIB Center for Cancer Biology, Leuven, Belgium.

Drug discovery faces a significant bottleneck where, despite millions of drug compounds and nanoformulations being synthesized and tested every year, only a small fraction progresses to clinical trials, and many of those fail during later stages due to safety concerns. This highlights the urgent need for improved systems that better replicate the human body, especially when it comes to mimicking the complex 3D structure of organs. Therefore, we present precision-cut tissue slices (PCTSs) as a promising ex-vivo 3D tissue culture method that addresses these challenges.

The key features of PCTS are; (1) its close mimicry of in-vivo environment: PCTSs maintain the natural tissue-matrix configuration of the organ, preserving important physiological and functional details. (2) Reproducibility: These slices offer consistent results, allowing for repeated experiments over an extended period. (3) Cost-effectiveness: They provide a more affordable alternative to in-vivo animal models. (4) Efficiency: Multiple slices can be derived from a single tissue sample, and the same sample can be used across multiple experiments. (5) Reduction in animal use: The PCTS system helps reduce the number of animals required for disease modelling, aligning with the 3Rs principle (Refinement, Reduction, and Replacement). And, (5) human relevance: It allows testing in systems that are closer to human physiology, especially for personalized medicine.

Thus, PCTSs were generated from healthy lung tissue and orthotopic lung cancer models in mice, and validated against in-vivo models through various methods, including high-throughput screening, viability tests, gene expression profiling, histology, and immunohistochemistry. The slices could be cultured for up to 21 days, which is significant for studying long-term drug responses. Culture conditions were further optimized through metabolomics studies, and tissues were successfully frozen and re-cultured after thawing, indicating the potential for long-term storage and reuse.

Conclusion: In our experimental conditions PCTSs appear to be a highly promising tool for advancing drug discovery, enhancing the predictability of pre-clinical studies, and reducing animal testing. Their ability to preserve tissue properties over time, coupled with their reproducibility and cost-effectiveness, positions them as highly valuable for testing personalized treatment regimens before clinical trials. By providing a reliable ex-vivo alternative, PCTSs contribute to more ethical and sustainable research practices by reducing reliance on animal models.

The Testicular Organoid as a Model to Study the Impact of Endocrine-disrupting Chemicals on Steroidogenesis

Katerina Papageorgiou[1,2], Samuel Silva[1]; Andrea Errico[3]; Ellen Goossens[1]; Tamara Vanhaecke[2]; Yoni Baert[1,2]

1. Biology of the Testis (BITE) laboratory, Genetics, reproduction and development (GRAD) research group, Vrije Universiteit Brussel, 1090 Brussels, Belgium; 2. In Vitro Toxicology and Dermato-cosmetology (IVTD) research group, Vrije Universiteit Brussel, Belgium; 3Biomedicine and Movement Sciences, Department of Neurosciences, University of Verona, Italy.

A decline in sperm count worldwide has been observed over the last decades. Exposure to xenobiotics such as environmental pollutants, drugs, food additives and synthetic polymers exhibiting endocrine-disrupting activities is considered a significant contributing factor. Addressing this decline necessitates the development of an effective human testicular model to reliably identify male fertility disruptors. In this context, we investigated the potential of human testicular organoids (TOs) as a promising in vitro approach to mimic steroidogenesis. TOs were derived from testicular tissue obtained from six adult transgender patients, and cultured up to 63 days. To characterize steroidogenesis in TOs, transcriptome profiling with microarray, qRT-PCR and immunostaining were performed to assess the expression of key markers for Leydig cells and the steroidogenic pathway. Testosterone and oestrogen production were measured in the culture medium of TOs by means of an electrochemiluminescence assay. The presence of Leydig cells and the expression of steroidogenesis-specific components in transgender TOs (n=3) were confirmed at both the gene and protein levels. The secretory function of Leydig cells tended to peak after two weeks, with an average testosterone concentration of 646.8620.1 ng/mL at day 14 (n=6), followed by a gradual reduction and stabilization, reaching a concentration of 128.2±74.2 ng/mL after one month (n=6). Testosterone production persisted for up to 63 days, with a concentration of 73.3± 51.9 ng/mL (n=6). Also, oestrogen was measured at day 14 and reached a concentration of 5.4± 2,2 ng/mL (n=5). TOs harbour functional Leydig cells, expressing all major factors important for steroidogenesis and producing hormones such as testosterone and oestrogen. This makes TOs a promising model for assessing the impact of endocrine-disrupting chemicals (EDCs) on steroidogenesis, facilitating the examination of EDC effects on male fertility. TOs hold promise for translating research findings into clinical implications or regulatory actions as a New Approach Methodology, contributing to the advancement of reproductive toxicology knowledge and guiding preventive measures.

