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Norwegian Society of Pharmacology and Toxicology

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Abstracts

Winter meeting at Beitostølen 2025

Sponsor of NSFTs winter meeting 2025:

BCPT
Basic & Clinical Pharmacology & Toxicology

Invited Presentations

Session	Presenter	Affiliation
Nordic symposium: Safe and sustainable by design	Dario Greco	BioMediTech Institute, Tampere University, Finland
	Lindvi Gudmundsdotter	Simplexia, Sweden
Parallell session I : Mammalian Toxicology	Anders Goksøyr	University of Bergen
	Anders Ruus	Norwegian Institute for Water Research
Parallell session I : Pharmacology	Audun Stubhaug	Oslo University Hospital
	Øyvind Svendsen	Diakonhjemmet Hospital
The Beito Lecture	Jean-Baptiste Woillard	University of Limoges, France
Parallell session II : Toxicology - Air pollution	Johan Øvrevik	Norwegian Institute of Public Health
	Eleonora Longhin	Climate and Environmental Research Institute
Parallell session II : Pharmacology - TDM/AI-tools	Anders Åsberg/Markus Hovd	Oslo University Hospital
	Peter McCourt	University of Tromsø
Plenary session: Neurotoxicology	Oddvar Myhre	Norwegian Institute of Public Health
	Cecilie Morland	University of Oslo

Oral presentations toxicology

Friday	Presenter	Affiliation
16.30-17.30	Ketil Hylland	University of Oslo
	Clare Andvik	University of Oslo
	Clare Andvik	University of Oslo
	Sara Zamani	University of Bergen
	María Fernández-Míguez	University of Bergen
	Eveline Munnikhof	Institute of Marine Research

Saturday	Presenter	Affiliation
16.50-17.50	Anna Lauvås	The National Institute of Occupational Health in Norway
	Merete Grung	Norwegian Institute of Water Research
	Peter McCourt	University of Tromsø
	Sina Velzi	Norwegian University of Life Sciences
	Ole Jakob Nøstbakken	Institute of Marine Research
	Hubert Dirven	Norwegian Institute of Public Health

Oral presentations pharmacology

Friday	Presenter	Affiliation
16.30-17.30	Ole Martin Drevland	University of Oslo
	Marte Grasdal	University of Oslo
	Kajangi Gnanachandran	University of Tromsø

Saturday	Presenter	Affiliation
16.50-17.50	Karl Martin Forbord	University of Oslo
	Tetyana Voloshyna	University of Tromsø
	Kjetil Wessel Andressen	University of Oslo

Poster presentations toxicology

POSTER #	Presenter	Affiliation
PT1	Emma Nilsen Granly	University of Oslo
PT2	Silje Modahl Johanson	Norwegian University of Life Sciences
PT3	Hubert Dirven	Norwegian Institute of Public Health
PT4	Line-Marie Berget	Norwegian Institute of Public Health
PT5	Mette Müller	Norwegian University of Life Sciences - Veterinary school
PT6	Hanne Friis Berntsen	The National Institute of Occupational Health in Norway
PT7	Tana-Helen Meyer-Becker	Institute of Marine Research
PT8	Eleonora Longhin	Norwegian institute for air pollution
PT9	Simona Kavaliauskiene	Norwegian University of Life Sciences
PT10	Elise Rundén-Pran	Norwegian institute for air pollution
PT11	Vegard Sætre Grytting	Norwegian Institute of Public Health
PE1	Malin Birkeland	University of Bergen
PE2	Elisa Puntervold Pereira	University of Bergen
PE3	Cecilie Farstad Vidme	University of Bergen
PE4	Mia Mathisen Nilsen	University of Tromsø
PE5	Idun Dysthe Sønderland	University of Oslo
PE6	Tengel Hvidsten	University of Oslo
PE7	Seta Noventa	Italian Institute for Environmental Protection and Research
PE8	Katrine Borgå	University of Oslo
PE9	Clare Andvik	University of Oslo
PE10	Clare Andvik	University of Oslo
PE11	Clare Andvik	University of Oslo
PE12	Merete Grung	Norwegian institute for water research

Poster presentations pharmacology

POSTER #	Presenter	Affiliation
PP1	Rebar Eredeni	University of Oslo
PP2	Ingrid Wendelborg	University of Oslo
PP3	Ludvig Husby Aftret	University of Oslo
PP4	Elisabeth Leite	Diakonhjemmet Hospital
PP5	Andreas Ellingsen	University of Oslo
PP6	Eskil Hofstad	University of Oslo
PP7	Dina Spigseth Hovland	University of Tromsø
PP8	Jakub Pospisil	University of Tromsø
PP9	Samaneh Shabani Åhrling	University of Oslo
PP10	Nora Lødøen	University of Oslo

Invited speakers

Integrated approaches in toxicology and pharmacology: from nanosafety to SSbD and One Health.

Dario Greco

Director Faculty of Medicine and Health Technology, BioMediTech Institute, Tampere University

Toxicology is going through profound changes as the focus of investigation is shifting from the observation of apical phenomena to mechanistic aspects of the exposure. Toxicogenomics aims at clarifying the mechanism of action (MOA) of chemicals by using omics assays. The Adverse Outcome Pathways (AOP) concept is also emerging to contextualise toxicogenomics-derived MOA.
At the Finnish Hub for Development and Validation of Integrated Approaches (FHAIVE) of Tampere University, we use advanced modelling of large amounts of data to anchor molecular assays to AOPs. We also combine big data science, artificial intelligence (AI), network science, toxicogenomics, molecular assays and cell technology to analyse a comprehensive knowledge graph comprising tens of millions of data points with the aim to develop AOP-derived New Methodology Approaches (NAMs).
In this talk, I will discuss how integrated data-driven approaches can be used to unify the currently fragmented comprehension of the chemical-biological interactions, while guiding the development of safe and sustainable by design (SSbD) and effective by design (EbD) chemicals, drugs, and materials.

Dario Greco (b. 1978) is professor of bioinformatics at the Faculty of Medicine and Health Technology, Tampere University, and the director of FHAIVE, The Finnish Hub for Development and Validation of Integrated Approaches (https://www.fhaive.fi). He is also professor of pharmaceutical bioinformatics, Faculty of Pharmacy, University of Helsinki, principal investigator at the Institute of Biotechnology, University of Helsinki, Finland, and the coordinator of the Finnish 3R Centre (https://www.fin3r.fi). To date, he published over 200 peer reviewed articles, reviews, and book chapters in the areas of nanotoxicology, toxicogenomics, drug discovery, network biology, data modelling and bioinformatics which were cited over 12,000 times (h-index 53). To date, he has completed the supervision of 10 PhD theses. FHAIVE is currently composed of over 35 people, including senior scientists, postdoctoral fellows, PhD and MSc students, technicians, and administrative staff. As principal investigator, Greco received funding from the Academy of Finland, the EU (HE, H2020, Green Deal, ERC, and IMI2 programs), the Novo Nordisk Foundation, the Finnish Red Cross, the Finnish Government for a total of over 16 M€ for the period 2013 – 2024. Among the ongoing projects, he leads the European Research Council (ERC Consolidator) project ARCHIMEDES (2022-2027), focused on developing in vitro and computational NAMs and IATA for pulmonary fibrosis. Moreover, Greco is the coordinator of the EU HE INSIGHT project (2024 – 2028), coordinator of the EFSA funded TULI project (2024 – 2027), and deputy coordinator of the EU HE CHIASMA project (2024 – 2028), focused on the development of an integrated SSbD framework and NAMs to replace animal experiments in biomedicine, toxicology, and pharmacology.

Sustainable by Design: Drug Development Process

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Sustainable by design (SbD) is more than a framework—it is a commitment to aligning health innovation with the planet's long-term well-being. SbD aims to minimize impact on the environment along the entire product life cycle. To apply the SbD method in drug development, it is essential to consider two interconnected "life cycle" concepts: 1) Product (drug, device and packaging) Sustainability Life Cycle: Encompasses the entire journey of the product, from sourcing raw materials to its end-of-life disposal or recycling. 2) Drug Development Life Cycle: Covers the progression of the drug from early discovery through to the commercial stage. By integrating sustainability principles into drug development, the pharmaceutical industry is evolving to foster innovation while minimizing environmental and societal impacts. This approach emphasizes embedding eco-friendly practices across the entire drug lifecycle—from discovery to manufacturing and distribution. Three synergistic workstreams form the foundation for achieving efficiency and quality goals without surpassing environmental and ecological boundaries:

1) Eco-design and Circular Economy: Minimizing the environmental impact of products through sustainable design and promoting resource circularity.
2) Operational Reductions: Cutting greenhouse gas emissions, water usage, and waste within product associated operations.
3) Value Chain Engagement: Collaborating with suppliers to lower indirect emissions upstream and downstream in the value chain.

By leveraging circular economy principles and advanced technologies such as AI and automation, sustainability by design not only mitigates risks but also drives efficiency and cost-effectiveness. It highlights the vital role of collaboration among regulators, industry leaders, and academia to establish a resilient, patient-focused, and environmentally responsible pharmaceutical ecosystem.

Whales and polar bear in a petri dish - *in vitro* approaches to probe marine mammal toxicity pathways.

Sofie Søderstrøm[1], Anya Mukundan[1], Maud Van Essche[2], Sara Zamani[1], Gwenaelle S. Noally[1], Demetri Spyropoulos[3], Heli Routti[4], Pierre Blévin[5], José Pablo Vázquez-Medina[6], Odd André Karlsen[1], Anders Goksøyr[1]

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Marine ecosystems, home to mid and top predators such as whales, seals, and polar bears, are because of their high energy intake often linked to elevated absorption of contaminants with bioaccumulating and biomagnifying properties, environmental contaminants such as persistent organic pollutants (POPs), per- and poly-fluoroalkyl substances (PFAS), and phthalates [1, 2, 3, 4, 5]. Due to challenges involved in studying these species in the wild [6] the Marma-detox project (NFR project No: 334739) employs *in vitro* models to investigate the toxicological defense systems against legacy POPs and chemicals of emerging concern (CECs) in marine mammals. Fibroblast cells were isolated from fin whale, killer whale, and polar bear skin biopsies, and endothelial cells from northern elephant seal placenta. Killer whale fibroblasts were used to explore the effects of PFAS on energetic metabolism and cellular state. A luciferase reporter gene assay (LRA) identified phthalates as modulators of the PPARG, THRB, and GR in fin whales. Exposure of fin whale fibroblasts to phthalates further demonstrated higher sensitivity of the GR pathway compared to PPARG and THRB (RT-qPCR). The low expression of the PPARG gene in fibroblasts made them less suitable for studying lipid metabolism-related toxicity. Exposure to a differentiation cocktail triggered polar bear fibroblasts to accumulate triglycerides indicative of adipogenesis. Elephant seal endothelial cells exposed to benzo(a)pyrene (BaP) showed a clear response in *CYP1A* expression, whereas phthalate exposure gave no clear response in *PPARG*. Exposing these cells to hypoxia, high pressure or a mixture of these seemed to affect the response to BaP, indicating interactions of multiple environmental stressors on the toxicological response. Preliminary findings suggest that the direct conversion of polar bear fibroblasts into adipocytes provides a more appropriate model for investigating pollutants' effects on lipid metabolism. These cell-based models offer new insights into the molecular mechanisms underlying pollutant toxicity in marine mammals, enhancing our understanding of environmental impacts on these elusive species.

References: [1]Andvik, Clare, et al. *Environ. Toxicol. Chem.* 40.7 (2021): 1848-1858; [2]Routti, Heli, et al. *Environ. Int.* 152 (2021): 106458; [3]Peterson, Sarah H., et al. *Sci. Total Environ.* 533 (2015): 144-155. [4]Routti, Heli, et al. *Sci. Total Environ.* 664 (2019): 1063-1083.[5]Dietz, Rune, et al. *Sci. Total Environ.* 696 (2019): 133792.[6]Vazquez, Juan Manuel, et al. *Front. mar. sci.* 11 (2024): 1466968.

Assessing the cumulative effect of multiple anthropogenic stressors on Norwegian killer whales – The MULTIWHALE project

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Killer whales (*Orcinus orca*) are apex marine predators. As many environmental contaminants are subject to biomagnification, killer whales are particularly exposed. Accumulated pollutants can be released into the bloodstream in times of stress when lipid reserves are utilised and may cause harmful effects such as impaired reproduction and immune function, potentially affecting the population. The aim of MULTIWHALE is to study the cumulative effects of three stressors in Norwegian killer whales: pollutants, human disturbance (boat traffic), and shifting prey availability/type.

Skin and blubber samples were collected from >100 killer whales, and divided for eight different analyses pertaining to contaminant concentrations, diet, stress levels, genetics etc. In addition, knowledge on feeding habits, family relationships, group affiliation and (approximate) age is available through a database on individual killer whales (Norwegian Orca Survey). This renders us with the possibility to study the cumulative stressor effects at an individual, as well as population level, also considering that some individuals prey on marine mammals, i.e. higher in the food chain.

The COVID-19 pandemic brought restrictions on tourism in Norway and provided us with a reference with less boat activity (human disturbance) during winter 2020. Currently, stress and health parameters are compared between whales that have experienced different levels of boat traffic stress. Some individuals were sampled both in seasons with low and high boat activity, allowing us also to analyse repeated measures. Preliminary results indicate that re-sampled whales have higher cortisol levels in seasons of high boat activity. Analyses are ongoing to further quantify health effects through transcriptomics and metabolomics, with the aim of including contaminants and nutritional stress in statistical models to assess the individual and combined effects of multiple stressors.

Novel techniques have been developed in the project, such as a new genetic marker that has given insights into social and ecological structuring. Furthermore, stable isotopic ratios of amino acids, and fatty acid analysis, have been applied as markers of diet and energy source. Our analyses have shown a high level of individuality in feeding habits, of which some features cause higher pollutant exposure. Furthermore, plasticity and overlap in feeding behaviour can explain lack of social and genetic structuring.

Teeth have also been obtained from killer whales harvested in Norway in the 1980s, and from killer whales found dead in 2015-2022, and teeth growth layers have been analysed for dietary markers and mercury concentrations. This connects results from the newly sampled killer whales with changes that may have occurred at larger time scales.

Clozapine and serum concentration dependent side effects.

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Objectives
Clozapine is considered the most efficient antipsychotic drug, but can cause severe side effects like agranulocytosis and metabolic syndrome. While the risk of agranulocytosis is known to be independent of clozapine serum concentration, less is known about the absolute neutrophil count (ANC) in users that do not develop agranulocytosis. The concentration dependency of metabolic side effects like dyslipidemia and insulin resistance is also scarcely studied. Our objective was to investigate how clozapine serum concentration is related to ANC, lipids, and glucose.

Methods
Routine blood samples from Diakonhjemmet Hospital were investigated retrospectively. We included samples in which serum concentration measurements of clozapine and its metabolite norclozapine was accompanied by analysis of ANC, triglycerides, HDL cholesterol, LDL cholesterol, glucose, or HbA1c.
Results
ANC, triglycerides, and glucose showed a positive correlation with clozapine and norclozapine serum concentrations, also after adjusting for age and sex. HDL and LDL cholesterol, as well as HbA1c, did not correlate with the serum concentration of clozapine and norclozapine.

Conclusion
First, the positive correlation between clozapine and ANC suggests a potential need to reassess current guidelines that advise against initiating clozapine in patients with low baseline ANC. Second, the positive correlation between clozapine and triglyceride levels, in the absence of such a relationship with HDL or LDL cholesterol, offers insights into the metabolic side effects' underlying mechanisms. We propose that clozapine induced reduction in liver sinusoidal endothelial cell porosity may be involved in these effects, warranting further in vitro investigation. Third, the positive correlation between clozapine and glucose levels aligns with this proposed explanation.

Smart Pharmacology: Machine Learning in Therapeutic Drug Monitoring

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The advent of machine learning (ML) offers new avenues to advance therapeutic drug monitoring (TDM) under the paradigm of "Smart Pharmacology." In this presentation, I will begin with a concise overview of ML techniques, establishing a foundation for their application in pharmacology. Building on this foundation, I will detail practical implementations developed by our research group aimed at finding the optimal first dose that maximize a target attainment or predicting drug exposure and informing TDM and dose individualization. These implementations will be illustrated through case studies involving anti-infectious and transplantation drugs.

Following this, I will briefly introduce the field of synthetic data generation in pharmacology, discussing the underlying principles of creating synthetic datasets. Examples will demonstrate how such data can augment limited clinical datasets and facilitate data sharing, thereby improving model robustness and generalizability.

Finally, I will explore the concept of digital pharmacological twins or multiscale and multisource models that integrate real-time data and predictive modeling. The work of the DIGPHAT (Digital Pharmacological Twins) consortium will serve as an example, illustrating how digital twins may revolutionize personalized medicine by paving the way for truly individualized healthcare solutions in pharmacology.

