WG3 WG4 Workshop "Omics technologies as a new tool in ecotoxicology "

26/03/2024

online

Workshop of the COST Action CA21111
One Health drugs against parasitic vector borne diseases in Europe and beyond
OneHealthdrugs

The event is open to PhD, young innovators and senior scientists from both academia and pharma

Prepared by Rolf During (WG4 leader) and Guy Cajon (WG3 Leader) and Maria Paola Costi

1. **MEETING LINK**
2. https://teams.microsoft.com/l/meetup-join/19%3aJdu4-YOGoTWvm2EtTXTcbi08m9LpmYFMY_vTAu_mQGU1%40thread.tacv2/1710247993842?context=%7b%22Tid%22%3a%22e787b025-3fc6-4802-874a-9c988768f892%22%2c%22Oid%22%3a%22ac391189-1971-4664-9abf-5dbf09f2a671%22%7d

3. **LIST OF ATTENDANTS**

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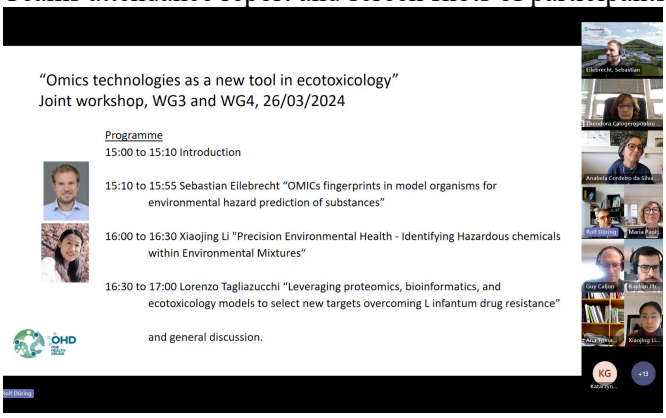
2. PROOF OF ATTENDANCE

Teams attendance report and screen shots of participants



4. DESCRIPTION OF THE ACTIVITIES

Description

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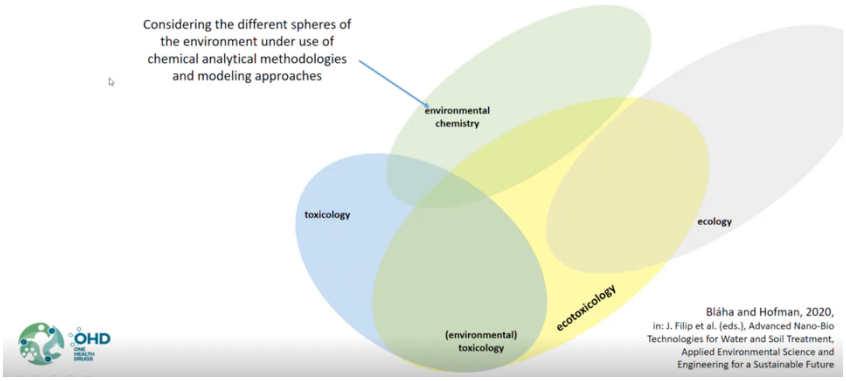
In the development of new active substances, consideration of possible effects on non-target organisms is becoming increasingly important. Assessment of environmental effects is still in its infancy in drug development. Here, the One Health approach often still falls short, as environmental health is an integral part of this concept, in addition to animal and human health. Efficient, innovative but also standardizable test methods and assays are needed to assess the behavior and effects of active ingredients released into the natural environment. This joint workshop of WG3 and WG4 will provide a platform to present and discuss the application of the "omics" approach in ecotoxicological testing.

E-COST invitations were sent to all working groups members.
The workshop was attended by 32 participants. Rolf During (WG4 leader) and Guy Cajon (WG3 Leader) and Maria Paola Costi organized the meeting. Prof. Rolf During introduced the workshop and its meaning in the ecotoxicology context. Prof M.P. Costi (CA21111 Chair) welcomed the participants. Subsequently, Prof. During chaired the session that comprised three lectures.

Testing in ecotoxicology and testing in pharmaceutical development, requires a combined approach and we should do it early in the process. The ecotoxicology is a combination of different disciplines:

Ecotoxicology:
Overlap of Different Disciplines, Propagation of Effects Through Biological Systems



It evaluates the presence of chemicals including drugs in Organisms, Organelle and cells