Advancing Nucleoside-based Therapeutics for Animal Trypanosomiasis: Balancing Efficacy with Environmental Safety

Kayhan Ilbeigi[1], Dorien Mabille[1], An Matheeussen[1], Rik Hendrickx[1], Mathieu Claes[1], Nick Van Reet[2], Roel Anthonissen[3], Fabian Hulpia[4], Cai Lin[4], Louis Maes[1], Clement Regnault[5], Phil Whitfield[5], Rajdeep Roy[6], Marzuq A. Ungogo[8,9], Yann G.-J. Sterckx[10], Hans De Winter[11], Birgit Mertens[3], Mirco Bundschuh[6,7], Harry P. De Koning[8], Serge Van Calenbergh[4], Guy Caljon[1*]

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Animal trypanosomiasis (AT) is a widespread disease caused by Trypanosoma spp. and has a devastating effect on animal husbandry all over the world due to the scarcity of efficient drugs and development of drug resistance, hence emphasizing the need for novel treatment options. Following previous identification of 3'-deoxytubercidin as a highly potent trypanocide with curative activity in mouse models of both stage-1 and stage-2 Human African Trypanosomiasis (HAT), we now present a comprehensive preclinical evaluation of new 6-amino substituted tubercidin analogues with promising activity against a broad range of AT species. Potent hits were identified in vitro across all important AT species, i.e. Trypanosoma brucei brucei, isometamidium (ISM)-resistant and -susceptible Trypanosoma congolense, Trypanosoma vivax, Trypanosoma evansi (type A and B) and Trypanosoma equiperdum. Selected 'hits' were further tested for in vitro metabolic stability (using bovine, horse and piglet liver microsomes), in vivo mouse models for each AT species, genotoxicity assays and mode-of-action studies (i.e. genome-wide RNA interference library screening, metabolomics). Analogue 3 was highly active in T. vivax, T. congolense, T. equiperdum, T. evansi and T. brucei curative mouse models. Furthermore, there was no indication of in vivo toxicity or in vitro genotoxicity in Vitotox®, micronucleus and comet assays. Mode-of-action studies for 3 revealed that the P1 nucleoside transporter and adenosine kinase are involved in drug uptake and activation, respectively. Ecotoxicological assessments on Daphnia and green alga Desmodesmus revealed that the compound has an acceptable ecotoxicological footprint. Given the preferred target product profile for a broad-spectrum drug against AT, analogue 3 represents an advanced lead candidate for treatment of animal trypanosomiasis, regardless of the causative species.

Effect-based Monitoring of Emerging Organic Micropollutant Mixtures in Conventional Wastewater Treatment Plants Effluents in Flanders, Belgium

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The increasing global contamination of freshwater systems with chemicals is an important environmental problem. Even though the existing treatment technologies for municipal wastewater significantly improve the quality of surface waters and comply with the current legislation on water-quality standards, the removal of many organic micropollutants (OMPs), including pharmaceuticals, pesticides, personal-care products, hormones and other industrial chemicals is incomplete. These OMPs are frequently detected in treated effluents that are subsequently discharged into surface waters and rivers, contributing to the water pollution. Due to the near-continuous presence of these substances, they can give rise to chronic effects because of long-term exposure in aquatic ecosystems. The adverse effects of wastewater treatment plant (WWTP) effluents on the water quality is therefore significant and cannot be underestimated. The current method of choice to monitor these WWTP effluents is chemical-based monitoring, however, it is faced with limitations regarding the range of compounds that can be detected and in the assessment of the potential effects caused by mixtures of pollutants. These mixture effects can be caused by either additive, antagonistic or synergistic interactions of the diverse components. Therefore, biological-based monitoring is gaining interest, as it allows the evaluation of the effective toxicity of these complex environmental samples. Ideally, fast-screening bioassays using model organisms are applied to provide toxicity information of water samples.