Trojan Horse Effect: On the role of organic chemicals in the toxicity of combustion particles

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In Greek Mythology Odysseus and a troop of Greek soldiers hid inside the Trojan Horse to enter the walled city of Troy and win the war. This has become a frequent metaphor of tricks to invite or smuggle something harmful into a protected area. In particle toxicology the "Trojan Horse effect" postulates that particles mediate much of their effects by acting as carriers of harmful soluble components such as organic chemicals and transition metals. However, instead of hiding inside, these soluble components cling highly "visibly" to the outside of what is often a tiny particle core. Moreover, while the original Trojan Horse facilitated transportation across the otherwise impenetrable walls of Troy, many soluble components easily slip through biological barriers often leaving the solid particle core behind, more like a conventional horse left outside the gates.

This talk will discuss the importance of lipophilic organic chemicals in mediating cardiopulmonary effects from combustion particles. Decades of toxicological research have shown that lipophilic compounds are rapidly taken up in cells through passive diffusion and may be transported into circulation within minutes through the transcellular route, faster and to a larger extent than the solid particles on which they arrived. Focus will be given on the role polycyclic aromatic hydrocarbons (PAHs) and the aryl hydrocarbon receptor (AhR) in intracellular signalling and regulation of inflammatory reactions in lung epithelial cells and vascular endothelial cells. These effects may not be restricted to classical AhR activating PAHs such as benzo[a]pyrene, but likely also involves lower-molecular weight species including pyrene and phenanthrene, which have received limited attention in toxicology due to low mutagenic activity. Finally, the implications of this will be discussed in relation to the role of mass versus size or surface area as metrics for combustion particle exposure, and implications for air quality assessment and regulation.
The talk is based previous and ongoing studies from our lab and others, including work from the ULTRHAS-project (Ultrafine particles from inflammation - health assessment of sources) funded under the EU's Research and Innovation program Horizon 2020 (Grant Agreement No. 955390; www.ultrhas.eu).

Investigating Air Pollution in Oslo: *In Vitro* Effects and Chemical Profiles

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Air pollution and particulate matter (PM) exposure poses a significant health burden globally, contributing to the onset of cardiovascular diseases, lung cancer, and respiratory conditions. Emerging studies connect PM to neurological and reproductive health outcomes. PM is a complex mixture of solid and liquid particles varying in size, composition, and origin. While it is widely acknowledged that reducing PM concentrations improves health outcomes, evidence indicates that not all particle types exert the same toxic effects. Numerous studies highlight that the physical and chemical properties of PM, such as oxidative potential and bioactive chemical compounds, are critical determinants of its impact on health.

Ongoing research at NILU focuses on the chemical profiles and toxicity of PM in Oslo, aiming at improving the understanding of its health impacts and sources. The research conducted in Oslo involved a one-year long sampling of PM10 (particles with aerodynamic diameter < 10 µm) and PM1 (aerodynamic diameter < 1 µm) at the urban background site of Sofienbergparken, coupling chemical and toxicological analyses. Toxicity assessments examined reactive oxygen species formation, release of pro-inflammatory markers, and DNA damage in a human cell model. The study also explored the particles chemical composition and source apportionment, trying to identify the main contributors to PM levels.

This research underscores the need for long-term monitoring and synergies between chemical analyses, modelling and toxicology to better understand PM effects and further guide air quality regulation.

Advanced therapeutic drug monitoring (TDM) at the Norwegian transplant center – perspectives and implementation

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In Norway, all transplantations are performed at a single center, located at Oslo University Hospital – Rikshospitalet. Each year approximately 250 patients receive a new kidney and require life-long immunosuppressive drugs to prevent rejection. The first eight weeks following transplantation patients receive follow-up at the transplant center, after which they return home to their local hospital for life-time follow-up.

The high volume and focus on kidney transplant recipients ensure that physicians become experienced with dosing of immunosuppressive drugs such as tacrolimus, which has a narrow therapeutic window. However, retrospective analysis of more than 15,772 samples revealed that only 68% were within the target range (4-7 μg/L). Can model informed personalized dosing (MIPD) make a positive impact on immunosuppressive drug target achievement?

Based on rich pharmacokinetic data from a series of clinical trials and standard therapeutic drug monitoring we have developed pharmacokinetic models that allow for accurate estimation of tacrolimus AUC. This model has been implemented in the clinical practice using a limited sampling strategy requiring only three samples taken at 0, 1, and 3, hours post-dose, and provides the physician with accurate information concerning systemic exposure of the individual patient.

In our presentation we aim to describe our implementation of MIPD in the transplant setting and give our perspectives on the future it may hold. We will also present other approaches of using pharmacometrics to improve the care of kidney transplant recipients, both in Norway and abroad.

Oral Nanotherapeutic Formulations of Low Dose Glucagon-like protein 1 receptor agonist (Liraglutide) Improve Metabolic Health in Ageing Mice

Nicholas J. Hunt[1], Amanda E. Brandon[1], Glen P. Lockwood[1], Lara J. Westwood[1], Lewin Small[2], Kazi S. Islam[2], Katrina M. Quizon[2], Harrison L. Ross[1], Qingyu Lei[2], Wojciech Chrzanowski[2], Peter A.G. McCourt[1, 3], Zdenka Kuncic[2], David G. Le Couteur[1,2], Victoria C. Cogger[1,2]

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Objectives:
To develop an oral nano-drug formulation of Liraglutide, a glucagon-like protein 1 receptor agonist, and determine its efficacy in improving the metabolic health of ageing mice.

Methods:
Liraglutide (Lira) was bound to the surface of carboxylic acid substituted Ag_2S quantum dots (QDs) using carbodiimide mediated coupling (Hunt et al. 2020) to produce QD-Lira. This was encapsulated in a chitosan/glucose (CS/GS) shell as described previously (Hunt et al. 2024) to produce QD-Lira-CS/GS and used for *in vitro* (hepatocytes) and *in vivo* studies (aged mice – 18-24 months). For the *in vivo* studies QD-Lira-CS/GS and control treatments were administered to mice either subcutaneously (s.c.) or via oral gavage. Blood, liver, spleen, kidney, lung, muscle, adipose tissue and duodenum were sampled from living/euthanized animals. Assays performed include oral glucose tolerance testing, insulin, cAMP, liraglutide, liver enzymes, blood lipids, proteomics, histology and elemental analysis.

Results:
Pharmacodynamic studies in young mice demonstrated that both injected and oral liraglutide were effective at reducing blood glucose in a dose dependent manner. In aged mice, 28-day treatment with low dose oral liraglutide improved metabolic parameters such as insulin resistance, free and total cholesterol, and glucose regulation with 8.4% weight loss. Injected liraglutide showed improved glucose regulation and 15% weight loss. Oral liraglutide also promoted greater upregulation of gero-therapeutic pathways, while both treatments promoted down regulation of *de novo* lipogenesis pathways.

Conclusion:
These studies demonstrate that the reformulation of an injectable medication for oral delivery using nanotechnology can also be applied for non-liver acting medications, and demonstrate a platform for oral GLP-1RA delivery.

References:
Hunt N. J. et al. 2020, ACS Nano 14, 1492-1507
Hunt N. J. et al. 2024, Nature Nanotechnology 19, 534-544

Development of a human neural progenitor cell model for neurodevelopmental toxicity testing – case study on opioids

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Objectives: There is a need to develop reliable new cell models for neurodevelopmental testing, and it is an expectation that the applicability domains of *in vitro* models are well characterized. Increased use of opioids among pregnant women is a societal concern, since it may lead to negative effects for the fetus despite considerable benefits for the mother. We hypothesized that exposure to methadone and buprenorphine leads to disturbed neurodevelopmental processes vital for normal brain development in a human neural progenitor cell (NPC) model undergoing differentiation.

Methods: This project is part of the EU funded projects ONTOX and PARC and aims to characterize a 2D human neural progenitor cell (NPC) model, derived from IMR90 human induced pluripotent stem cells (hiPSCs), undergoing differentiation for up to 28 days using RNA sequencing, EPIC Array (epigenomics), and high content imaging (protein marker quantification). Secondly, to assess whether the NPCs are fit-for-purpose to test effects of u-opioid receptor agonists we tested repeated exposures to methadone and buprenorphine.

Results: For the cell differentiation process, RNAseq enrichment analysis from four different databases revealed that the more enriched terms were related to neurodevelopment and function. Transcriptomics data showed that gene markers for astrocytes and neuronal subpopulations (glutamatergic, GABAergic, serotonergic, cholinergic, noradrenergic, glycinergic and dopaminergic) are present in the cultures at all differentiation timepoints. Presence of microglia and astrocytes in the co-culture were shown. The genome becomes hypomethylated with differentiation, however, chromosomes 3, 15, 21 and X exhibit hypermethylation with differentiation. Enrichment analysis revealed terms related to neurodevelopmental processes in addition to terms for more common processes and multicellular system development. High content imaging confirmed presence of u-opoid receptors in the NPCs, and functionality of the receptor was shown by a concentration-dependent decrease in cell viability after exposure to methadone and buprenorphine. Selected results on neurite outgrowth and synaptogenesis will be presented.

Conclusion: This study enhances our understanding of the applicability domain for this 2D NPC model including fit-for-purpose to test u-opioid receptor agonists and thereby contributing to its regulatory readiness. Exposure to the opioids methadone and buprenorphine resulted in neurodevelopmental toxicity at low concentrations at all differentiation stages tested.

Exercise, lactate signaling, and brain health enhancement -lessons from animal models.

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Exercise is widely recognized for its physical health benefits, but also for its ability to enhance brain function and induce resilience against neurological conditions. Neurogenesis, the process of generating new neurons in the brain, is enhanced by exercise and is vital for learning and memory, as well as the brain's ability to recover from injury or adapt to changes. Neurogenesis is further believed to play a crucial role in maintaining cognitive health throughout the lifespan and resilience against neurodegenerative diseases. Similarly, the formation of new capillaries in the brain is suggested to counteract the reduced cerebral blood flow associated with aging and age-related neurodegenerative disorders. Using animal models and cell cultures, our research delves into the cellular and molecular processes connecting physical activity to brain health, focusing on the lactate receptor HCA_1.

A key question in the field is whether high-intensity or more moderate intensities are more effective at protecting the brain. We investigate how different exercise intensities, particularly high-intensity interval training (HIIT) and medium-intensity interval training (MIIT), influence neurogenesis and angiogenesis in brain regions relevant to brain aging and neurodegeneration. We further explore whether the mechanisms activated by exercise hold promise for prevention and/or protection against cerebral stroke.

Abstract session I: Toxicology

Effect-based monitoring of contaminants in the oceans: does it work?

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Objective: To review our current understanding of the use of health-related responses in marine organisms to monitor effects of environmental contaminants, including chemicals of emerging concern (CECs).

Background: Effect-based monitoring was introduced in the 1980s in some countries and more widely in Europe in the 1990s. There has been significant scientific progress since then, both in terms of method development and not least in our understanding of biological effect methods (biomarker) application and interpretation. Biomarkers are rarely used singly, simply because there is always more than one contaminant in any marine ecosystem and there is a need to monitor different mechanisms of toxicity. This is particularly important with contaminants with uncertain mechanism of toxicity, such as contaminants of emerging concern (CECs).
There has been a focus on biomarkers that indicate potentially population-relevant effects, such as neurotoxicity, cardiotoxicity, endocrine disruption, genotoxicity, and that has a track record of reproducibility. Exploratory strategies using metabolomics, proteomics, transcriptomics and epigenomics are clearly relevant to identify processes affected by contaminants. Their main use until now has however been to identify potential markers for for more in-depth studies. At the moment, there is no standardisation for their use in monitoring.

Results and conclusion: There is general agreement that effects need to be determined in situ, preferably using organisms from natural populations, but there are ongoing discussions on whether we are actually looking for the correct responses, whether we monitor the most sensitive species and life-stages and the ultimate health consequences of observed effects. National and international monitoring programmes over the past decades have documented the usefulness of biomarkers, but also highlighted challenges.

Intercorrelations of short-, medium- and long-chain chlorinated paraffins, dechloranes and legacy POPs in 10 species of marine mammals from Norway, in light of dietary niche

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Objectives: Short-, medium- and long-chain chlorinated paraffins (SCCPs, MCCPs and LCCPs) and dechloranes are chemicals of emerging concern, however, little is known of their bioaccumulative potential compared to legacy contaminants in marine mammals. As part of our Arktis2030 project on new contaminants in Norwegian whales, we investigated the co-occurrence of CPs and dechloranes, with legacy persistent organic pollutants (POPs) and a selection of unregulated brominated flame retardants (BFRs), in 10 marine mammal species of differing diets. Factors potentially explaining concentrations and patterns such as diet, species, sex, age class, and carcass decomposition state were also explored.

Methods: Blubber samples were obtained from 9 species of marine mammals that had stranded along the coast of Norway, and 1 species that was harvested from the Barents Sea. We analyzed SCCPs, MCCPs, LCCPs, 7 dechloranes, 4 emerging brominated flame retardants (BFRs) and 64 legacy contaminants including polychlorinated biphenyls (PCBs) in the blubber of 46 individual marine mammals, representing 10 species, from Norway. Dietary niche was modelled based on stable isotopes of nitrogen and carbon in skin/muscle. CPs and dechloranes were analysed at NILU Kjeller, and other POPs at the Laboratory of Environmental Toxicology at NMBU. Stable isotopes of N and C were analysed at UiO.

Results: SCCPs and dechlorane-602 strongly positively correlated with legacy contaminants and were highest in killer (*Orcinus orca)* and sperm (*Physeter macrocephalus*) whales (median SCCPs: 160 ng/g lw; 230 ng/g lw and median dechlorane-602: 3.8 ng/g lw and 2.0 ng/g lw, respectively). In contrast, MCCPs and LCCPs only weakly correlated to recalcitrant legacy contaminants and were highest in common minke whales (*Balaenoptera acutorostrata*; median MCCPs: 480 ng/g lw and LCCPs: 240 ng/g lw). The total contaminant load in all species was dominated by PCBs and legacy chlorinated pesticides (63%–98%), and MCCPs dominated the total CP load (42%–68%, except only 11% in the long-finned pilot whale *Globicephala melas*).

Conclusion: Surprisingly, there was no relation between contaminant concentrations and dietary niche, suggesting other large species differences such as lifespan and elimination capacities mask effects of diet. CP and dechlorane concentrations were higher than in other marine mammals from the (sub)Arctic, and were present in a killer whale neonate, indicating bioaccumulative properties and a potential for maternal transfer in these predominantly unregulated chemicals.

Multiple stressors in Norwegian killer whales: The combined effects of pollutant load and whale watching on steroid hormone levels

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Objectives: Marine mammals are exposed to multiple stressors. Killer whales (*Orcinus orca*) from Norway have high concentrations of legacy and emerging contaminants and are at risk from toxic effects. Boat traffic from winter whale watching in northern Norway has increased dramatically since 2015, with the activity known to cause physiological and nutritional stress in whales. However, no research has been conducted on how unregulated whale watching in Norway impacts killer whales, or how it may interact with pollutant load. This study aimed to assess differences in cortisol levels between killer whales sampled in seasons of low boat traffic (spring/summer months and winter 2020, with reduced boat traffic due to COVID-19 travel restrictions) and seasons of high boat traffic (winters 2019, 2021 and 2022), in conjunction with other steroid hormones and contaminants.

Methods: Blubber and skin biopsy samples were taken from 87 photo-identified killer whales, of varying age and sex, in both seasons of low and high boat traffic. Killer whale individuals were identified using photographs, and five of the 87 individuals were sampled in both seasons. Hormone and contaminant analysis were conducted at the Norwegian University of Life Sciences (NMBU), and total mercury analysis at the University of Oslo.

Results: We found no effect of season (boat traffic), age class, sex, or mercury levels on cortisol levels across all 87 sampled whales. There was a high variation in cortisol levels within each season, indicating a high level of inter-individual variation. However, season became significant when focusing on the five individuals sampled twice over the course of the study (Wilcoxon signed rank test: $p < 0.05$), with higher cortisol levels in seasons of high boat traffic than low boat traffic for all re-sampled whales. Analysis of contaminant concentrations in blubber are ongoing, and will be included in the models in January 2025.

Conclusion: Our data suggest large inter-individual differences that, without resampled individuals, would have masked the association between higher cortisol levels and seasons of higher boat traffic. Accounting for individual history and variations is thus vital in understanding effects of multiple stressors. Integrating contaminant results into our models will soon give further insights into the combined effect of multiple stressors in killer whales.