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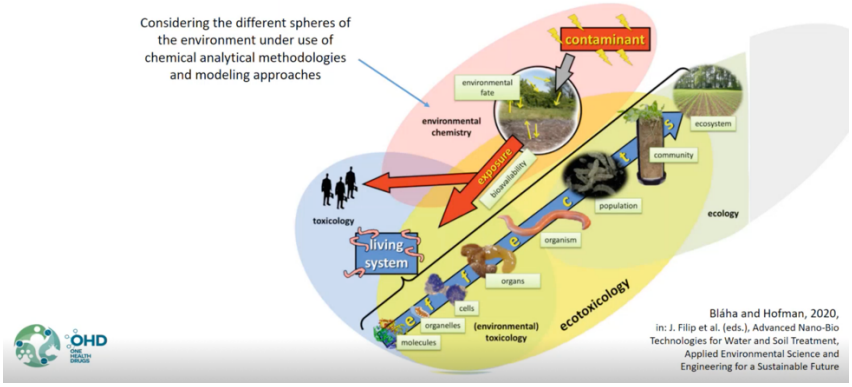
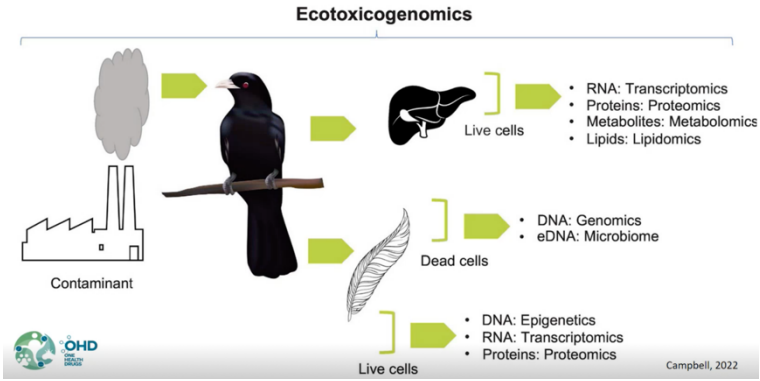


As an example, a bird is exposed to a contaminant and we can observe an effect and this can be observed in cells and organs and these are the samples of analysed by the omics technologies. Different omics technologies are available today:



Omics approaches in ecotoxicology

Omics approach	Advantages	Disadvantages
eDNA	Rapidly identify species present in the environment without exhaustive field surveys.	Protocol standardization lacking; type I and type II errors. A type I error rejects a true null hypothesis, while a type II error accepts a false null hypothesis.
Transcriptomics	Thousands of potential biomarkers assessed.	Transient expression; type I and type II errors. Relevance to biological function uncertain. High variability among individuals.
Proteomics	Proteins are functional units of the cell. More long-lived than transcripts (days versus hours).	Can be expensive to obtain data. Limited number of proteins identified; often those most abundant in tissue.
Metabolomics	Metabolites (nutrition-derived, synthesized in body) are shared and ubiquitous across species (e.g., glucose, cholesterol). Targeted versus untargeted approaches do not require *a priori* knowledge of molecular composition of species (e.g., their genes). Detection of metabolites derived from a specific chemical are a 'smoking gun' for exposure.	Sensitive to diet and other environmental perturbation.
Lipidomics	High relevance to metabolism and oxidative stress.	Uncertain what changes mean as the function of many lipids unknown.
Epigenomics	Multi-generational perspective.	Difficult to conduct studies in wild populations.

Campbell, 2022

With the present workshop we wanted to link the different field together by linking WG3 and WG4. See the WG3 and 4 objectives.

WG3 Objectives

Promoting and strengthening of innovative technologies required in the translation of leads and candidates from animal to humans and vice versa to ensure the progression of qualified leads and candidates to the end of the pre-clinical phase and de-risk studies in clinical phase 1. This is restricted to advanced leads and candidate.

WG4 Objectives

Coordination of the R&D programs innovative strategies and compliance with the overall environmental impact to provide a sharable guideline-like document. This may inform the compounds probability of exposure, an information derived from a more detailed understanding on the substances environmental fate. The validation against the ecological interpretation of selected indicators (see below) is important to properly inform drug designer and managers of environmental risks compared to societal benefits.

The presentations and their contents are reported below.

1. OMICs fingerprints in model organisms for environmental hazard prediction of substances

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Pesticides, biocides or pharmaceuticals can display adverse effects in non-target organisms. This may threaten populations with far-reaching consequences for the ecosystem. Therefore, European legislation requires manufacturers to provide data for environmental risk assessment of active substances for registration. The commonly applied OECD tests are time and cost consuming and utilize a substantial number of animals. Thus, they are conducted only in the final stage of substance development, bearing the risk of failing registration due to adverse environmental effects. The Eco'n'OMICs project aims at an early ecotoxicological risk prediction for development compound classes based on their induced molecular changes in aquatic model organisms. We apply OMICs to identify gene expression changes as molecular biomarkers for a number of ecotoxicologically well characterized model substances covering a broad range of modes-of-action (MoA). We combine recent transcriptomic and proteomic techniques with ecotoxicological approaches to generate a data base linking substance-specific gene expression signatures with adverse effects on the organism and the population. Therefore, aquatic model organisms such as fish larvae [1,2,3,4,5,6], water flea [7] or aquatic plants [8,9] are exposed to sublethal concentrations of reference substances in an environment based on standard OECD test guidelines. The reference substances are selected to be ecotoxicologically well characterized chemicals with known adverse effects, covering a broad range of MoAs. Our data base covers the substance's MoA, adverse effects on the organism and the population as well as the OMICs-generated molecular fingerprint in aquatic non-target organisms and is used to develop targeted molecular screening approaches in order to rank members of development compound classes based on their ecotoxic potential.