In this study, toxicity information for different WWTP effluents collected across Flanders is obtained by a combination of model organisms, ensuring that different types of toxic effects are assessed and sensitivity differences between organisms are considered. Both growth inhibition on cyanobacteria, and behavioural effects in zebrafish larvae are assessed and compared between the models. In addition to the toxicity evaluation, liquid chromatography coupled to high-resolution mass spectrometry (LC-HRMS) analysis is used to gather information on the chemical composition of the effluents. Different classes of OMPs (pharmaceuticals, pesticides, PFAS and hormones) are identified in a targeted screening approach. Finally, the link between the toxicological effects and the chemical composition of the samples is investigated using iceberg modelling. This approach can help identifying the main drivers of toxicity in these complex samples, to better understand the impact of these effluents on the aquatic environment.

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Y1: Assessment of Safe & Sustainable by Design Substitutes for BPA in Medical Devices

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The properties of Bisphenol A (BPA), including strength, transparency, heat and chemical resistance and ease of processing, make it an ideal chemical for the manufacturing of certain high-performance plastics used in medical devices. Applications of BPA in medical devices include medical tubing, haemodialysers, newborn incubators and dental sealants and fillings. However, BPA can leach from plastics, especially under conditions of heat, sterilization, or acidic/basic environments, which are common in medical settings. Moreover, BPA has been identified as substance of very high concern due to its endocrine disrupting properties which cause probable serious adverse human health effects. Even low doses of BPA exposure might interfere with reproductive, neurological, and immune systems. Concerns are especially high with more vulnerable populations in neonatal and paediatric care settings, where devices are used on more sensitive patients and people with long-term exposure, such as patients on dialysis or frequent users of specific medical devices. To minimize exposure for patients and healthcare workers, medical devices containing BPA-Free alternatives are opted, when available. However, BPA-free medical devices may still contain "regrettable substitutes" such as Bisphenol S (BPS), Bisphenol F (BPF) or Bisphenol A Diglycidyl Ether (BADGE) that initially appeared to be safer but later were proven to have similar or unforeseen risks. This has raised questions about the safety of "BPA-free" polymers in medical devices. A more cautious approach, with a focus on comprehensive testing, transparency, and the development of non-bisphenol, bio-based alternatives, may help to avoid repeating the cycle of regrettable substitution in the future.

As part of two EU funded initiatives, RADAR & BisGO, scientists at the unit of experimental toxicology (UET) at Sciensano have partnered up with the University of Leuven and Ghent University to work towards a solution. In both initiatives, Sciensano will develop non-clinical safety testing strategies to support the development of lignin-based bisguaiacol compounds in a Safe and Sustainable by Design (SSbD) approach. Lignin-based bisguaiacol is a promising alternative to BPA in medical devices since it retains the properties that are crucial in medical applications, including strength and physiochemical stability, while showing less possible health risks compared to BPA. Apart from OECD test methods, Sciensano will combine New Approach Methodologies (NAM) and human-relevant microphysiological systems to identify biomarkers of toxicity and support risk assessment of BPA alternatives. Ultimately, our non-clinical safety testing strategy will contribute to a blueprint for the SSbD development of polycarbonate plastics aimed for use in medical devices.