Uncoupling effects of per- and polyfluoroalkyl substances on mitochondrial metabolism in killer whale fibroblasts

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Objectives: Killer whales (KW) are highly exposed to contaminants due to their position as apex predators in marine food chains. Among these contaminants, per- and polyfluoroalkyl substances (PFAS) are persistent chemicals known for their strong bioaccumulation and biomagnification. This study investigates the metabolic effects of specific PFAS, i.e. PFOA, GenX, PFOSA, PFOS, and PFECHS, on KW fibroblasts.

Methods: The fibroblasts derived from KW skin biopsy were seeded in a 24-well plate for 24h. Metabolic assays were performed using the Agilent Seahorse Analyzer to investigate the metabolic alteration of these fibroblasts following 24h and 20 min exposure to PFAS.

Results: Four non-cytotoxic PFAS concentrations (3.125 μM, 6.25 μM, 12.5 μM, and 50 μM) were tested in this study. Initial screening showed a significant increase in oxygen consumption rate (OCR) after 24h exposure to 12.5 μM and 50 μM of PFOSA, as well as to 50 μM of PFOA. In terms of extracellular acidification rate, a significant increase was observed with 12.5 μM of PFOA, PFOSA, and PFOS. Subsequent analysis examined the effects of these compounds on mitochondrial parameters. Results revealed that 50 μM of PFOSA and PFOA significantly affected proton leak, ATP production, and non-mitochondrial respiration. Additionally, exposure to 12.5 μM of PFOA led to a significant increase in basal and compensatory glycolysis. Since PFOSA and PFOA are suggested as mitochondrial uncouplers, their effects on OCR were compared. A significant, dose-dependent increase in OCR was observed, with 6.25 μM of PFOSA reaching the same OCR level within 20 min as 0.5 μM of FCCP, a wellknown uncoupler of the electron transport chain. However, no increase in OCR was detected during 20 min of PFOA exposure.

Conclusion: Our metabolic screening underscores PFOSA as the most toxic PFAS, changing the metabolic shift toward energetic and aerobic profiles. This study highlights the strong uncoupling effects of PFOSA, which disrupt mitochondrial membrane potential, increase proton leak, and stimulate oxidative respiration, similar to FCCP. PFOSA's hydrophobicity enhances its ability to penetrate mitochondrial membranes. This contributes to its potent uncoupling effect, as evidenced by the dose-dependent increase in OCR. In contrast, PFOA exhibited weaker uncoupling effects, inhibiting mitochondrial respiration and shifting metabolism towards glycolysis as a compensatory mechanism.
These findings underscore the distinct mechanisms of PFOSA and PFOA as uncouplers in the KW fibroblasts, requiring further studies to explore their effects on mitochondrial metabolic potential and mitochondrial metabolite production to illustrate their uncoupling behaviour in more detail.

This study is part of the Marma-detox project (Project No. 334739) funded by the Research Council of Norway.

Decoding the Spill: Multi-Omics Insights into Crude Oil's Impact on Fish Reproduction

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Understanding the effects of crude oil exposure on critical life stages in fish is essential for safeguarding the reproductive health and sustainability of fish populations. Previous studies suggest that polar cod's spawning period may be a sensitive life event to crude oil exposure by causing an advancement on the spawning and transfer of toxic compounds to the spawned eggs. However, the toxicity mechanism compromising the reproductive success of fishes is not well understood. Through a series of experiments on polar cod (*Boreogadus saida*), we aimed to map biological pathways affected by crude oil and identify key molecular events. Following three weeks exposure of fish under vitellogenesis to weathered crude oil water-soluble fraction (WSF), we examined the altered transcripts (RNA-seq) in the pituitary, gonad and liver tissues, as well as protein (proteomics) and lipid (lipidomics) composition in spawned eggs.

Transcriptomic analysis of liver samples following ten days of exposure, revealed an upregulation of genes related to the cytochrome P450 family, proteasome and catabolic processes involved in contaminant detoxification. In the gonads, genes associated with lipid metabolism were enriched. Spawned eggs showed a reorganization of lipid synthesis and membrane composition. Plasma metabolomics and transcriptome analyses in key tissues sampled close to spawning will further elucidate key events and mechanisms underlying reduced gamete quality. A forthcoming transgenerational zebrafish experiment, using epigenetic markers, will offer crucial insights into the long-term effects of acute oil exposure on future generations. Ultimately, this data will help identify toxicity mechanisms of crude oil WSF on the reproductive success of adult fish and link them to the consequences for fitness and survival across successive generations.

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Fate and effects of per- and polyfluoroalkyl substances (PFAS), their precursors and alternatives in male Salmon hepatocytes

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Objectives: The widespread contamination of per- and polyfluoroalkyl substances (PFAS) in the environment and living organisms has been linked to point sources such as firefighting foam. PFAS are highly persistent, bio accumulative and exhibit toxic effects on marine organisms including fish. In response to growing concerns, the European Chemicals Agency (ECHA) even proposed restrictions on over 10,000 PFAS compounds.
As a result, PFAS are increasingly being replaced by (ultra) short chain PFAS or fluorine free alternatives. However, limited information is available regarding the toxicity of these replacements. Therefore, further research is needed to better understand their biological implications and how they perform compared to the to the previously widely used long-chain PFAS.

Materials and methods: Various representative PFAS were evaluated for their toxicity using primary Atlantic salmon hepatocytes. This included the long-chain PFAS perfluoroundecanoic acid (PFUnDA), the 6:2 fluorotelomer sulfonate (6:2 FTS), the ultra-short-chain trifluoroacetic acid (TFA) alongside the replacement component 4-DoDA, which is used in the fluorine free firefighting foam. In addition, the toxicity of the fluorine free Rehealing (RH) aqueous firefighting foam was also evaluated. Consequently, the effect on metabolic activity along with the membrane fluidity were assessed. Exposed cells were collected for RNA sequencing and cells and cell medium were analysed with target and non-target approaches using high-resolution liquid chromatography-mass spectrometry (LC-HRMS).

Results and conclusion: PFUnDA , TFA, 4 DoDA were found to significantly decrease the metabolic activity at 400µM compared to the control, whereas 6:2FTS was found to increase the membrane fluidity at 50 µM. Initial data from the LC-HRMS indicates that the cell pellet contains both long chain, short chain PFAS and the PFAS precursor after exposure. Additionally, new metabolites were detected in the cell pellet, indicating the cells are capable of metabolizing some PFAS precursors. The results for the fluorine free alternatives and the ultra-short chain PFAS on the LCMS still need to be processed. RNA sequencing results will provide more insights what pathways are affected by the different PFAS compounds.

Abstract session II: Toxicology

Occupational exposure to aerosolized viruses and health effects in Norwegian wastewater treatment plants

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Objectives: In this study, we aim to assess occupational exposure to aerosolized viruses and associated health outcomes in Norwegian wastewater treatment plants. We also aim to evaluate seasonal variations and the use of the Pepper Mild Mottle Virus as an indicator for viruses aerosolized from sewage.

Methods: 31 personal and 62 stationary air samples were collected in three wastewater treatment plants in the inner Oslo fjord area in the period winter 2022 – summer 2023. The inhalable aerosol fraction was collected using Conical inhalable sampler cassettes at 3.5 L air/min (31 personal and 30 stationary) and larger air volumes were collected with Coriolis at 200 L air/min (26 stationary). Human adenovirus, Norovirus GI and GII, Influenza A, and Pepper mild mottle virus were quantified with digital droplet PCR. The participating workers provided information on the time spent in the plant, workstations, and tasks. Health outcomes were evaluated by questionnaire-based self-reported symptoms in 20 exposed workers and 22 unexposed controls.

Results: The exposed workers experienced episodes of chest tightness and wheezing in the chest, dry skin, fever, congested nose, and diarrhea significantly more often than unexposed controls. The human pathogenic viruses were detected in 22% of the samples; the highest observed concentration was Norovirus GII at 762 genome copies per m3 air. Pepper mild mottle virus was detected in 67% of all samples with concentrations ranging from 18 to 9700 genome copies per m3 air. Both the pathogens and pepper mild mottle virus were detected most often at the grids, biological cleansing, sedimentation basins, and sludge treatment/de-watering stations. Tasks such as flushing, cleaning, routine work, and general maintenance were identified as tasks most often associated with the positive detection of viruses.

Conclusion: Our results indicate that workers in Norwegian wastewater treatment plants are at risk of exposure to aerosolized viruses through their work. Although the targeted pathogens were detected in a proportion of the samples, the results indicate that workers may risk exposure to human pathogenic viruses at sufficient levels to cause disease. The presence of Pepper mild mottle virus, which is ubiquitously expressed in human solid waste, in most of the air samples, indicates a significant potential for exposure to aerosolized viruses. The increased prevalence of diarrhea in exposed workers may be caused by exposure to enteric viruses such as adenovirus and norovirus.

MILKYS (contaminants in coastal waters), a wealth of data provided since 1981.

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Objectives: The Norwegian environmental program MILKYS investigates contaminants in samples of blue mussel, cod, dogwhelk, common periwinkle, and common eider on an annual basis. NIVA has performed the monitoring since 1981 on commission from the Norwegian Environment Agency. MILKYS examines the levels, trends, and effects of contaminants along the Norwegian coast, fjords, and Svalbard. The program provides a basis for assessing the state of the environment in Norwegian coastal waters. The monitoring makes an important contribution to national administration and to international organizations such as OSPAR.

Methods: MILKYS presently monitors the concentration of contaminants in blue mussel (*Mytilus edulis*) at 24 stations, Atlantic cod (*Gadus morhua*) at 18 stations, dogwhelk (*Nucella lapillus*) at 7 stations, common periwinkle (*Littorina littorea*) at 2 stations, and common eider (*Somateria mollissima*) at one station. The stations are located both in areas with known or presumed point sources of contaminants, in areas of diffuse loads of contaminants such as city harbour areas, and in more remote regions with presumed low exposure to pollution.

Results: Levels: Mercury, sumPCB7 and sumBDE6 were the contaminants that exceeded environmental quality standards (EQS) as outlined in the Water Framework Directive. The EQS were most frequently exceeded in urban areas and harbours. The highest sum of risk quotients was observed in cod from the Inner Oslofjord followed by the Inner Sørfjord. NIVA has developed a statistical method to assess high background levels of contaminants (PROREF). Exceedances of PROREF were higher in blue mussel than in cod, and the highest exceedances were observed in blue mussel from Inner Oslofjord.
Time trends: Downward time trends dominated both recent (≤ 10 years) and historically where trends could be statistically detected. Most occurrences of upward whole and recent trends were found for blue mussel in the Inner Oslofjord, and for cod at Lista and Sandnessjøen. Upward whole trend for mercury was found in cod fillet from the Inner Oslofjord where several contaminants occur at higher concentrations than other areas along the coast.
Effects: Biological effect parameters (biomarker analysis) showed no effects of TBT in snails but confirmed exposure of PAH and lead in cod in the Inner Oslofjord and the Inner Sørfjord.

Conclusions: A wealth of data has been provided in MILKYS since 1981, some recent examples will be presented.

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N-Acetyl-Cysteine (NAC) mitigates oxidative stress damage to liver endothelium

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Objectives: We investigated the effects of hydrogen peroxide (H_2O_2)-induced oxidative stress on liver sinusoidal endothelial cells (LSEC) to evaluate the protective potential of N-acetyl cysteine (NAC) and glutathione (GSH) against reactive oxygen species (ROS) mediated damage. LSEC are directly exposed to all pharmaceuticals in the bloodstream as well as potentially toxic metabolites, making them a therapeutic target for potential hepatoprotective agents. Given their critical role in liver function and vulnerability to oxidative stress, understanding LSEC responses to ROS is crucial for developing strategies to mitigate drug-induced liver injury and other hepatotoxic effects.

Methods: Isolated rat LSEC were exposed to H_2O_2 (0.5-1000 µM) for 10-120 min. The protective effects of NAC and GSH were evaluated in pre- and co-treatment experiments. Cell viability, reducing potential, scavenging function, intracellular ROS levels, and morphological changes were assessed using lactate dehydrogenase release and resazurin assays, radiolabelled formaldehyde-treated serum albumin uptake, and advanced imaging techniques.

Results: H_2O_2 exposure increased intracellular ROS levels leading to decreased LSEC function, such as cell viability, reducing potential, and scavenging function, in a dose- and time-dependent manner. LSEC facilitate passive filtration in the liver via transcellular pores a.k.a. fenestrations which showed a dynamic response, initially closing but partially reopening when exposed to H_2O_2. Scavenging function was irreversibly reduced after just 10 minutes of exposure, primarily affecting degradation pathways rather than receptor-mediated uptake. Pretreatment with NAC, but not GSH, reduced the negative effects of ROS exposure, suggesting that LSECs do not store excess GSH but can readily produce it under oxidative stress conditions

Conclusion: The observed thresholds in dose and time-dependent changes, as well as the differential effects of NAC and GSH treatments suggest that LSEC do not store excess GSH but can readily produce it under oxidative stress conditions and confirm the existence of a ROS-depleting system in LSEC. These findings and may have implications for developing strategies to protect liver function in various pathological conditions associated with increased ROS production.

Establishing *ex vivo* brain-pituitary and gonadal models for PFAS toxicity assessment

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Objectives: Reproduction is a fundamental biological process regulated by the Brain-Pituitary-Gonadal axis, via the secretion of hormones like gonadotropin-releasing hormone, follicle-stimulating hormone, and luteinizing hormone. Chemicals like per- and polyfluoroalkyl substances (PFAS), can disrupt hormonal balance, leading to reproductive dysfunctions. PFAS, known for their persistence and bioaccumulative nature, are widely used in consumer products and remain prevalent in the environment despite regulatory controls. This study, as part of the EU-funded CHIASMA project[1], aims to establish brain-pituitary and gonadal *ex vivo* models as new approach methodologies (NAMs) to evaluate how PFAS and other relevant compounds may affect the neuroendocrine system and reproductive functions.

Methods: In the brain-pituitary model, the brain-pituitary complex of adult Japanese medaka is cultured for up to seven days. The use of existing transgenic medaka lines with fluorescent markers driven by *lhb* and *fshb* promoters, alongside the existing 3D pituitary atlas and pituitary single-cell data, will facilitate detailed endocrine cell analysis through tissue labeling, imaging techniques, and transcriptomic approaches. This will allow us to assess cell proliferation, transdifferentiation and apoptosis, as well as gene expression changes. In the gonadal model, medaka testes are cultured for up to seven days, with sperm morphology and viability assessed using flow cytometry and computer-assisted sperm analysis (CASA) systems. Additionally, *in vivo* studies will complement *ex vivo* approaches, enabling validations of the models and further Integrated Approaches to Testing and Assessment (IATA) modeling.

Expected results: These models are expected to reveal neuroendocrine toxicity induced by PFAS. The brain-pituitary *ex vivo* model will likely show changes in cell proliferation, transdifferentiation, and apoptosis, providing insights into disruptions in neuroendocrine cell populations. The use of transgenic medaka lines, the 3D pituitary atlas, and the planned transcriptomic analysis will allow precise mapping of affected cells. In the gonadal *ex vivo* model, alterations in sperm morphology and viability are anticipated and identified through flow cytometry and CASA analyses, complementing the brain-pituitary findings. Comparing *in vivo* and *ex vivo* results will validate these methods, establishing a robust framework for evaluating the effects of PFAS on the neuroendocrine system.

Conclusions: Ongoing optimization shows that our *ex vivo* models are promising tools for studying endocrine disruptive potential of toxic chemicals, such as PFAS. As NAMs, the models offer valuable alternatives, contributing to a deeper understanding of chemical-biomolecule interactions while reducing reliance on *in vivo* toxicity studies for chemical evaluation and risk assessment.

[1]CHIASMA - Accessible Innovative Methods for the Safety & Sustainability Assessment of Chemicals & Materials (Grant Agreement (GA) No.: 101137613); Call Topic: HORIZON-CL4-2023-RESILIENCE-01-21.

Interactions between marine oils and persistent organic pollutants in C57BL/6 mice.

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Objectives: Seafood, particularly oily seafood, contains high levels of n-3 unsaturated fatty acids, which are recommended to be included in a balanced, healthy diet. The Norwegian recommendation is to include 200 grams of oily fish in the diet per week. Still, seafood, and particularly oily seafood, is the main source of several persistent organic pollutants (POPs) such as dioxins, polychlorinated biphenyls (PCBs) and brominated flame retardants. Hence, consuming seafood as a part of a balanced and healthy diet also implies exposure to POPs at the same time. Although the detrimental effects of POPs and the health effects of n-3 fatty acids have been studied extensively separately, few studies have investigated their combined effects, and even fewer have examined potential interaction effects between n-3 fatty acids and POPs.

Methods: In this study, we have fed adolescent male C57BL/6 mice diets including marine oils, combined with elevated levels of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), or a mix of the seafood-relevant POPs: TCDD, PCB 138, PCB 153, PBDE 47 and PBDE 99. We used a fractional factorial design to evaluate potential interaction effects of the compounds. The mice were exposed through their diet for 90 days before sacrifice. Following sacrifice, we evaluated a host of relevant endpoint analyses such as: POP levels in liver, body weight and -composition, organ weight, sperm quality, plasma testosterone levels, liver toxicity (through enzyme analyses such as ALAT, ALAS and histological analyses), fatty acid composition in the liver and transcription of relevant markers for POP-specific toxicity in liver and epididymis such as CYP1a induction, redox stress and vitamin A metabolism.

Results: Preliminary results show few interaction effects between n-3 fatty acids and POPs on toxic endpoints, despite findings of toxicity in gross physiology such as reduced body weight and changes in body composition. No POP-induced toxicity effects were observed on sperm quality, testosterone or oxidative stress in epididymis. However, clear signs of liver toxicity were observed both for TCDD alone and to an even greater extent for the mix groups. Liver toxic effects included: increased liver weight, elevated levels of ALAT and ALAS, oxidative stress and changes in histology of the liver.

Conclusion: Despite a POPs exposure sufficient to induce clear toxicological effects, few interaction effects with marine oils were observed. This suggests that interaction effects between n-3 fatty acids and POPs do not occur at the endpoints tested in our study.

The Norwegian football field study: exposure to micro and nanoplastics and acute effects on the immune system.

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To assess the health risks of micro and nanoplastics (MNP) for humans, data is needed on both exposure and the hazards. The POLYRISK project is studying the human exposure to micro and nanoplastics in three real-life scenarios.

In one of the real-life scenarios, NIPH have studied the exposure to MNPs and effects on the immune system in 36 young adults (25 males, 11 females, aged 17-21) that played a football match on an indoor football field with artificial turf with rubber granules as infill. The same players also played a match on an indoor football field with olive sand infill. Exposure to MNPs and effects on the immune system were compared.

Aerosol samples were collected for 1) mass determinations, 2) the level of rubber and for 3) Scanning Electron Microscopy with subsequent particle chemical analysis for polymer identification by Raman-Microscopy. Blood samples were collected before the match, 2h after the match and 18h after the match for 1) immune cell profiling by mass cytometry (CyTOF) and for 2) cytokine profiling by Olink proteomics. Rubber and polymer mass in blood will be determined in blood by pyrolysis GC-MS.

Preliminary data indicate that in both halls particles can be found in the air samples, including rubber. The rubber concentration was higher in the hall with rubber granulates compared to the olive sand hall. Airborne spores were detected in the olive sand hall and is being further investigated.

While immune changes were observed due to the exercise, no evident biologically significant changes in immune cell profiling or cytokine profiling were detected that could be attributed to the higher exposure to rubber MNPs. Data on the levels of MNPs in the blood samples are pending.

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Abstract session I: Pharmacology

Development and clinical validation of a volumetric absorptive capillary microsampling method for quantification of MPA and MPAG in kidney transplant recipients

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Objectives: Mycophenolic acid (MPA) is the antiproliferative immunosuppressant of choice following solid organ transplantation. Obtaining drug concentrations through traditional venous blood sampling at outpatient clinics is time-consuming and often not feasible, both on clinical visit days and in a research setting. Home-based blood sampling is a patient-friendly method, facilitating limited sampling strategies for predicting total drug exposure. We aimed to develop and clinically validate an assay for determining MPA and its metabolite mycophenolic acid glucuronide (MPAG) by utilizing dried volumetric absorptive capillary microsamples (VAMS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Methods: An assay based on VAMS and LC-MS/MS was bioanalytically and clinically validated in accordance with European Medicines Agency guidelines. Agreement between VAMS and plasma samples was investigated in kidney transplant recipients (KTR) on mycophenolate mofetil (MMF) therapy. Paired VAMS and plasma samples were obtained prior to (0hr) and 0.5hr and 2hr post MMF-dosing. The samples were split in a development (75%) and validation (25%) dataset. Conversion from VAMS to plasma concentrations was established using a regression model.

Results: Twelve KTR (median age 49 years, range 21-70, 7 male) provided a total of 69 paired VAMS and plasma samples. The between-series mean accuracy was 90 to 106% with coefficients of variation < 7% in the plasma concentrations range from 0.25 to 32 mg/L (MPA) and 2.5 to 320 mg/L (MPAG). The conversion equation based on the regression model was successfully applied and validated in an independent datatset. The mean relative differences between corrected VAMS samples and plasma samples were 1.9% for MPA and 2.7% for MPAG, with less than 5% outside ±20% for both analytes. The levels of MPA and MPAG in dried VAMS samples were stable for 3 months at ambient temperature.

Conclusion: The VAMS method demonstrated acceptable performance. MPA and MPAG can be reliably quantified using VAMS and is suitable for patient self-sampling in clinical PK studies in KTR.

Patiromer administered three hours post-tacrolimus does not affect tacrolimus pharmacokinetics in kidney transplant recipients

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Objectives: Hyperkalemia is common in kidney transplant (KTx) recipients. Patiromer, a potassium-binding polymer used to treat acute and chronic hyperkalemia, has the potential to bind charged particles in the gastrointestinal tract and thereby potentially affect the absorption of coadministered drugs. The immunosuppressive drug tacrolimus (Tac) has a narrow therapeutic window, is susceptible to drug-drug interactions (DDIs), and a potential gastrointestinal interaction with patiromer could elevate the risk of allograft rejection. We aimed to investigate the potential DDI between patiromer and Tac pharmacokinetics in KTx recipients with hyperkalemia by sampling capillary blood using volumetric absorptive microsampling (VAMS).

Methods: Thirteen KTx recipients on Tac twice daily with plasma potassium levels between 4.6 and 5.6 mmol/L were included. Two 12-hour pharmacokinetic investigations were performed. The first pharmacokinetic investigation was performed after 7 days of 8.4 mg patiromer/day, followed by a 6–7 day washout period before the second pharmacokinetic investigation. Oral Tac dose remained unchanged at the two pharmacokinetic investigations, and patiromer was administered 3 hours after the Tac dose. Tac sampling was self-conducted using VAMS after mastering the technique. Potential DDI was evaluated by calculating the geometric mean ratio and 90% confidence interval (CI) and compared with specified ranges outlined in the European Medicines Agency guidelines for bioequivalence.

Results: Ten patients provided 2 evaluable pharmacokinetic profiles. The Tac area under the curve $(AUC)_{0–12}$ ratio ($AUC_{Tac+patiromer}/AUC_{Tac}$) was 0.99 (90% CI, 0.86-1.14), and the C_{max} ratio was 1.01 (90% CI, 0.86-1.19). Tac C_0 and C_{12} fulfilled the bioequivalence criteria with a ratio of 0.98 (90% CI, 0.90-1.07) and 0.93 (90% CI, 0.83-1.04), respectively.

Conclusion: When patiromer is administered 3 hours after the Tac morning dose, it has no clinically relevant impact on Tac pharmacokinetics. We demonstrate that VAMS is a well-suited sampling method to simplify the execution of DDI studies.

Studying the adverse effects of polypharmacy on liver sinusoidal endothelial cells (LSEC) in mouse liver-derived *in vitro* models: advancing from 2D to 3D systems

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Objectives: Polypharmacy, the simultaneous use of ≥4 medications, is linked to increased risks of drug-induced liver injury and drug-drug interactions (Burato S. *et al.*, 2020). Liver sinusoidal endothelial cells (LSEC) play a key role in drug clearance and the maintenance of hepatic microarchitecture. The aim of our study is to investigate the adverse effects of polypharmacy on LSEC, advancing from traditional 2D monolayer cultures to physiologically relevant 3D spheroid systems.

Methods: Multicellular liver spheroids derived from primary mouse hepatocytes and liver non-parenchymal cells have been shown to be a good model for fatty liver disease (van Os E.A. et al., 2022). In our study, we use spheroids formed by hepatocytes and LSEC in the polypharmacy context. Both monolayer of LSEC and spheroids were treated with a combination of therapeutic drugs, such as citalopram, oxybutynin, metoprolol and oxycodone. The steady state concentrations of these drugs were based on the *in vivo* experiments done in mice by Mach J. et.al., 2021. The viability of the cells was assessed by using resazurin assay, while the endocytic activity of LSEC was tested via a radioactive scavenging assay by using ^{125}I-FSA. To further study the morphology of LSEC, scanning electron microscopy (SEM) was used.

Results: Treatment with clinically relevant concentrations of the single drugs and a high DBI drug cocktail had no significant impact on cell viability and scavenging activity of LSEC in 2D cultures. The results from spheroids, show an effect on the viability and endocytosis upon treatment with oxybutynin drug, while in presence of all the other drugs this compound seem to not affect the overall behaviour of the spheroids. Morphological analysis via SEM of the LSEC monolayers revealed a notable loss of fenestrations, a key feature associated with LSEC function, following treatment with all the drugs except for metoprolol. This aspect needs to be explored more in the spheroids, where the fenestrations were visible in the untreated samples.

Conclusion: Our findings highlight the relevance of the 3D multicellular liver spheroids in capturing the complexity of polypharmacy's impact on LSECs and suggest them as a good *in vitro* model for assessing polypharmacy-induced liver toxicity. Integrating insights from both 2D and 3D systems provides a deeper understanding of the critical role of LSECs in liver health under polypharmacy conditions. Future studies will explore multiple drug concentrations to better understand the impact of initial doses pre-first-pass metabolism on LSEC function and morphology.

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Abstract session II: Pharmacology

Paracrine roles of the protease legumain in osteogenic differentiation

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Objective: Expression of the cysteine protease legumain has previously been shown to inversely correlate with osteoblast maturation and matrix mineralization both *in vitro* and *in vivo*, indicating potential as a putative therapeutic target in metabolic bone disease, such as osteoporosis. Legumain is secreted by cells in the bone microenvironment and circulates in plasma, suggesting a possible paracrine role. Thus, legumain overexpression could influence neighboring cells by increased processing of extracellular matrix (ECM) proteins such as fibronectin, a known promotor of osteogenic differentiation. The aim of this project was to explore potential paracrine influence of legumain in regulation of osteogenic differentiation.

Methods: Osteogenic cells were differentiated from an immortalized human bone marrow-derived mesenchymal stem cell line. Osteogenic commitment was analyzed by rt-qPCR. Matrix mineralization was analyzed by BoneTag™ (LI-COR, UK) and Alizarin Red staining. Protein concentration in conditioned media was measured by enzyme-linked immunosorbent assay. Protein expression and qualitative assessment of proteolytic products were analyzed by immunoblotting. Legumain activity was measured by calculating kinetics from the fluorescence increase during the time-dependent cleavage of the peptide substrate Z-Ala-Ala-Asn-AMC in cell lysates, with results normalized to total protein concentration.

Results: Extracellular prolegumain internalization and activation was shown during *in vitro* osteoblast differentiation accompanied by reduced matrix mineralization, indicating legumain-mediated paracrine regulation of osteoblast maturation. Activation of both endogenous and internalized prolegumain was induced by transforming growth factor β1 (TGF-β1), resulting in an additive inhibitory effect on matrix mineralization, which was rescued by pharmacological inhibition of legumain. This suggests that inhibition of matrix mineralization was mediated through the proteolytic activity of legumain. Overexpression of legumain during osteoblast differentiation led to an increase in truncated fibronectin products and sequestration of prolegumain within the ECM. Differentiation of osteoblasts on ECM produced by legumain-overexpressing osteogenic cultures reduced cellular legumain expression and activity, and increased matrix mineralization. These findings substantiate the inverse correlation between legumain activity and matrix mineralization and indicate an additional legumain-mediated paracrine effect in osteoblast differentiation through ECM modifications.

Conclusions: The results introduce legumain as a putative paracrine regulator of osteoblast maturation, acting both directly through prolegumain secretion, internalization and activation, and indirectly via ECM modifications. This work shows that TGF-β1 is a regulator of legumain and that TGF-β1-mediated activation of legumain inhibit mineralization. Collectively, these findings underscore the potential of targeting legumain in osteoporosis treatment.

The effect of antipsychotic remedies on liver sinusoidal endothelial cell function and morphology.

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Objectives: The superior antipsychotic, clozapine, is associated with rapid weight-gain and metabolic disturbances. The mechanisms underlying these serious side-effects are unclear, calling for experimental studies to reveal the pathophysiology. Liver sinusoidal endothelial cells (LSEC) line the liver sinusoids and perform a dual function in physiology – they are perforated with patent nanopores (fenestrations of 50-350 nm diameter) that facilitate the bi-directional passage of biomolecules such as lipoproteins between plasma and hepatocytes, and they are the body's most potent cellular scavengers of soluble waste and colloids < 200nm. Impaired LSEC function is increasingly recognized as a significant factor in the pathophysiology of metabolic syndrome. We set out to determine the effects of clozapine at its main metabolite norclozapine on LSEC scavenging function and porosity.

Methods: Freshly isolated (3h post-op) and cultured LSEC from mice were challenged with the antipsychotic medication clozapine, and its metabolite norclozapine, for 60-120 minutes at clinical and supra-therapeutic concentrations. The effects on LSEC morphology and scavenging properties were assessed with electron microscopy and 125I-formaldehyde treated BSA (FSA) uptake assays.

Results: The challenge of LSEC with clozapine and norclozapine resulted in reduced porosity (loss of fenestrations) and was dose dependent. Regarding scavenging, clozapine had no effect on FSA uptake, while norclozapine caused reduction in LSEC mediated scavenging, albeit at concentrations higher than would be seen in patients.

Conclusion: The results from *in vitro* studies suggest that the superior antipsychotic clozapine can have negative effects on LSEC function. If these findings translate to *in vivo* settings, this would have profound implications for the pathophysiology of patients using these medications and could lead to new treatment strategies.

cGMP in HFpEF: only unsuccessful trials or a new target?

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Introduction: Heart failure is a significant global health burden, which can be coarsely categorized into heart failure with preserved ejection fraction (HFpEF) and heart failure with reduced ejection fraction (HFrEF). HFpEF is a complex disease where traditional drugs, such as β-blockers, ACE-inhibitors and ARBs have failed in clinical trials. To date, only SGLT2 inhibitors have been proven effective and there is a need for developing new drug classes. The second messenger cGMP was early proposed as a target for HFpEF, but PDE5 inhibitors and soluble guanylyl cyclase stimulators and activators have failed in clinical trials. Our group has previously shown that C-type natriuretic peptide (CNP) stimulation of the guanylyl cyclase receptor B (GC-B) increased cGMP in cardiomyocytes, leading to faster relaxation and phosphorylation of titin, resulting in decreased passive stiffness of cardiomyocytes.

Aim: Examine whether long-term administration of CNP improves cardiac function in a mouse model of HFpEF.

Methods: HFpEF in C57BL/6N mice was induced by a combination of a high-fat diet and the nitric oxide synthase inhibitor L-NAME in the drinking water. Miniosmotic pumps delivering CNP, or vehicle (NaCl) were implanted in mice for 6 weeks. Tail-cuff plethysmography, transthoracic echocardiography, pressure-volume loop acquisition, and morphometrical analysis were used to investigate cardiac function in this study.

Results: After 6 weeks of CNP administration to HFpEF mice, echocardiography and invasive hemodynamic analysis showed that cardiac diastolic function parameters, such as e', E/e,' left atrial diameter, end-diastolic pressure, and end-diastolic pressure-volume relationship were all markedly ameliorated compared to vehicle-treated HFpEF mice. HFpEF mice displayed progressed cardiomyocyte hypertrophy accompanied by increased left ventricular wall thickness and myocardial fibrosis, while those changes were significantly suppressed in CNP-treated HFpEF mice.

Conclusion: The results demonstrate that CNP treatment improves cardiac dysfunction and reduces myocardial hypertrophy and fibrosis in HFpEF mice, suggesting its prospective effectiveness for treating HFpEF.

Poster session: Toxicology

Aryl Hydrocarbon Receptor Differentially Regulated Gene Expression Profiles in Resting and Polarized Bone Marrow-Derived Macrophages

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Background: The aryl hydrocarbon receptor (AHR) is best known for regulating the toxic effects of many environmental contaminants. More recent studies show that AHR is an important regulator of metabolism and immune responses and is highly expressed in macrophages. Resting macrophages (M0) can be polarized into pro-inflammatory M1 or anti-inflammatory M2 phenotypes in response to environmental stimuli. AHR preferentially regulates M2 macrophages, but how loss or inhibition of AHR affects polarization and gene expression profiles in macrophages is poorly understood.

Objectives: Determine the role of AHR in bone marrow-derived macrophage (BMDM) polarization and identify AHR-dependent genes that are differentially expressed in M0, M1, and M2 macrophages.