Y2: Establishment of a Rabbit Embryonic Stem Cell Line for Developmental Toxicity Testing

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The teratogenic potential of new drugs and chemicals is traditionally assessed using animal studies, primarily involving pregnant rabbits and pregnant rats. Due to the high animal usage, costs and time demands associated with these in vivo studies, there is a clear need for in vitro alternatives. Several in vitro alternatives for developmental toxicity testing have been developed over the past years, such as the mouse Embryonic Stem Cell (ESC) Test, the rat Whole Embryo Culture, and the Zebrafish Embryo Developmental Toxicity Assay. However, replacement of the traditional in vivo models with these approaches for regulatory purposes will only be accepted when these alternatives are properly qualified and predict adequately the human risk. Therefore, one of the newer approaches for developmental toxicity testing is the use of human in vitro models, such as human ESCs or human induced pluripotent stem cells (iPSCs). However, as the in vivo effects in humans, particularly in pregnant women, are unknown for most drug candidates and chemicals, the results from these human in vitro models need to be compared with animal in vivo data, raising concerns about species differences. To overcome this problem, a rabbit and rat stem cell-based assay could provide a solution. Rat ESCs are commercially available, but rabbit ESCs are not. Therefore, the aim of this study is to establish a stable rabbit ESC-line that can be further developed into a rabbit embryonic stem cell (ESC)-based developmental screening assay with a sensitivity and a specificity equal to or greater than existing in vivo models. In this assay, rabbit ESCs will be exposed to known teratogenic and non-teratogenic substances and the impact on their differentiation potential (into cells from endodermal, mesodermal and ectodermal origin) will be assessed morphologically and with biomarkers. Alterations in predefined biomarkers will be tracked throughout different stages of cell development via q-PCR and potential deviations due to teratogenic exposure will be detected at a biochemical level. At this point, we already successfully isolated rabbit ESCs and confirmed their identity through immunofluorescent visualization of pluripotent biomarkers present in these ESCs. In the end, we aim to adopt this assay across multiple species (human, rat, rabbit), enabling both in vitro comparisons of teratogenicity across species and in vitro-in vivo comparisons using existing animal data.

Y3: Speciation Analysis of Nutritional Cr(III) and Carcinogenic Cr(VI)

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Chromium (Cr) is a transition element that exists in oxidation states ranging from - 2 to +6. The common stable ones in the environment are trivalent Cr(III) and hexavalent Cr(VI) chromium. Cr(III) is an important micronutrient for the human body, while Cr(VI) is highly toxic and carcinogenic. The environmental concentrations of both oxidation states are low. Due to the differences in toxicity between Cr(VI) and Cr(III) compounds, speciation of Cr is very important. Therefore, an improved sensitive and robust method for the simultaneous determination of Cr(III) and Cr(VI) in water samples has been developed. The method uses a hyphenated micro liquid chromatography (µLC) system coupled to inductively coupled plasma mass spectrometry (ICP-MS). The optimised method incorporates a pH adjusted EDTA complexation step to stabilise Cr(VI) and Cr(III). The µLC system uses an anion exchange micro-sized column to separate the Cr species. Cr(III) and Cr(VI) were separated with different retention times at 170 and 230 sec, respectively. The method was optimized and validated by spiking Cr(III) and Cr(VI) in various water samples. Furthermore, the method was validated using a drinking water proficiency testing material sample. The developed method can be used for rapid routine determination of chromium species with high precision and reliability.

Y4: In vitro Screening for Immunotoxicity Assessment of Nanomaterials, a Case Study using Synthetic Amorphous Silica

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Manufactured Synthetic Amorphous Silica nanomaterials (SAS-NMs) are the second largest nanomaterials produced worldwide. They are widely used in various applications including cosmetics and food market. Nanomaterials have been recently identified as immune adjuvant possibly involved in the outcome of allergic reactions to environmental allergens. Consequently, a better evaluation of their immunotoxicological potential is needed. In line with the 3Rs principle, development of new in vitro approach is becoming increasingly relevant.

The evaluation of the adaptive immune response can be based on simple tests involving key cells such as Dendritic Cells (DCs), which capture and present antigens in an immunogenic or tolerogenic manner. In the presence of 'immune danger signals', they undergo maturation, resulting in the expression of co-stimulation and activation molecules, and migrate to draining lymph nodes where they activate naïve T lymphocytes.