Methods: BMDMs isolated from wildtype (WT) and *Ahr*$^{-/-}$ mice were matured into macrophages using L929 containing medium for 7 days. On day 7, BMDMs were polarized into M1 and M2 phenotypes. During the polarization, cells were treated with dimethyl sulfoxide (DMSO) and 1µM BAY2416964, an AHR antagonist, for 18 h. RNA sequencing was used to identify AHR-dependent and differentially expressed genes (DEGs) in M0, M1 and M2 macrophages. Western blotting and real-time qPCR analyses confirmed AHR and DEG levels identified from the RNA sequencing data.

Results: Western blot and RT-qPCR revealed that AHR expression was highest in M2 macrophages. No AHR was detected in *Ahr*$^{-/-}$ BMDMs. Compared with M0, 81% of the 4755 WT and 5436 *Ahr*$^{-/-}$ DEGs in M1 were common to both genotypes. Similarly, 71% of the 1998 WT and 1230 *Ahr*$^{-/-}$ DEGs in M2 were shared between genotypes. When normalized to WT, there were 257 DEGs in M0, 521 in M1, and 847 in M2 in *Ahr*$^{-/-}$ macrophages. Pathway analysis revealed decreased interferon signaling and cytokine activation in M0 and M2 but increased cytokine activation in M1 in *Ahr*$^{-/-}$ compared with WT BMDMs. The expression levels of serine peptidase inhibitor, Kunitz type 1 (Spint1), G protein-coupled receptor 141 (GPR141), and AHR repressor (AHRR) were strongly downregulated in *Ahr*$^{-/-}$ BMDMs and after BAY2416964 treatment and independent of polarization. We identified several AHR response elements in the regulatory region of *Spint1*. Studies characterizing the AHR-dependent regulation of *Spint1* are currently ongoing.

Conclusion: These data highlight AHR's role in gene regulation, function, and polarization of macrophages and identify AHR as an essential regulator of Spint1 expression. The results of this study further highlight the importance of AHR in macrophages and as a key regulator of inflammation and immune cell function.

Changes in the plasma metabolome following single or combined exposure to benzo[a]pyrene and *N*-nitroso compounds in A/J Min/+ mice

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Introduction and objectives: The consumption of processed meat has been linked to an increased risk of colorectal cancer. Several components are proposed as causes to this carcinogenic effect, including the presence of polycyclic aromatic hydrocarbons (PAHs) and *N*-nitroso compounds (NOCs) (Bouvard et al. 2015). Cancer cells have dysregulated metabolism supporting the demand for uncontrolled proliferation. Thus, studying alterations in the metabolome (metabolomics) in response to carcinogen exposure can provide a better understanding of the mechanisms behind cancer (Patty et al. 2012, Gold et al. 2022). The present study aimed to investigate the dose-dependent effect on the plasma metabolite composition following exposure to NOCs and benzo(a)pyrene (BaP; a well-known PAH) in A/J Min/+ mice, a human relevant model for colorectal cancer.

Methods: A/J Min/+ mice were divided into 5 groups (N=20 per group, both genders): Control, NOCs Low, BaP Low, NOCs+BaP Low and NOCs+BaP High. All groups were fed an experimental gel diet (nocturnal exposure) 3 days/week from 4 to 13 weeks of age. The Low doses were calculated from human estimated daily intake levels (95^{th}-$97{,}5^{th}$ percentiles x10 000), and the High doses were x100 to the Low concentrations. Plasma was extracted for targeted metabolomics analysis using the AbsoluteIDQ® p400 HR kit and high-resolution mass spectrometry. Data var analyzed by ANOVA multivariate orthogonal partial least squares analysis.

Results: The targeted metabolomics kit quantified up to 365 lipids and 43 small molecules. The BaP Low and the NOCs+BaP High diets significantly changed the plasma metabolic profiles compared to Controls. Mice exposed to the NOCs+BaP high diet had alterations in cellular membranes, lipid and energy metabolism. On the other hand, exposure to the NOC+BaP Low diet did not affect the plasma metabolome.

Conclusion: The combination of high dose BaP and NOCs affected several metabolic pathways in A/J Min/+ mice. The study highlights the complex host-diet interplay when exposed to a combination of multiple dietary carcinogens. Further studies should include analysis of enzyme induction or activity to facilitate the interpretation of mechanistic effects.

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Assessing the effects of chemicals used in plastic on the immune system

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Objective: Plastic use continues to increase as time passes, with production nearly doubling in the last 20 years, reaching 435 million tonnes in 2020 and a prospective increase of 70% by 2040[1]. At the same time, more research is starting to show the negative impact of plastics and chemicals leaching from plastics on the environment and human health. This study delves into the effects of five commonly used chemical additives—PPD, 6PPD, IPPD, UV-328, and UV-622—on the immune system, using human primary peripheral blood mononuclear cells (PBMCs) as a model. These chemicals, found in everyday items like car tyres, coatings, cosmetic appliances, and food and medical packaging, are a cause for concern. We also investigate the impact of rubber granules made from car tyres and the chemical leachate of these granules.

Methods: The potential immunomodulatory effects of these chemicals and rubber granules will be assessed by measuring cytotoxicity, cytokine release (using Luminex assay), and the expression of various surface markers (using flow cytometry). Five chemicals will be tested on cells collected from six individuals of varying ages and sexes, while the rubber granules and their leachates will be tested on cells from four donors.

Results: Our initial trial run with the five chemicals revealed a trend of decreased cell viability at low concentrations for PPD, IPPD, and 6PPD. Meanwhile, the results for UV-328 and UV-622 did not point toward a clear trend at the concentrations tested. However, these findings are preliminary and require validation with samples from multiple donors.
We also observed a negative effect on the viability of the PBMCs when exposed to rubber granules and their chemical leachate at various concentrations. The cell supernatants collected for the LUMINEX analysis are yet to be evaluated.

Conclusion: Our current results suggest that the chemical additives used in plastic appear to negatively affect PBMCs, which might correlate to adverse health effects in humans when exposed to these chemicals. Furthermore, it appears some of these chemicals are capable of leaching out of the plastic, as shown in the rubber granule leachate experiment, increasing the risk of exposure.

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Processed foods and colorectal cancer: Effect of protein source, processing and dietary patterns (CRC-3p)

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Colorectal cancer (CRC) is the third most commonly diagnosed cancer and the second leading cause of cancer death, responsible for more than 1.9 million new cases and almost 904 000 deaths in 2022 worldwide (Bray *et al.* 2024). Recent studies report a global rise in CRC incidence rates among younger adults (before age 50 years) in several high-income countries (Sung *et al.* 2024). Norway is among the countries with the highest CRC incidence, especially in women. Norwegian women have a particularly high intake of processed meat compared to other European women, and in two Norwegian prospective studies frequent consumption of sausages was the only dietary factor associated with increased risk of CRC (Parr *et al*. 2013). In 2015, The International Agency for Research on Cancer (IARC) categorized processed meat as carcinogenic to humans (IARC 2015). The associations are mainly drawn from epidemiological studies in populations that have a Western diet characterized by high intake of energy-dense and processed foods. Furthermore, the cause-and-effect relationships and the key carcinogenic drivers are yet to be documented. At present, an increasing number of plant-based meat alternatives have become more common on the market. These products undergo a high degree of processing. The long-term health effects of these products have not yet been evaluated.

Objectives: The CRC-3p project aims to generate new knowledge on the link between processed food and CRC, by tracing the carcinogenic potential of red meat and plant-based meat alternatives to reveal the significance of the protein source and the degree of processing (unprocessed, processed, ultra-processed) on CRC development. Additionally, CRC-3p seeks to elucidate how dietary patterns may influence the carcinogenic potential of the different processed foods.

Methods: Animal -and plant protein diets with varying processing degrees and combinations with high-fat, high-sugar components will be tested in the A/J mouse model for colorectal carcinogenesis. The significance of protein source, processing, and dietary pattern on CRC will be examined using host-diet-microbiota interactions, such as intestinal lesion scoring, microbiota composition, inflammatory biomarkers, immunological parameters, plasma metabolomics and intestinal tissue omics. In addition, data from established Norwegian prospective epidemiological studies and from the National CRC screening study will be collected and analysed to determine CRC risk of intake of processed food.

Results and conclusion: The project plan and preliminary results will be presented at the symposium.

The CRC-3p-project is funded by FFL/JA, and the consortium is a close collaboration between NMBU, Nofima, NVI, UiO, and the Norwegian meat industry (Animalia, Nortura, Grilstad and kjøtt og fjørfebransjens landsforbund (KLF).
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Veterinary surgical smoke: exposure from calf-disbudding and hot-shoeing of horses

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Objective: Surgical smoke is created when the use of medical tools raise intracellular temperatures in tissues to above 100 °C. Surgical smoke is known to be composed of 95 % water, and 5 % of compounds such as volatile organic compounds (VOCs), polycyclic aromatic hydrocarbons (PAHs), and aromatics such as benzene, toluene and xylene, as well as particles and bioaerosols. Isocyanic acid (ICA) has been detected in smoke from cauterisation of nitrogen-containing tissues and disbudding of calves. Smoke of surgical type is generated during disbudding of calves performed by veterinarians, and hot-shoeing of horses by farriers. Veterinarians and farriers may be working under conditions with poor ventilation, and without the use of personal protective equipment. As such, they are potentially exposed to various classes of hazardous compounds, present in the smoke, generated during the heating process. In the present study, the objectives were to characterize the main constituents of the smoke generated during pyrolysis of animal tissues, and to assess the exposure of technicians during disbudding and hot-shoeing processes. The study design was conducted in three stages, where the generation of surgical smoke in a smoke chamber was used as the basis for the selection of chemicals to be measured in a pilot study, giving further indications of core chemicals to be measured in a field study of exposure.

Methods: Surgical smoke was simulated in the laboratory using a smog-chamber by pyrolysis at 550 °C of calf hide and deer feet fragments, and the chemical composition of the smoke was determined. Air samples were collected from the smog chamber using off-line active air sampling techniques, followed by analysis using LC-MS. For the field-studies, personal air sampling was performed during disbudding or hot-shoeing procedures. Respirable dust, VOCs and ICA, among other chemicals, were then quantitatively determined from the samplers and exposure was evaluated.

Results: ICA was detected in the surgical smoke generated both during disbudding and horseshoeing. For disbudding, the levels generated were variable, and occasionally above 20% of the 8 hours occupational exposure limit (OEL). In one instance it was found to be twice the OEL. For horseshoeing the levels were generally higher, up to six times the OEL, for the shoeing of one horse. In addition, respirable dust was detected in the samples, and although it was below the OEL, in one of the dehorning samples the concentration exceeded 10 % of the OEL, indicating a need for preventive measures. Several VOC, some of which are known irritants, such a phenol and p-cresol were also detected in the smoke at variable concentrations.

Conclusion: The respiratory irritant ICA, as well as respirable dust and irritant VOCs were detected in the surgical smoke created during disbudding and horseshoeing processes. Using respiratory protective equipment, as well as sufficient ventilation should be considered when performing these procedures.

Effects of Beauvericin and Enniatin B on the heme biosynthesis pathway in salmon and human cell lines

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Objectives: Enniatin B (ENNB) and Beauvericin (BEA) are emerging, non-regulated mycotoxins commonly found in human food and animal feed, including salmon feed. These secondary metabolites, produced by moulds that are found in agricultural crops, are introduced into Atlantic salmon diet through the use of plant-base feed ingredients. Previous in vitro and in vivo studies with Atlantic salmon have identified that these ionophoric mycotoxins can induce anaemia, impair bone formation and growth, cause liver damage and disrupt heme biosynthesis at concentrations found in commercial feeds. The main objective of this project is to investigate the mechanisms of action (MOA) underlying the effects on anaemia and heme biosynthesis in Atlantic salmon using primary Atlantic salmon hepatocytes and assess whether similar impacts occur in humane liver cells (HepG2) and a liver epithelium cell line from Rainbow Trout (SOB15).

Method: A comparative cytotoxicity analysis was done of BEA and ENNB in HepG2, SOB15 and primary hepatocytes from Salmon. The cells were exposed to different mycotoxin concentrations and cell viability was measured using impedance measurements (Real-time cell analysis (RTCA)) or mitochondrial activity (MTT assay). Mycotoxin exposed cell lines and primary cells were further used to analyse effect on the metabolite protoporphyrin IX in the heme biosynthesis pathway through auto-fluorescence with a fluorescence microscope and plate reader. Further, an iron-heme assay was utilised to assess if ferrochelatase binds ferrous iron (Fe^{2+}) to protoporphyrin IX to form heme.

Results: The comparative cell viability assessment determined a dose response for all cells, however toxicity was observed at different concentrations and time points during exposure. The primary hepatocytes exhibited auto-fluorescence, suggesting that these cells contained protoporphyrin IX, however no dose response was observed. HepG2 and SOB15 did not show auto-florescence suggesting no protoporphyrin IX. SOB15 did not have free iron and there were low indications of heme bound iron in the mitochondria, slightly higher levels were measured in the cytosol.

Conclusion: There was observed a dose response to both BEA and ENNB in all the cell models which, indicates that the mycotoxins have a dose dependent effect, and that all cells used were sensitive. The mycotoxins did not affect the levels of protoporphyrin IX in exposed salmon hepatocytes. The lack of auto fluorescence in HepG2 and SOB15, as well as the lack of heme iron and free iron in SOB15 may suggest that SOB15 and HepG2 are not good cell models for analysing the heme biosynthesis pathway.

The ANALYST project: Strengthening the integrated approach of holistic impact assessments for Safe and Sustainable by design plastic value chain

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Building on the early principle of Safe by Design (SbD), the concept of Safe and Sustainable by Design (SSbD) has evolved over the years, shaped by various European projects and activities into the comprehensive framework we know nowadays. In response to growing global environmental concerns, SSbD expanded the SbD principles to incorporate sustainability, creating a more holistic approach. This evolution culminated in the European Commission's 2023 formal recommendation on SSbD, which provides structured guidelines and methodologies to support its implementation.

In this context, the Horizon Europe project ANALYST, focuses on advancing the transition toward safer and more sustainable industrial value chains, particularly within the plastic sector. It contributes to the advancing of the SSbD framework by developing robust methodologies and tools for holistic impact assessments covering environmental, health, social, and economic factors. Key innovations include the ANALYST-BOX, a methodology for PVC impact assessment; ANALYST-DIGI, a digital platform for decision-making support; and ANALYST-T-PACK, a validation program with real-world use cases in the automotive and construction sectors. The project aims to support informed investment, align with EU policies like the Green Deal, and foster a paradigm shift towards cleaner and more sustainable industrial practices.

Intelligent testing strategies and a tiered approach to the SSbD framework are essential for generating new data and addressing the complexity of chemical and material innovation. Non-animal-based new approach methodologies (NAMs), including *in vitro* and *in silico* methods, are pivotal for innovative, exposure-led, and hypothesis-driven hazard and risk assessments. However, clear guidelines on which methodologies and tools to use, whether alone or in combination, during different stages of the SSbD assessment are still lacking.
As part of the ANALYST project, the state of the art in hazard and risk assessment methodologies relevant to SSbD was scoped through a review of published and grey literature, as well as unpublished research presented at conferences and workshops. This foundational work is driving the creation of the ANALYST-toolbox, a collection of methodologies and guidelines designed to integrate SSbD practices into industrial workflows. The toolbox will be tested across three practical cases within the PVC and plastic value chain, aiming to operationalize the SSbD framework effectively.
By facilitating this integrated approach, the ANALYST project seeks to empower the industrial sector to transition towards inherently safe and sustainable chemicals, materials, and products, supporting a broader paradigm shift.

CHIASMA - Accessible Innovative Methods for the Safety & Sustainability Assessment of Chemicals & Materials

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Objectives: The shift to ethical and sustainable toxicology is one of the goals set by EU Commission for the next generation hazard and risk assessment of chemicals and materials. However, the shift is hindered by the reliance on animal testing, lack of validated alternatives, standardized methods, stakeholder coordination, and accessible data. To address this, a large collaborative project namely CHIASMA - Accessible Innovative Methods for the Safety & Sustainability Assessment of Chemicals & Materials[1] – has been funded by the European Union's Horizon Europe Research and Innovation program and will run during 2024-2027. The project includes more than 20 partners from around the globe and aims at developing and implementing a comprehensive set of New Approach Methodologies (NAMs) for a user-friendly, reliable, and robust evaluation of human and environmental safety within regulatory context.

Methods: NAMs will be based on both *in silico* and experimental methods, including *in vitro* methods, human *ex vivo* methods and non-mammal *in vivo/ex-vivo* methods. The *in silico* and experimental NAMs will be 'in-project' validated against three groups of chemicals and materials: (i) Polyfluoroalkyl Substances (PFAS), (ii) (nano)-pesticides, and (iii) 2D materials for energy applications, which were chosen based on their high health impact, environmental persistence, and large industrial use. At the Norwegian University of Life Sciences (NMBU), we will use an *ex vivo* Brain-Pituitary-Gonadal axis system based on model fish (Zebrafish and Medaka) to develop NAMs within CHIASMA framework (ref. abstract and presentation by Sina Velzi et al.).

Anticipated Results: By combining chemometric and biometric models, optimized experimental NAMs and data integration, the CHIASMA project will create Safe and Sustainable by Design (SSbD) Assessment (for safety- and environmental-assessment), which will enable risk assessors, commercial enterprises and regulators to address relevant endpoints using human-centric and 3R-compliant approaches.

[1] CHIASMA - Accessible Innovative Methods for the Safety & Sustainability Assessment of Chemicals & Materials (Grant Agreement (GA) No.: 101137613); Call Topic: HORIZON-CL4-2023-RESILIENCE-01-21.

Development of PFAS-free coatings in a safe and sustainable by design (SSbD) approach- the PROPLANET project

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Poly- and perfluoroalkyl substances (PFAS) have been widely used for their exceptional water- and oil-repellence properties, high thermal stability, and durability. However, their environmental persistence and potential adverse health effects have led to stringent regulatory restrictions, and initiatives to phase out PFAS compounds. Development of novel PFAS-free coating materials that address environmental and human safety challenges, while enabling chemical innovations and circular value chains, is the aim of the Horizon Europe project PROPLANET. The project focuses on developing innovative PFAS-free coatings for the industrial textile, glass and food-packaging machines sectors by applying the principles of Safety and Sustainability by design (SSbD).

The SSbD approach integrates safety considerations throughout the development process of and the life cycle of the PFAS-free coating materials. This is promoting the shift towards safer and more sustainable chemicals, fostering innovation and competitiveness in the chemical industry, while aligning with the EU´s Chemicals Strategy for Sustainability. A stage-gate approach for safety evaluation along the development process ensures safer products, facilitates regulatory preparedness, and optimizes cost and time efficiency. Existing data on the toxicological potential of the individual coating components have been collected from the early stages of the innovation process, and data gaps identified. These gaps have been reviewed and gap-filling experiments conducted using a tiered approach aligned with the material´s development and life-stages.

The testing strategy addresses key toxicological endpoints in line with regulatory requirements and incorporates Adverse Outcome Pathways (AOPs) linking key events to the potential toxicity of the developing coatings. Gap filling is performed using new approach methodologies (NAMs), including both *in vitro* and *in silico* methods. Quantitative Structure-Activity Relationship (QSAR) models can contribute to identifying safer PFAS-free formulations. The formulations developed are tested using *in vitro* methods for human hazard assessment. A tiered approach is applied, where the traditional cellular models (or 2D models) and screening assays are used during the earlier development phases of the project, while advanced (3D) models will be used to evaluate the final products. The hazard assessment is done on several formulations, and spans a broad range of toxicity endpoint, including cytotoxicity (AlamarBlue assay), genotoxicity (comet assay), inflammation (pro-inflammatory markers by ELISA), carcinogenicity (cell transformation assay) and reproductive toxicity (*c.elegans*).

In conclusion, the coatings are evaluated based on their technical performance while ensuring the safety and sustainability throughout their life cycle. This work also supports the international effort to advance the regulatory acceptance of NAMs for next generation risk assessment (NGRA) and further the development of the SSbD framework.

The contribution of chemical constituents and the particle core to the toxicity of diesel exhaust particles

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Objectives: Exposure to air pollution particulate matter (PM) is one of the leading environmental risk factors for morbidity and mortality. Particles less than 2.5 µm in aerodynamic diameter (PM2.5), which often derive from combustion and transport processes, is the main driver of the disease burden. Particle toxicity is affected by physical characteristics, such as size, shape and surface area, as well as chemical characteristics such as soluble compounds and surface reactivity. One of the key objectives of the ULTRHAS (Ultrafine particles from transportation – Health assessment of sources) project is clarifying whether physical or chemical characteristics are the main drivers of effects of particles from transport mode emissions. The present study assesses whether the adverse effects of diesel exhaust particles (DEP) can be attributed to adsorbed chemical constituents or to the carbon particle core.

Methods: A 3D cell culture model of the human airways, consisting of Calu-3 bronchial epithelial cells, THP-1-dervied macrophages and Ea.hy926 endothelial cells, was exposed to corresponding concentrations of DEP, extracts of DEP (DEPe), and the residual washed particles (DEPr). RNA was harvested separately from the apical and basolateral side of the cell culture, representing the airway and circulatory compartments, and RNA sequencing (RNAseq) was performed to identify changes in gene expression and biological processes.

Results: The results show that the adverse effects of DEP were largely driven by the soluble chemical constituents, evident in the large overlap in the responses induced by DEP and DEPe. DEP and DEPe induced 207 and 387 differentially expressed genes (DEG), respectively, of which 159 were shared between the treatments. Conversely, only a single gene was differentially expressed after exposure to DEPr. Similarly, 17 and 19 DEG were induced by DEP and DEPe in the basolateral compartment, of which 7 overlapped, while no significant effects were detected after exposure to DEPr. Notably, CYP1A1 and CYP1B1 was among the most differentially expressed genes for all treatments, suggesting a potential role for polycyclic aromatic hydrocarbons and the aryl hydrocarbon receptor pathway in the observed effects.

Conclusions:

This study highlights the role of chemical constituents, rather than the particle core, in the toxicity of DEP.

Using precision-cut liver slices for assessing hepatic toxicity of components of crude oil on Atlantic cod (*Gadus morhua*)

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Increased oil activity in the Arctic is of special concern to the arctic marine ecosystems, particularly to ecologically and commercially important species such as the Atlantic cod (*Gadus morhua*). Crude oil and its water accommodate fractions (WAF) are complex mixtures, with toxicity often associated with polycyclic aromatic hydrocarbons (PAHs) and the aryl hydrocarbon receptor (AhR) pathway. This study aimed to investigate the toxicity pathways of specific chemical fractions of crude oil WAF using cod precision-cut liver slices (PCLS) as an *ex vivo* exposure method. The liver is the main site for detoxification of crude oil related contaminants mainly through the cytochrome P450 (Cyp) family enzymes, where the metabolism of crude oil contaminants can be monitored through the Cyp1a/Ahr pathway activity. The liver also plays an important role in reproduction through the production of vitellogenin (Vtg), as well as in lipid metabolism where it is the main site for lipid storage in cod.
In this study, five different WAF fractions were assessed (saturates, monoaromatic hydrocarbons, naphthalenes, polycyclic aromatic hydrocarbons, and resins) in addition to the full WAF extract. Liver slices were prepared from three males and three females, acclimatized in culture medium and exposed for 48 hours to different dilutions of each fraction/extract. The mRNA levels of genes related to the xenobiotic metabolism and reproduction (i.e. *cyp1a* and *vtg*, respectively) was quantified through reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR).

Preliminary results showed significant increase in *cyp1a* expression in liver slices exposed to the total WAF extract and resin fraction by 20 fold and 5 fold, respectively, which may suggest the presence of AhR agonists. Chemical analyses demonstrated that the resin fraction was composed of 250 different compounds where the most abundant compounds were 7-methyl-1-indanone, anthrone, and dimethyl-tetrahydroanthracene. No significant changes in *cyp1a* mRNA levels were found in the other fractions. The lack of significant effects in the PAH fraction may be attributed to its low concentration relative to the other fractions, and the predominance of phenanthrene, a weak AhR agonist. Future work will include the analysis of genes related to the lipid metabolism and oxidative stress, to identify key toxicity pathways activated by individual WAF fractions and the whole WAF mixture. This study is highly important to elucidate the mixture toxicity of crude oil WAF and its main drivers.

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Modulation of peroxisome proliferator-activated receptor alpha (Ppara) activity in zebrafish (*Danio rerio*) by long- and short-chained perfluoroalkyl substances (PFAS)

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Objectives: Per- and polyfluoroalkylated substances (PFAS) are man-made fluorinated compounds that due to their water and grease repellent properties are widely used in industrial applications and consumer products. Their chemical characteristic make PFAS bioaccumulate in organisms and biomagnify in food chains. Exposure to PFASs have been associated with developmental and reproductive toxicity, liver toxicity, and metabolic dysfunction in both mammals and fish. Their ubiquitous presence in nature and biota have raised environmental concerns globally. Long-chained PFAS, such as perfluorooctanesulfonic acid (PFOS) and perfluorooctanoic acid (PFOA), have therefore been included in the Stockholm Convention on Persistent Organic Pollutants. Consequently, restrictions and limitations on their use have prompted the search for potential replacement compounds, such as short-chained precursors of PFOS and PFOA. Previous studies on PFAS compounds have demonstrated a correlation between exposure and activation of the key regulator of lipid homeostasis, the peroxisome proliferator-activated receptor alpha (Ppara). The main objective in this thesis is to investigate how legacy and emerging PFAS can modulate the activity zebrafish Ppara subtypes, including zfPparaa and zfPparab.
Methods: The ligand-binding domain of zfPparaa and zfPparab were amplified from zebrafish larvae using PCR and further cloned into a eukaryotic expression vector, fused to the DNAbinding domain of the yeast GAL4 protein. Interestingly, a novel splice variant was found for the zfPparaa subtype, which was included in downstream assays. The ligand-binding properties of the three zebrafish Ppara subtypes towards different PFAS were assessed *in vitro* by performing UAS/GAL4-DBD based luciferase reporter gene assays in a COS- 7 cell line. In addition to investigating agonistic effects, also co-exposures with the PPARA agonist WY14643 and PFAS were performed with the luciferase reporter gene assay to unveil potential antagonistic or potentiating effects.
Results: Seven PFAS pollutants have been tested, representing a structurally diverse group of both long-chained, short-chained, and branched PFAS. Ligand activation profiles suggest an activation for both receptor subtypes when exposed to PFOA, with a maximum of 6- and 15-fold activation of zfPparaa and zfPparab, respectively. Also, perfluoro-2-methyl-3-oxahexanoic acid ammonium (GenX), a branched PFAS, significantly activated zfPparaa (17-fold). Interestingly, PFOS and perfluoro-3-methoxypropanoic acid (PrMoPra) did not seem to result in activation alone, although co-exposure to WY14643 increased activation for PFOS and PrMoPra for Pparaa. It is noteworthy that the splice variant of zfPparaa did not demonstrate any significant activation for the PFAS ligands or the WY14643, suggesting that the xx amino acid truncation in exon is located in the region that is essential for protein dimerization.
Thus, exposure of both long-chained and short-chained PFAS pollutants to zebrafish could potentially modulate the lipid metabolism through directly interfering with the different Ppara subtypes, making the current replacement compounds not necessarily a safer alternative.

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Development of estrogen receptor TR-FRET assays for assessment of estrogenic effects of bisphenol compounds

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Objectives: A notable group of chemicals used in plastic production are bisphenols, which are extensively used in the production of carboxy polymers and exposy resins. Bisphenol A (BPA) is recognized as an endocrine disruptor due to its xenoestrogenic activity. As public concern over the use of BPA has grown, products labelled "BPA-free" have become more common. However, the industry often substitutes BPA with other bisphenols, such as bisphenol F, bisphenol S, etc., which possess similar structures and chemical properties and may also exhibit endocrine disruptive effects on humans and other organisms.
This study aims to establish estrogen receptor time-resolved fluorescence resonance energy transfer (TR-FRET) assays to evaluate the estrogenic effects of various bisphenol compounds. In comparison to traditional cell-based reporter gene assays, TR-FRET is a cell-free alternative that is less labour-intensive and less time consuming, and a promising new approach methodology (NAM) for toxicity assessment with nuclear receptors. The estrogen receptor alpha from human (hERa), zebrafish (zfEra), and cod (codEra) have been selected as models for developing this assay

Methods: To clone zfEra-LBD (ligand binding domain), RNA was isolated from zebrafish liver using The RNeasy® Plus Mini Kit. Extracted zebrafish RNA was reverse transcribed into complementary DNA (cDNA) using the Invitrogen superscript IV kit**.** zfEra-LBD were consequently cloned into the pSC-B vector using The StrataClone Blunt PCR cloning kit (Agilent). hERa-LBD and codEra-LBD were cloned previously. The zfEra-LBD, codEra-LBD and hERa-LBD were subcloned into a bacterial expression vector for expression in *Escherichia coli* BL21. The recombinantly expressed proteins were purified using an ÄKTA chromatography system, using a His-Trap column (IMAC) and a size exclusive chromatography (SEC) column. The purified Era/ERa-LBD proteins were subjected to a Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET) assay to evaluate their interaction with target molecules.

Results: The initial TR-FRET assays, with the human, zebrafish and cod Era/ERa-LBD and using 17α-ethynylestradiol (EE2) as the agonist, have shown promise as there is a clear response in the FRET signal upon ligand activation. Results from additional optimization and exposure to other ligands like 17ß-estradiol and bisphenol A analogs will be presented and discussed.

This work is part of the XENOSENSE project (project no. 342186) is funded by the Research Council of Norway.

Impact of an organochlorine mixture on development of type 2 diabetes in an Arctic population: A multipollutant analysis from The Tromsø Study

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Objectives: When evaluating environmental pollutants and their health effects, traditional "one-at-a-time" analyses often fail to account for the high correlations and possible interactions between the compounds, thereby not capturing the complexity of pollutant mixtures as exposures. Moreover, such approaches increase the probability of Type I errors, due to multiple testing. To address these limitations, we used a multipollutant approach to investigate the effect of an organochlorine mixture on the development of type 2 diabetes (T2D) in an Arctic population using repeated pre-diagnostic measurements from The Tromsø Study.

Methods: We conducted a nested case-control study within The Tromsø Study, including 260 participants who underwent three repeated pre-diagnostic assessments over a 15-year period. Among these participants, 116 developed T2D after the last measurement, while 144 participants remained free of diabetes. To investigate the association between the organochlorine mixture and future T2D diagnosis, as well as the contribution of each compound into the mixture, we used Weighted Quantile Sum Regression (WQS) at each timepoint. The results were compared to those obtained from traditional univariate analyses (Charles et al. 2021).

Results: The results from the WQS partially confirms the positive associations seen at the last two timepoints for certain compounds in the univariate analyses (Charles et al. 2021). However, the odds ratios are elevated, which underscores the impact of the entire mixture on future development of T2D, rather than individual compounds. Additionally, the WQS provide clearer insights into which compounds that really contributes to the association with T2D, and which compounds that appear in univariate analyses due to high correlations.

Conclusion: This study highlights the importance of using more advanced statistical methods to assess the health effects of environmental pollutants, allowing us to identify the risk of disease associated with exposure to the entire mixture, as well as identifying the "bad actors" in the mixture.

Reference: Charles D, Berg V, Nøst TH, Bergdahl IA, Huber S, Ayotte P, Wilsgaard T, Averina M, Sandanger T, Rylander C. Environ Res. 2022 Mar;204(Pt B):112129. doi: 10.1016/j.envres.2021.112129. Epub 2021 Sep 28. PMID: 34597662.

Colonization by meiofauna of sediments contaminated with the fluroquinolone antibiotic ciprofloxacin

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Objectives: Investigate the potential effects of the fluroquinolone antibiotic ciprofloxacin on marine sediment biodiversity. Explore potential difference in responses to the contaminant from a sewage impacted meiofauna assemblage and a reference assemblage.

Methods: Examination of meiofauna communities following a 25-day colonization experiment. Microcosms were set up using source meiofauna assemblages collected in the outer Oslofjord and colonization trays of defaunated sediments, which were either ciprofloxacin contaminated or uncontaminated. Ciprofloxacin (0.05 g/L) was dissolved in seawater and added to freeze-dried homogenized sediments of colonization trays. Controls had seawater only. Source meiofauna materials originated from sediment close the outfall from Fuglevik sewage treatment plant or 1000 meters distant at a reference location at similar depth.

Meiofauna assemblages were extracted from sediments of the microcosms using a floatation method and major meiofaunal taxa were quantified on a Dollfuss plate under a stereoscopic microscope. Permutational multivariate analysis of variance (PERMANOVA) was used to analyse differences in community structure of taxa between sediments from treatments and field origins. Multidimensional scale analysis (MDS) was used to test for dissimilarity in community structure.

Results: No difference in community structure, or single taxa between sediments of the ciprofloxacin-contaminated and control colonization trays was shown with PERMANOVA. Field origin affected community structure/ single taxa of meiofauna in the sediments of the colonization trays. The MDS showed that the communities of the source meiofauna assemblages differed between the two field origins.

Conclusion: There were no significant differences in colonization of ciprofloxacin-contaminated and control sediments by meiofauna. There was low abundance of colonizers in all colonization trays. A lack of added food in the sediments of the colonization trays may have influenced low meiofauna abundances.