We have already demonstrated using a human in vitro co-culture model, that SAS-NMs behave as an immunological danger signal by increasing DCs maturation and T-cell response. We addressed the hypothesis that SAS-NAMs surface chemistry could be at the origin of the propagation of these immune danger signals. Indeed, the surface of SAS-NMs contains silanol groups whose reactivity depends on their propensity to establish hydrogen bonds with the polar head of zwitterionic phospholipids in the cell membrane. Surface silanol groups, already identified as critical for cytotoxicity, could also play a determining role in the activation of DCs. To explore this hypothesis, we compared the effects of SAS-NMs by assessing different manufacturing routes and tailoring surface chemistry on the expression of co-stimulatory molecules. As a surrogate model of human DCs, the monocytic THP-1 cell line was exposed for 16 hours to the materials. Cytotoxicity and expression of CD54 and CD86, markers of THP-1 activation, were measured for each condition. Our results showed that both SAS-NMs increased CD54 surface marker expression with a greater extent for pyrogenic SAS-NMs compared to precipitated SAS-NMs and reducing silanol density led to a downregulation of CD54 expression. We hypothesize that surface chemistry is at the core of the interactions with SAS-NMs leading to DCs maturation. To further investigate membrane interactions, SAS-NMs were also tested on phospholipid bilayer models mimicking cell membrane.

Our work will allow us to develop a relevant strategy to assess immunotoxicity of NMs, considering precise characterizations of surface chemistry, and combining in vitro tests using cells and synthetic biomimetic membrane models.

Y5: Microplastic Pollution: A Hidden Driver of Antibiotic Resistance Gene Transfer?

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Microplastics (MPs) have been detected globally, from beaches and ocean depths to air and sediment pollution. The widespread use of antibiotics in treating bacterial infections has increased their presence as emerging contaminants in aquatic environments, as wastewater treatment systems often fail to remove them. Antibiotics add selective pressure to bacteria, promoting antibiotic resistance and contributing to infections that undermine modern medicine.

Microplastics, due to their small size, large surface area, hydrophobicity, and low degradability, serve as vectors for microbial colonization and biofilm formation. Compared to natural materials like rocks and leaves, MPs have a greater ability to support microbial communities. Additionally, MPs can adsorb environmental contaminants, including antibiotics, which makes biofilms on MPs hotspots for horizontal gene transfer. This process facilitates the spread of antibiotic resistance genes (ARGs) to potentially pathogenic bacterial species within microbial communities.

Bacteria can exchange resistance genes easily via horizontal gene transfer, a process that is enhanced in biofilms due to the proximity of cells. We investigated how varying concentrations of polyvinyl chloride (PVC) and polystyrene (PS) MPs affect the frequency of ARG transfer in laboratory conditions. Furthermore, we explored how different MPs influence biofilm formation and ARG transfer efficiency.

Escherichia coli cultures were grown in lysogeny broth (LB) medium, and MPs were added at concentrations ranging from 0 to 800 mg/L. After 24 and 48 hours, particles were collected, and bacteria attached to the particles were cultured on plates. MPs were characterized using dynamic light scattering (DLS) and scanning electron microscopy (SEM) to study their effect on biofilm growth. Surface roughness of MPs was found to influence biofilm formation, and the efficiency of antibiotic gene transfer varied significantly with the type of MPs. Compared to the control group without MPs, biofilms formed on MP surfaces significantly enhanced bacterial survival rates and increased the

efficiency of ARG transfer. Future research will include field sampling to study ARG transfer dynamics in natural aquatic environments, offering insights into how MPs contribute to the spread of antibiotic resistance in real-world conditions.

Y6: Physicochemical characterization and genotoxicity assessment of nanomaterials using new approach methodologies

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Nanotechnology applications are widely incorporated into the food sector. Due to their smaller size and increased volume-specific surface area, nanoparticles may possess unique properties compared to their bulk counterparts, making them useful for applications such as food colouring and plant protection products. Manufacturers bringing food-based nanotechnology applications to the European market must comply with the European Union's regulatory framework, including a nano-specific risk assessment [1,2]. Currently, hazard and risk assessment still heavily relies on conventional animal testing. Use of new approach methodologies (NAMs) can fill certain data gaps and complement the available safety studies, thereby avoiding the need for conducting additional in vivo studies. Although many NAMs are available, experience in using them to support risk assessment is scarce and the majority of the NAMs have not yet been validated. The integration of NAMs for the physicochemical characterization and genotoxicity assessment of inorganic nanomaterials and materials containing a fraction of nanoparticles, applied in the food chain, is particularly promising as illustrated in case studies examining iron (hydr)oxides applied as food additive, and copper oxides applied as plant protection product and feed additive [3].