Modulating gene expression in foraminifera exposed to contaminants

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Objectives: Establish a protocol for isolation and quantification of mRNA from the foraminifera *Globobulimina turgida*
Measure a response in the foraminifera *Globobulimina turgida* gene expression due to different environmental stressors

Methods: Petri dishes with pooled foraminifera were exposed to ciprofloxacin (50 µg/L), copper (Cu) (10 µg/L), or left untreated as control for 24h. After the exposure experiment, RNA was isolated and then sequenced by High Throughput Sequencing (HTS). The sequenced reads were then assembled into transcriptome. A differential expression (DE) analysis was then conducted to identify genes expressed under the different exposure conditions. The function of the genes was then predicted to assess their potential as biomarkers for marine pollution.

Results and conclusion: This study presents a protocol that gives mRNA of sufficient quality for a de novo transcriptome assembly of *Globobulimina turgida*. However, only a few genes showed DE in the two exposure conditions. Foraminifera exposed to ciprofloxacin showed expression of genes predicted to be involved in the cell cycle, while those exposed to Cu showed expression of genes related to an oxidative stress response. Both examples illustrate that it is feasible to use transcriptomics to identify DE of genes due to different environmental stressors. However, more research and data are needed on the molecular biology of foraminifera to conclude that the presented genes in this study could be used as reliable biomarkers for marine pollution.

Advancing mixture risk assessment in aquatic ecosystems: the CHARM project's integration of NAMs and probabilistic modelling

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The project CHARM - *Computational characterization of ecological hazard and risk of environmental mixtures* focuses on two key priorities in advancing toward a new-generation environmental risk assessment (ERA): i) transitioning to assessing mixtures, and ii) utilizing mechanism-based hazard assessment obtained via New Approach Methodologies (NAMs).
The project is supported by the European Food Safety Authority (EFSA) under the European Food Risk Assessment Fellowship (EU FORA) Programme. Through a case-study application, it explores an enhanced component-based risk assessment (CBMRA) framework, integrating high-throughput-screening (HTS) bioactivity data combined with a quantitative adverse outcome pathway (qAOP) approach for hazard assessment.
The project is based on quantitative AOP #263, which models growth inhibition through the uncoupling of mitochondrial oxidative phosphorylation by chemicals, using model aquatic organisms. As a case-study, it uses pesticides concentration data from the European Environmental Agency's Pesticide Indicator dataset (Waterbase and WISE statistics) in freshwater environments. Bioactivity profiles from ToxCast and Tox21 are mapped to the OECD endorsed AOP #263 to derive equipotent mixture concentrations compared to the model chemical, providing input data to the qAOP model. Statistical modelling is used to estimate uncertainty across various risk components, which is then integrated and propagated throughout the whole risk characterisation process using Bayesian network modelling. The resulting probabilistic risk characterisation is compared against predictions from other lines of evidence.
This poster presents the background and foundation of the CHARM project, which started in September 2024. It illustrates the project's alignment with EFSA's guidance on cumulative risk assessment, and highlights conceptual, technical and modelling innovations.

Respiratory and Genotoxic Effects of Imidacloprid Exposure within the Upper Optimal Thermal Range of Springtails (Collembola)

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Objectives: Ambient temperature shapes pesticide toxicity, reflected in increased pesticide sensitivity in springtails (Collembola), even within their optimal thermal range. The neonicotinoid imidacloprid toxicity is increased by the increasing temperature, resulting in sublethal exposure turning lethal, and in impaired life history trait responses that affect population growth. We have previously shown that imidacloprid toxicity is reflected differently in springtails depending on their thermal adaptation, with temperate populations being more sensitive than Arctic. We here assess if the impaired life history traits (e.g. adult growth, egg production) in the sensitive temperate springtails within the optimal thermal range is reflected in underlying respiratory and/or genotoxic responses.

Methods: We used two methodologies that are novel for springtails - continuous oxygen measurements to examine metabolic changes (respiration), and the Fast Micromethod to assess double-stranded DNA damage.
Adult *Hypogastrura viatica* were dietary exposed to sublethal imidacloprid concentrations (0, 0.04, 0.16, 0.64 mg/kg) at 15°C and 20°C for 7 days, followed by 24h continuous oxygen measurements, and DNA damage measurement. The imidacloprid concentration range was confirmed sublethal at both temperatures, with no mortality at 15°C, and a 99% survival at 20°C.

Results: As expected for ectotherms, the respiration rate increased with elevated temperature, but at a lower rate when exposed to the highest imidacloprid concentration. Exposure to lower imidacloprid concentrations increased respiration compared to control, both at 15°C and 20°C. DNA damage also increased with elevated temperature, but there was no effect of imidacloprid at any temperature.

Conclusion: Thus, the imidacloprid related changes in oxygen consumption was probably not linked to genotoxicity, apart from potential DNA repair mechanisms requiring energy resources affecting the respiration. The interactive effects of temperature and acute imidacloprid exposure on oxygen consumption may partly explain the impaired life history responses observed in earlier long-term observations. Our findings contribute to the growing knowledge of the effects of multiple stressors on soil fauna, and importance of acknowledging thermal adaptation when assessing how temperature shapes toxicity at different levels of biological organization.

Per- and polyfluoroalkyl substances (PFAS) and metals in 10 species of marine mammals from Norway

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Objectives: Per- and polyfluoroalkyl substances (PFAS) are highly persistent, and have been linked to estrogenic, reproductive and endocrine effects in wildlife. Both PFAS and toxic metals like mercury (Hg) have increased in concentrations in wildlife due to human activity. Furthermore, PFAS and Hg can bioaccumulate in food chains, reaching high levels in marine mammals. The aim of this study was to calculate the concentrations of PFAS and metals in marine mammals from Norway, and explore the factors potentially explaining concentrations and patterns, such as diet, species, sex, age class, and carcass decomposition state.

Methods: Skin, muscle and liver samples were obtained from 9 species of marine mammals that had stranded along the coast of Norway, and 1 species that was harvested from the Barents Sea. We analysed 14 PFAS in 29 muscle samples and 13 liver samples (29 unique individuals) at the Laboratory of Environmental Toxicology at NMBU, and 17 metals in 29 muscle samples, 13 liver samples and 29 skin samples (29 unique individuals) at the Faculty of Environmental Sciences and Natural Resource Management (MINA) at NMBU.

Results: We found highest PFAS levels in a long-finned pilot whale (*Globicephala melas*; ∑PFAS 80 ng/g ww in muscle and 180 ng/g ww in liver), followed by sperm whale (*Physeter macrocephalus;* muscle: 56 ng/g ww and liver: 150 ng/g ww) and killer whales (*Orcinus orca;* muscle: 10 ng/g ww and liver: 63 ng/g ww). For most species, perfluorooctane sulfonic acid (PFOS) was the dominating PFAS congener, with perfluorooctanesulfonamide (PFOSA) also high in killer whales, harbour seals (*Phoca vitulina*) and sperm whales. Hg levels were highest in pilot, sperm and killer whales (liver levels 17 ng/g dw, 3 ng/g dw and 2.5 ng/g dw, respectively). We found large differences between the species, but no effect of diet, sex or decomposition state. We found indications of maternal transfer in common minke whales (*Balaenoptera acutorostrata*).

Conclusion: Pilot, sperm and killer whales had higher levels of PFAS and metals than the other species, and levels were high enough to indicate a risk of adverse health effects. The presence of these contaminants in remote Arctic areas further indicate the high persistence and mobility of these substances.

Estimating the biomagnifying potential of mercury and chlorinated paraffins in a Norwegian Arctic food web and in killer whales (*Orcinus orca)*

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Objectives: Mercury (Hg) and chlorinated paraffins (CPs) are environmental contaminants found even in remote regions. They can bioaccumulate in marine food webs, potentially reaching harmful concentrations in apex predators. Little is known, however, about the occurrence and bioaccumulation potential of these contaminants in Arctic Norway, where the killer whale (*Orcinus orca)* is a top predator. The aim of this study was to investigate inter- and intraspecific variability in contamination levels, and assess the biomagnifying potential, of Hg and CPs in the Arctic Norwegian food web.

Methods: Tissue samples were taken from 107 photo-identified killer whales, of varying age, sex, and dietary preference. In addition, the following species were sampled from the Andfjord and Skjervøy regions: Atlantic cod (*Gadus morhua,* n=5*),* Atlantic halibut (*Hippoglossus hippoglossus*, n=5) Atlantic herring (*Clupea harengus*, n=40) Atlantic mackerel (*Scomber scombrus*, n=3) Blue mussel (*Mytilus edulis*, n=3), Capelin (*Mallotus villosus*, n=5) Haddock (*Melanogrammus aeglefinus*, n=5), Harbor porpoise (*Phocoena phocoena*, n=8), Harbor seal (*Phoca vitulina*, n=4), Lumpfish (*Cyclopterus lumpus*, n=5), Megrim (*Lepidorhombus whiffiagonis* n=3), Monkfish (*Lophius piscatorius,* n=4), Saithe (*Pollachius virens*, n=4) and Witch flounder (*Glyptocephalus cynoglossus*, n=3). Stable isotope analysis was conducted to give an indication of the trophic position of species in the food web, and to calculate trophic magnification factors (TMFs) for the investigated contaminants (total Hg and short-, medium- and long-chain CPs), based on tissue concentrations.

Results: Total Hg analysis showed that killer whale intraspecific differences in contaminant concentrations are influenced by sex, age and diet and that total Hg biomagnifies in this Arctic marine food web (TMF = 4). Analysis of short, medium, and long-chained CPs revealed significant variability in contamination levels within and between species. None of the CP-classes biomagnified in the studied food web, however, short and medium-chain CPs biomagnified when only poikilotherm species were considered (TMFs of 2.7 and 3 respectively).

Conclusion: This study is the first to calculate TMFs for THg and CPs in a Norwegian Arctic food web whilst also assessing interindividual variability in contamination levels for killer whales.

Temporal trends of dietary patterns and mercury in norwegian killer whales (*Orcinus orca*) from contrasting ecosystem states

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Background: Killer whales are long-lived, occupy a high trophic position, and are valuable bioindicators to assess marine ecosystem health and exposure to persistent contaminants. Norwegian killer whales were formerly categorized as fish specialists strongly associated with Norwegian Spring Spawning Herring (*Clupea harengus*) (NSSH), but are now known to incorporate other prey, including marine mammals, into their diet. The NSSH stock collapsed in 1970 due to overfishing but have since recovered.

Objectives: This study aimed to evaluate historic and contemporary dietary variations possibly correlated to changes in preferred prey availability, and to assess changes in Mercury (Hg) concentrations between and within individuals over time by (1) investigating annual patterns of inter and intraindividual dietary trends and Hg accumulation over both time periods, (2) evaluating whether shifts in preferred prey resources caused by fishing pressures played a role in killer whales integrating more marine mammals in their diet, (3) determining if feeding on higher trophic prey resulted in a greater body burden of Hg, and (4) to identify temporal trends of Hg intake in response to changes in emissions.

Methods: Bulk stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), and Hg concentrations were analysed from annual growth layers of killer whale teeth (n=21) from two groups living in contrasting ecosystem states. Teeth were sectioned longitudinally with a diamond saw and two slices (circa. 1 mm width) were cut from each half. The left slice was ground into a powder mixing all GLGs, and the right piece was decalcified in a 0.25 M HCl solution. Each growth layer was extracted and minced using a scalpel. Samples were prepared for $\delta^{13}C$ and $\delta^{15}N$ analysis performed by the Institute for Energy Technology (Kjeller, Norway). Hg analysis is scheduled for December 2024 at the University of Oslo.

Results: Preliminary results show the contemporary group exhibits higher $\delta^{15}N$ values and a greater range than the historical set. This could indicate a higher variability in dietary patterns and a larger consumption of marine mammal prey. $\delta^{15}N$ values for powdered tooth samples representing all combined GLGs were greater for the contemporary set ($\delta^{15}N$: Max=15.59 ‰, Min=11.82 ‰) than the historical samples ($\delta^{15}N$: Max=13.14 ‰, $\delta^{15}N$ Min=11.40 ‰).

Conclusion: This project offers six decades of insight on how Hg has been accumulating in wildlife pre- and post-emission reductions brought forth by the Minamata Convention.

MILKYS (contaminants in coastal waters), a wealth of data provided since 1981.

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Objectives: The Norwegian environmental program MILKYS investigates contaminants in samples of blue mussel, cod, dogwhelk, common periwinkle, and common eider on an annual basis. NIVA has performed the monitoring since 1981 on commission from the Norwegian Environment Agency. MILKYS examines the levels, trends, and effects of contaminants along the Norwegian coast, fjords, and Svalbard. The program provides a basis for assessing the state of the environment in Norwegian coastal waters. The monitoring makes an important contribution to national administration and to international organizations such as OSPAR.

Methods: MILKYS presently monitors the concentration of contaminants in blue mussel (*Mytilus edulis*) at 24 stations, Atlantic cod (*Gadus morhua*) at 18 stations, dogwhelk (*Nucella lapillus*) at 7 stations, common periwinkle (*Littorina littorea*) at 2 stations, and common eider (*Somateria mollissima*) at one station. The stations are located both in areas with known or presumed point sources of contaminants, in areas of diffuse loads of contaminants such as city harbour areas, and in more remote regions with presumed low exposure to pollution.

Results: Levels: Mercury, sumPCB7 and sumBDE6 were the contaminants that exceeded environmental quality standards (EQS) as outlined in the Water Framework Directive. The EQS were most frequently exceeded in urban areas and harbours. The highest sum of risk quotients was observed in cod from the Inner Oslofjord followed by the Inner Sørfjord. NIVA has developed a statistical method to assess high background levels of contaminants (PROREF). Exceedances of PROREF were higher in blue mussel than in cod, and the highest exceedances were observed in blue mussel from Inner Oslofjord.
Time trends: Downward time trends dominated both recent (≤ 10 years) and historically where trends could be statistically detected. Most occurrences of upward whole and recent trends were found for blue mussel in the Inner Oslofjord, and for cod at Lista and Sandnessjøen. Upward whole trend for mercury was found in cod fillet from the Inner Oslofjord where several contaminants occur at higher concentrations than other areas along the coast.
Effects: Biological effect parameters (biomarker analysis) showed no effects of TBT in snails but confirmed exposure of PAH and lead in cod in the Inner Oslofjord and the Inner Sørfjord.

Conclusions: A wealth of data has been provided in MILKYS since 1981, some recent examples will be presented.

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Poster session: Pharmacology

Development of a method to determine microbiome-derived metabolism of tacrolimus in fecal lysates from kidney transplant recipients

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Introduction: Following organ transplantation, life-long immunosuppressive therapy is necessary to reduce the risk of organ rejection, with tacrolimus being the cornerstone of the immunosuppressive regimen. Dosing of tacrolimus is challenging due to large inter- and intra-individual variability in pharmacokinetic properties. The gut microbiome may be a significant contributor to one of the factors explaining the variability in tacrolimus pharmacokinetics. Studies have shown that administration of oral tacrolimus alters the gut microbiome composition. Vice versa, the gut microbiota has been found to affect tacrolimus pharmacokinetics both through direct and indirect mechanisms. The aims of this study are to develop a LC-MS/MS method to quantify tacrolimus in fecal lysates and to develop a method to determine the microbiome-derived metabolism of tacrolimus.

Methods: Initially, different matrices for the calibration curve were tested: methanol (MeOH), Ringer´s solution (an isotonic solution of electrolytes), extraction buffer, and 50:50 combination of the three solutions. The calibration curve ranged from 1.5 ng/ml to 1500 ng/ml. To each sample, 50 µL of precipitation solution with internal standard (ACN/MeOH (90:10) with 20 ng/ml ascomysin) and 30 µL of ZnSO4 was added. The samples were vortexed for one minute and left at room temperature for five minutes before being placed in the freezer (-20| |C) for one hour. After centrifugation at 4000 RPM, 4ºC for 10 minutes, 50 µL of the supernatant was transferred to a 96 well plate and mixed with 50 µL of mobile phase A (10 mM ammonium formate) before injection into the UPLC-MS/MS system. The final method was validated according to the European Medicine Agency (EMA) for bioanalytical method validation.

Results and conclusion: MeOH was chosen as the best matrix for the calibrators and quality controls based on signal-to-noise ratio, peak shape, and analyte stability. Within-series and between-series performance were assessed with resulting coefficient of variation < 8.5% and <17.6%, respectively, and the mean accuracy ranged between 96% to 110 %. The validated method will be used to investigate the microbiome-derived metabolism of tacrolimus in fecal samples from kidney transplant recipients.