A detailed physicochemical characterization of the test materials is an essential first step in the risk assessment. It includes measuring the particle size distribution, surface charge, specific surface area, shape, solubility and dissolution rate, agglomeration state, chemical composition and crystal structure. Data obtained by electron microscopy-based methods on the physicochemical characterization of iron (hydr)oxide and copper oxide NMs applied in the food chain will be presented.

Moreover, an overview will be provided of the NAMs that will be used to investigate the genotoxic potential of the characterized iron (hydr)oxides and copper oxides. These cover both the in vitro genotoxicity tests that are currently recommended by EFSA which are adapted for nanomaterials and materials containing a fraction of nanoparticles [4], as well as new innovative NAMs such as the transcriptomics-based biomarker, called GENOMARK. This NAM uses prediction models to classify analyzed materials as genotoxic or non-genotoxic based on gene expression data. In addition, a high-content method to simultaneously assess several genotoxicity parameters (e.g. ɣH2AX, PH3,…) with the Cytek-Amnis ImageStream technique will be developed.

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F1: How special are Nanoplastics: Studying the Difference between Nanoparticle and Nanoplastic Cellular Response under Long-term Repeated Dose Exposure Scenario's

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The ubiquitous presence of small micro- and nanoplastics (MNP, <1mm) is raising concerns on their negative impact for human health. Understanding the cellular mechanisms involved in nanoplastic effects is imperative for evaluating their environmental and human health risks. One critical question that remains unresolved is whether the observed cellular responses to nanoplastics stem from their general nanoparticle properties, i.e. the presence of a foreign particle, or from specific toxicological attributes intrinsic to nanoplastics. Addressing this question is pivotal for delineating the risks associated with nanoplastic exposure.

In this study, we aimed to explore the underlying mechanisms behind the impact of nanoplastics on cells, specifically focusing on the distinct effects between nanoparticle and nanoplastics exposure. For this we used a mixed exposure scenario using nanoplastics (polystyrene and polydisperse polyethylene nanoplastics (<800nm)) and manufactured nanomaterials, silica dioxide nanoparticles (150nm). Silica dioxide nanoparticles are one type of manufactured nanomaterials with high production volume and wide applicability which were included in the priority list of OECD reference materials for which risk assessment is urgently needed. The three nanoparticles were added in different ratio's per treatment (1:1:1; 1:0:1, 1:0:0, etc.) with two constant total particle concentrations (10^2 and 106 nanoparticles/mL). Caco-2 cells were exposed to the particle mixtures during 12 days under a repeated dose exposure scenario. Oxygen consumption rate was determined as a proxy for the bioenergetic state of the cells using an extracellular flux assay on day 2, 6 and 12 of exposure. Additionally, sulforhodamine B assay was performed to measure total protein content as a proxy for cellular viability.

Particle exposure caused a significant decrease in protein content compared to the untreated cells from 6 days exposure onwards, indicating that particle exposure did cause either decreased cell growth or lower protein production per cell. The bioenergetics of the cells were affected by nanoparticle exposure with an observed shift towards glycolysis. There was no observed difference between the plastic and non-plastic nanoparticles indicating similarities in modes of action or downstream effects.

In conclusion, nanoplastic and non-plastic nanoparticle exposure affect cellular responses of Caco-2 cells in a similar way indicating it is rather a cellular response to a foreign particle then a plastic-specific response. Importantly, a crucial characteristic of nanoplastics are their heterogeneity with different shapes, sizes and functional groups. This was not yet included in the study and the effect of these characteristics should be studied in future research.

F2: Advancing the Replacement of Animal Testing in Toxicology via the RE-Place Platform

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The 3Rs Principle, aimed at advancing the Replacement, Reduction, and Refinement of animal testing, has gained significant importance in recent years due to scientific, economic and ethical concerns related to animal use. In the field of Toxicology, the development of alternative methods contributing to the partial and/or full replacement of animal testing has been, and still is, a key priority. By incorporating these alternative methods into scientific research, the reliance on animal testing can be reduced, leading to more relevant, reliable, and ethically sound research results.

Alternative methods linked to the replacement of animal testing are also referred to as 'New Approach Methodologies (NAMs)'. In vitro models, like organ-on-chip technology and human-derived cell lines and tissue cultures, are increasingly used to assess toxicity. These models can provide valuable insights on the cellular and molecular pathways involved in adverse effects. Also in silico approaches, including Quantitative Structure-Activity Relationship (QSAR) models and Artificial Intelligence (AI), are deployed to predict toxicity without the need for animal testing. Although NAMs should not involve the direct use of live laboratory animals, the term is sometimes also used to refer to methods that use less animals compared to the original set-up.

The importance of NAMs is growing interest worldwide, but their (regulatory) acceptance and implementation is lagging behind. Encouraging the use of NAMs may benefit from a bottom-up approach like the 'RE-Place' project, which collects the existing expertise on the use of NAMs in Belgium in one central, open-access database. This database, available via www.RE-Place.be, provides an up-to-date overview of the existing NAMs and links this to the names of experts and institutes where the methods were developed and/or are currently applied.

In October 2024, the RE-Place database contained about 290 models in biomedical research, regulatory testing and education. Among these, about one third is linked to the field of toxicology including spheroids, organ-on-chip, machine learning, (Q)SAR and AI. Despite the growing expertise on NAMs in Belgium, only a fraction is represented in the RE-Place database. RE-Place therefore encourages scientists to share their knowledge, collaborate and facilitate the exchange of best practices. Only by working together, the full potential of NAMs can be reached. RE-Place is thus fully committed to advancing and promoting the use of NAMs in the fields of toxicology by offering valuable resources and opportunities to researchers and helping them in increasing the visibility of their work on NAMs.

F3: A Comparative Risk Assessment of Fluoroquinolone Ciprofloxacin and its Greener Alternative, CIP-Hemi

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The fluoroquinolone antibiotic ciprofloxacin (CIP) is a pseudo-persistent contaminant detected in water bodies across the globe. It is a widely prescribed human and veterinary medicine used to treat various bacterial infections by inhibiting bacterial deoxyribonucleic acid (DNA) replication, which ultimately halting the growth of bacteria. However, as CIP is insensitive to biodegradation and thus, it tends to persist in the environments and cause adverse ecotoxicological effects to non-target organism. To address this problem, the European Union (EU) project 'Transforming into a sustainable European pharmaceutical sector' (TransPharm), has been dedicated to developing alternative compounds for fluoroquinolone antibiotics with reduced in environmental impacts in their entire life circle since 2022 under the 'Benign by design' concept. For CIP, a greener alternative, namely ciprofloxacin -hemi (CIP-Hemi), has been developed, which demonstrates greater degradability and lower ecotoxicology than the parent compound, CIP. This current study conducted a comparative environmental risk assessment (ERA) of CIP and CIP-Hemi. We combined experimental methods—antimicrobial susceptibility testing and cyanobacteria toxicity testing—with a physiologically based pharmacokinetic model (PK-sim) to predict the environmental occurrence and risk of the two substances. Our results showed that, when administrated at the same dose, CIP-hemi exhibits a lower environmental impact due to its increased degradability.

F4: Unique Transcriptomic Responses of Rat and Human Alveolar Macrophages to Specific Types of Titanium Dioxide and Carbon Black Particles

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Inhalation of poorly soluble, low toxicity (PSLT) particles (e.g., titanium dioxide and carbon black) can lead to chronic inflammation and neoplastic manifestations in rats. These adverse outcomes occur at doses that induce lung particle overload, characterized by impaired macrophage mobility and reduced overall clearance capacity. Although PSLT-induced overload has been observed in various experimental species (rat, mouse, and hamster), pre-neoplastic and neoplastic responses have only been documented in rats. However, whether the rat adverse responses are predictive for humans exposed to PSLT particles remains a subject of debate. For this reason, some PSLT particles are currently classified as possibly carcinogenic to humans.