Effects of antimicrobial drugs on microbiome-derived reactivation of mycophenolic acid

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Objectives: The gut microbiome may be a significant contributor to variations in the pharmacokinetics of mycophenolic acid (MPA). MPA is metabolized in the liver by uridine diphosphate glucuronosyltransferase enzymes through glucuronidation, producing an inactive form of MPA, known as mycophenolate glucuronide (MPAG). MPAG is subsequently excreted into the gastrointestinal (GI) tract via the biliary ducts. Within the GI tract, bacterial β-glucuronidase (GUS) enzymes converts MPAG back into MPA, which then can be reabsorbed. This enterohepatic recycling process result in a secondary plasma peak occurring 6–12 hours after oral administration and contributes to the overall systemic MPA exposure. Antimicrobial drugs are commonly used following kidney transplantation, and treatment with these drugs is associated with shifts in the gut microbiome. The objective of this project is to investigate whether various antimicrobial drugs influence the microbiome-derived reactivation of MPA in fecal samples.

Method: Fecal samples were obtained from a previously conducted clinical trial in healthy volunteers (IntraCYP). Fecal samples from two single donors were inoculated in anaerobic Gifu media, with six different antimicrobial drugs, ampicillin, erythromycin, vancomycin, ciprofloxacin, trimethoprim, and sulfamethoxazole each individually added to separate samples, followed by a 24-hour incubation. Concentrated lysates were prepared from the fecal cultures to isolate proteins through bead beating, sonication, and filtration. Total protein concentration in the lysates was determined using a Bradford assay and diluted to a concentration of 0.2 mg/ml. To assess the reactivation rate of MPA in these samples, MPAG was added to the lysates, followed by incubation in a water bath at 37°C. Samples were collected at specific time points: 0 minutes, 15 minutes, 30 minutes, 1 hour, 1.5 hours, and 2 hours. Concentrations of MPA and MPAG were quantified by using UHPLC-MS/MS. The reactivation rate of MPA was determined using a linear regression function of cumulative MPA concentrations over time.

Results and conclusions: Lysates have been prepared from fecal cultures that were created by inoculating fecal samples containing six antimicrobial drugs in varying concentrations. These samples were obtained from two donors. These initial experiments have aimed to determine appropriate antimicrobial drug concentrations that mimic the intestinal levels. The reactivation rates of MPA in the lysates will be determined and compared. Preliminary results will be presented at the meeting.

Bioavailability impairment of immunosuppressive drugs with concurrent administration of calcium supplements in kidney transplant recipients – an interaction study

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Objectives: Kidney transplant recipients (KTR) have an increased risk of bone diseases. A preexisting hyperparathyroidism together with the mandatory immunosuppressive regimen contribute to increased bone fragility. Calcium supplementation is an easy and inexpensive treatment to limit bone loss in KTRs. Calcium supplementation has been shown to interact with the immunosuppressive drug mycophenolate mofetil (CellCeptÒ). A time-shifted administration of 2-3 hours is therefore recommended, but that could negatively impact adherence in KTRs. Tacrolimus (Prograf®) may theoretically also be subjected to the same interaction with calcium. The aim of the study is to investigate the possible interaction between calcium supplementation and immunosuppressive drugs (CellCeptÒ and PrografÒ) to determine if a time-shifted administration is necessary in KTRs.

Method: A total of 26 kidney transplant recipients in an immunosuppressive drug steady-state with a drug regimen of CellCeptÒ, PrografÒ and prednisolone will be included. Pharmacokinetic 12-hour investigations will be performed before and after a one week twice daily regimen with Calcigran ForteÒ 500 mg/400 IU. Additionally, the patients will undergo a visit with intravenous administration of their immunosuppressive drugs to determine absolute bioavailability. Blood samples will be obtained by volumetric absorptive microsampling (VAMS). The primary endpoint is calculation of AUC0-12 and Cmax of tacrolimus and mycophenolic acid with:without (calcium supplementation) ratio. The ratio limits are calculated as according to EMAs bioequivalence guidelines.

Results and conclusion: So far 2 patients have finished the course of the study. The results from the first patients will be presented at the meeting.

The impact of CYP3A5 genotype on clozapine serum concentration in patients with treatment-resistant schizophrenia

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Objectives: Despite clozapine being the antipsychotic with the superior clinical efficacy against schizophrenia, its use is not indicated until the disease is categorized as treatment resistant schizophrenia (TRS) due to risk of severe side effects. Most side effects of clozapine are dependent on serum concentration, which may be determined by genetic variability in metabolism. CYP3A5 genotype has been linked to individual variation in clozapine metabolism. The aim of this study was therefore to investigate the impact of CYP3A5 genotype on clozapine serum concentration.

Method: This study was based on data from the therapeutic drug monitoring (TDM) service at Center for Psychopharmacology, Diakonhjemmet Hospital. Patient using clozapine, who had been genotyped, were included retrospectively during the period between January 2005 and November 2024. The serum sample were included if both clozapine and the metabolite desmethylclozapine were detected in blood. Criteria for inclusion comprised available dose, smoking status, and sample time in between 10-30 hours after last dose intake. The patient was excluded if use of an interacting antipsychotic drug was reported at any time. Information about age and gender were available. For included patients CYP3A5 was genotyped by TaqMan probe based real-time PCR analysis.

Results and conclusion: In total, 602 patients were included in the study. These patients amounted to 9754 blood samples included in the dataset. Data on the impact of CYP3A5 genotypes on serum concentrations will be presented. Adjustments will also be made for smoking habits.

Bioavailability impairment of immunosuppressive drugs with concurrent administration of calcium supplements in kidney transplant recipients – an interaction study

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Objectives: Kidney transplant recipients (KTR) have an increased risk of bone diseases. A preexisting hyperparathyroidism together with the mandatory immunosuppressive regimen contribute to increased bone fragility. Calcium supplementation is an easy and inexpensive treatment to limit bone loss in KTRs. Calcium supplementation has been shown to interact with the immunosuppressive drug mycophenolate mofetil (CellCept®). A time-shifted administration of 2-3 hours is therefore recommended, but that could negatively impact adherence in KTRs. Tacrolimus (Prograf®) may theoretically also be subjected to the same interaction with calcium. The aim of the study is to investigate the possible interaction between calcium supplementation and immunosuppressive drugs (CellCept® and Prograf®) to determine if a time-shifted administration is necessary in KTRs.

Method: A total of 26 kidney transplant recipients in an immunosuppressive drug steady-state with a drug regimen of CellCept®, Prograf® and prednisolone will be included. Pharmacokinetic 12-hour investigations will be performed before and after a one week twice daily regimen with Calcigran Forte® 500 mg/400 IU. Additionally, the patients will undergo a visit with intravenous administration of their immunosuppressive drugs to determine absolute bioavailability. Blood samples will be obtained by volumetric absorptive microsampling (VAMS). The primary endpoint is calculation of $AUC_{0\text{-}12}$ and C_{max} of tacrolimus and mycophenolic acid with:without (calcium supplementation) ratio. The ratio limits are calculated as according to EMAs bioequivalence guidelines.

Results and conclusion: So far 2 patients have finished the course of the study. The results from the first patients will be presented at the meeting.

Population pharmacokinetic modelling of intrathecal iodixanol for the estimation of glymphatic clearance

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Objectives: Currently, there is no definitive model describing the clearance of substances from the central nervous system (CNS), which hinders the development of individualized dosing strategies for CNS disorders. The glymphatic system, a waste clearance pathway in the brain, is thought to play a critical role in the progression of neurodegenerative diseases. It removes waste products like β-amyloid and tau proteins via the cerebrospinal fluid. Accumulation of these proteins is seen in patients with Alzheimer's and Parkinson disease. Measuring an individual's glymphatic clearance may thus serve as a possible diagnostic tool for patients at risk at developing these conditions. This study aims to develop a predictive model for glymphatic clearance and compare and contrast to an existing gadobutrol-based clearance model.

Method: 810 mg of the contrast agent Iohexol is administered intrathecally as a bolus into the cerebrospinal fluid (CSF) and measured in blood samples over a 50-hour period. The model was split into a development- and validation-set in a 3:1 ratio. The elimination coefficient and half-life are then compared to the gadobutrol-based clearance model. All calculations are done in PMetrics, an R-library, using non-parametric adaptive grid (NPAG), Bayesian statistics, and maximum likelihood functions to generate the models.

Results and conclusions: Forty-six patients, with a total of 497 measurements, were included in the study. Preliminary results indicate that the pharmacokinetics can be well described by the two-compartmental model, with a root-mean-square-¨prediction error (RMSE) of 6.27, a mean predicted error (MPE) of -1.565 and an absolute mean prediction error (AMPE) of 2.747.

Studying the adverse effects of polypharmacy on liver sinusoidal endothelial cells (LSEC) in mouse liver-derived *in vitro* models: advancing from 2D to 3D systems

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Objectives: Polypharmacy, the simultaneous use of ≥4 medications, is linked to increased risks of drug-induced liver injury and drug-drug interactions (Burato S. *et al.*, 2020). Liver sinusoidal endothelial cells (LSEC) play a key role in drug clearance and the maintenance of hepatic microarchitecture. The aim of our study is to investigate the adverse effects of polypharmacy on LSEC, advancing from traditional 2D monolayer cultures to physiologically relevant 3D spheroid systems.

Methods: Multicellular liver spheroids derived from primary mouse hepatocytes and liver non-parenchymal cells have been shown to be a good model for fatty liver disease (van Os E.A. et al., 2022). In our study, we use spheroids formed by hepatocytes and LSEC in the polypharmacy context. Both monolayer of LSEC and spheroids were treated with a combination of therapeutic drugs, such as citalopram, oxybutynin, metoprolol and oxycodone. The steady state concentrations of these drugs were based on the *in vivo* experiments done in mice by Mach J. et.al., 2021. The viability of the cells was assessed by using resazurin assay, while the endocytic activity of LSEC was tested via a radioactive scavenging assay by using ^{125}I-FSA. To further study the morphology of LSEC, scanning electron microscopy (SEM) was used.

Results: Treatment with clinically relevant concentrations of the single drugs and a high DBI drug cocktail had no significant impact on cell viability and scavenging activity of LSEC in 2D cultures. The results from spheroids, show an effect on the viability and endocytosis upon treatment with oxybutynin drug, while in presence of all the other drugs this compound seem to not affect the overall behaviour of the spheroids. Morphological analysis via SEM of the LSEC monolayers revealed a notable loss of fenestrations, a key feature associated with LSEC function, following treatment with all the drugs except for metoprolol. This aspect needs to be explored more in the spheroids, where the fenestrations were visible in the untreated samples.

Conclusion: Our findings highlight the relevance of the 3D multicellular liver spheroids in capturing the complexity of polypharmacy's impact on LSECs and suggest them as a good *in vitro* model for assessing polypharmacy-induced liver toxicity. Integrating insights from both 2D and 3D systems provides a deeper understanding of the critical role of LSECs in liver health under polypharmacy conditions. Future studies will explore multiple drug concentrations to better understand the impact of initial doses pre-first-pass metabolism on LSEC function and morphology.

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AI-Powered Segmentation of Scanning Electron Micrographs: Unravelling Pharmaceutical Effects on Liver Sinusoidal Endothelial Cells

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Objectives: Liver sinusoidal endothelial cells (LSECs) facilitate the bidirectional transport of small substances between the blood and the liver parenchyma via transcellular pores – fenestrations – ranging from 50 to 350 nm in diameter (Zapotoczny et al., 2022). The porosity of LSECs (total fenestration area per cell area) is critical for proper liver function. Loss of fenestrations ("de-fenestration") contributes to dyslipidaemia, insulin resistance, and reduced clearance of pharmaceuticals. This process may be reversible by numerous existing medicines. Quantitative analysis of the LSEC porosity is crucial for understanding of pharmaceuticals' impact on LSECs. Here, we propose a fully automatic neural network-based fenestration segmentation algorithm for *in vitro* scanning electron microscopy micrographs of LSECs.

Methods: Scanning electron microscopy (SEM) micrographs of LSECs were segmented using a convolutional neural network (CNN)-based algorithm with a U-Net architecture (Ronneberger et al., 2015). In this work, 44 manually annotated micrographs were used for training the CNN, and 22 micrographs for performance evaluation. The performance of the segmentation method was evaluated against manually annotated masks using a Dice score metric. The proposed CNN-based method was compared to the semi-automatic segmentation method that is based on micrograph intensity thresholding (Szafranska et al., 2021).

Results: Proposed U-Net CNN-based segmentation method demonstrated performance with a Dice score of 92.8% ± 2.2%. In comparison, the thresholding method achieved a Dice score of 88.8% ± 2.1% after excluding the outliers, 81.5% ‖ 15.1% when outliers were included.

Conclusion: The proposed U-Net CNN-based segmentation method achieves approximately 4% higher performance compared to the thresholding method. More importantly, the presented approach significantly outperforms on low contrast micrographs, where the thresholding method's Dice score falls below 80%.

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Differential Effects of Loss and Pharmacological Inhibition of Aryl Hydrocarbon Receptor on Mammary Tumor Growth

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Objectives: The aryl hydrocarbon receptor (AHR) is a key regulator of immune cell function and is associated with immunosuppression. In the context of cancer this leads to pro-survival processes, enabling cancer cells to evade immune detection. In this study we determined how loss or inhibition of AHR affected mammary tumor growth in wildtype and *Ahr*$^{-/-}$ mice, and if treatment with the AHR antagonist, BAY2416964, improved the effectiveness of immune checkpoint inhibition by anti-PD1.

Methods: We characterized the AHR signaling pathway in Py8119 and EO771 murine breast cancer cell lines *in vitro*. EO771 are naturally *Ahr* deficient, and we generated *Ahr* knockout Py8119 cells using CRISPR-Cas9. To determine AHR's role in tumour progression and in the immune microenvironment, we injected Py8119 and EO771 cells into the mammary fat pads of wildtype (WT) and *Ahr*$^{-/-}$ mice. We monitored tumor growth and did histological, immunohistochemical and RNA sequencing of tumor tissue. WT mice injected with Py8119 cells were also treated with BAY2416964 and anti-PD1 alone, and in combination.

Results: Ahr loss or long term (> 4 weeks) treatment BAY2416964 decreased proliferation of Py8119 cells and decreased the expression of AHR target genes. In tumour studies, we found that Py8119 and EO771 tumors grew more quickly in *Ahr*$^{-/-}$ compared with WT mice. For Py8119 tumors, we observed reduced infiltration of CD45^{+} cells in *Ahr*$^{-/-}$ compared with WT mice. Py8119 tumors had significantly more CD45^{+} cells than EO771 tumors. RNA sequencing of tumors identified 817 altered unique genes in WT mice compared with 566 genes specific for *Ahr* $^{-/-}$ mice. Co-treatment with BAY2416964 and anti-PD-1 significantly reduced tumor progression compared with either treatment alone.

Conclusion: Our data show the loss of *Ahr* increases tumor progression which may be due to altered immune cell function, but this needs to be further investigated. We also report that pharmacological inhibition of AHR in combination with anti-PD-1 reduces tumor growth compared with anti-PD-1 alone. This suggests that targeting AHR might lead to effective cancer treatment by elevating immunosuppressive signals to improve immune checkpoint therapy.

Enantioselective UHPLC-MS determination of 9-hydroxyrisperidone in plasma samples for risperidone-treated patients

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Objective: The antipsychotic drug risperidone (RIS) is mainly metabolized via CYP2D6 and CYP3A4 to 9-hydroxyrisperidone (9-OH-RIS), which is equipotent to the parent compound. A previous study indicated that the R- and S-enantiomers of 9-OH-RIS is selectively formed by CYP2D6 and CYP3A4, respectively. The aim of this study is to develop a method for quantification of R-and S-enantiomers of 9-OH-RIS in plasma samples of risperidone-treated patients, and later investigate the connection between CYP2D6-genotype and plasma concentration of the two enantiomers.

Method: Plasma samples from patients requested at Diakonhjemmet hospital was collected to be reanalyzed with UHPLC-MS to separate R- and S-9-OH-RIS. A robust UHPLC-UV method has been developed and optimized for enantioselective separation of the enantiomers. This method has later been introduced to UHPLC-MS to quantify the concentration of the enantiomers. A chiral UHPLC-column, Chiralpak® IB N-3, was used to separate and determine the enantiomers in patient samples. TDM full-scan high-resolution mass spectrometry was used to quantify and determine the concentrations of R- and S-9-OH-RIS. TaqMan probe based real-time PCR analysis was used to determine the patients CYP2D6-genotype.

Results and conclusion: Method development so far using racemic 9-OH-RIS in methanol on HPLC-UV has shown separation of the two enantiomers in both UHPLC-UV and UHPLC-MS. Also, so far, a total of seven patient samples has been analyzed on UHPLC-MS using the developed method. The results from the analysis have indicated the same results as from the study, considering the ratio between R-and S-enantiomers in the samples. Preliminary results will be presented at the meeting.