To further investigate the potential carcinogenic hazard of PSLT in humans, we compared the in vitro responses to PSLT overload in human and rat alveolar macrophages using untargeted transcriptomics. Primary alveolar macrophages from the two species were exposed to specific type of titanium dioxide (P25) or carbon black (Printex90) particles at control, non-overload or overload doses. Four days post-exposure, the genome expression profile was assessed and compared across species. Following P25 or Printex90 particle overload, rat alveolar macrophages exhibited greater sensitivity compared to human macrophages, with hundreds of genes significantly differentially expressed. Among these differentially expressed genes, we identified 18 genes significantly modulated in the same way by both particle types, specifically under overload conditions (compared to control and non-overload conditions). Most of these genes are associated with inflammation, macrophage clearance functions, and cancer development, aligning with in vivo observations in rats. This suggests that these genes may act as key mediators in the rat inflammatory and carcinogenic lung responses. Most of these 18 genes were similarly modulated in human macrophages, but the magnitude of the response was markedly lower. In addition, a specific 16-gene signature was observed in human macrophages upon overload, which was not observed in rats. Overall, these findings provide new insights into the mechanisms of lung overload in rats, and highlight similarities and differences in transcriptomic responses of rat and human alveolar macrophages.

F5: Genotoxicity Assessment and Potency Ranking of Enniatins and Alternaria Toxins with the In Vitro Micronucleus Assay

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Enniatins and Alternaria toxins are emerging mycotoxins produced by fungal strains that can contaminate food and feed. Like for other natural toxins, there is no manufacturer or supplier responsible for providing hazard data for these mycotoxins, and consequently, there are a lot of data gaps. Additional toxicity tests urgently need to be performed to address the data gaps for different toxicological endpoints, including genotoxicity. Here, the results of the in vitro micronucleus (MN) assay are presented. Together with the Ames test, this assay forms the in vitro testing battery recommended by the European Food Safety Authority (EFSA) to assess genotoxicity. First, a selection out of the large number of enniatins and Alternaria toxins was made based on the review of existing toxicological data and other criteria such as availability and price. The selected mycotoxins are the enniatin (ENN) B, B1, A and A1, beauvericin (BEA), altenuene (ALT), alternariol (AOH), alternariol monomethyl ether (AME), altertoxin-I (ATX-I), tentoxin (TEN) and tenuazonic acid (TeA). Then, these mycotoxins were tested in the in vitro cytokinesis-block MN assay in mammalian cells (i.e. TK6) with and without metabolic activation according to the OECD test guidelines No 487. The in vitro MN test results were negative in each condition for all the selected ENNs, BEA, ALT and TEN. In contrast, AME, AOH, ATX-I and TeA showed an increase in MN formation after 24h exposure without metabolic activation, indicating the induction of structural and/or numerical chromosome damage. In the 3h exposure with metabolic activation scenario, an increased MN formation was so far only observed for AME. These results will be combined in a next step with data from other genotoxicity tests such as the Ames test and new approach methodologies (e.g. γH2Ax or GENOMARK, a transcriptomics-based biomarker for genotoxicity). Finally, the benchmark dose (BMD) approach was applied for mycotoxins with a positive result in the in vitro MN assay allowing a genotoxic potency ranking of these mycotoxins. The BMDs were calculated with the PROAST software based on the data of at least three independent repeat experiments. Preliminary results indicate that ATX-I is more potent than AOH and AME. Afterwards, these BMDs will be used to assess the genotoxic effects of mycotoxin mixtures. The research is part of the work package ‘Hazard Assessment’ of the European Partnership for the Assessment of Risks from Chemicals (PARC) project (GA No 101057014) and the ENNIATOX project (RF 23/18).

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