References

[1] Reinwald, Hannes, et al. "Toxicogenomic fin (ger) prints for thyroid disruption AOP refinement and biomarker identification in zebrafish embryos." Science of the Total Environment 760 (2021): 143914.

[2] Reinwald, Hannes, et al. "Toxicogenomic profiling after sublethal exposure to nerve-and muscle-targeting insecticides reveals cardiac and neuronal developmental effects in zebrafish embryos." Chemosphere 291 (2022): 132746.

[3] Essfeld, Fabian, et al. "Transcriptomic profiling of clobetasol propionate-induced immunosuppression in challenged zebrafish embryos." Ecotoxicology and Environmental Safety 233 (2022): 113346.

[4] Ayobahan, Steve U., et al. "Comprehensive identification of gene expression fingerprints and biomarkers of sexual endocrine disruption in zebrafish embryo." Ecotoxicology and Environmental Safety 250 (2023): 114514.

[5] Luckner, Benedikt, et al. "Transcriptomic profiling of TLR-7-mediated immune-challenge in zebrafish embryos in the presence and absence of glucocorticoid-induced immunosuppression." Ecotoxicology and Environmental Safety 266 (2023): 115570.

[6] Marghany, Fatma, et al. "Transcriptomic and proteomic fingerprints induced by the fungicides difenoconazole and metalaxyl in zebrafish embryos." Environmental Toxicology and Pharmacology 105 (2024): 104348.

[7] Pfaff, Julia, et al. "Toxicogenomic differentiation of functional responses to fipronil and imidacloprid in Daphnia magna." Aquatic Toxicology 238 (2021): 105927.

[8] Loll, Alexandra, et al. "Short-term test for toxicogenomic analysis of ecotoxic modes of action in Lemna minor." Environmental Science & Technology 56.16 (2022): 11504-11515.

[9] Hanfland, Jost, et al. "Short-term test for the toxicogenomic assessment of ecotoxic modes of action in Myriophyllum spicatum." Science of The Total Environment (2024): 171722.

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2. Precision Environmental Health - Identifying Hazardous chemicals within Environmental Mixtures

Xiaojing Li School of Biosciences, Research fellow
Centre for Environmental Research and Justice (CERJ) focus on integrating multi-omics for toxicogenomic studies and evolutionary ecology.

From bringing perspectives from multiple disciplines including state-of-art machine learning methods, a research program is formed under the framework of Precision Environmental Health that centers on identifying systematic biological response to environmental stressors. Dr Li is a member of the Centre for Environmental Research and Justice (CERJ).

Within the concept precision environmental health, precision toxicology stands out as one of the most important field. The goal of *PrecisionTox* is to overcome conceptual barriers to replacing traditional mammalian chemical safety testing by accelerating the discovery of evolutionarily conserved toxicity pathways that are shared by descent among humans and more distantly related animals. An international consortium is systematically testing the toxicological effects of a diverse set of chemicals on a suite of five model species comprising fruit flies, nematodes, water fleas, and embryos of clawed frogs and zebrafish along with human cell lines. Multiple forms of omics and comparative toxicology data are integrated to map the evolutionary origins of biomolecular interactions that are predictive of adverse health effects, to major branches of the animal phylogeny. These conserved elements of adverse outcome pathways (AOPs) and their biomarkers are expected to provide mechanistic insight useful for regulating groups of chemicals based on their shared modes of action. *PrecisionTox* also aims to quantify risk variation within populations by recognizing susceptibility as a heritable trait that varies with genetic diversity. This initiative incorporates legal experts and collaborates with risk managers to address specific needs within European chemicals legislation, including the uptake of new approach methodologies (NAMs) for setting precise regulatory limits on toxic chemicals.

The detailed description of the parameters that are taken in consideration. The assessment of persistence (P), bioaccumulation (B), and toxicity (T) of a chemical is a crucial first step at ensuring chemical safety and is a cornerstone of the European Union's chemicals regulation REACH (Registration, Evaluation, Authorization, and Restriction of Chemicals). Existing methods for PBT assessment are overly complex and cumbersome, have produced incorrect conclusions, and rely heavily on animal-intensive testing. We explore how new-approach methodologies (NAMs) can overcome the limitations of current PBT assessment. We propose two innovative hazard indicators, termed cumulative toxicity equivalents (CTE) and persistent toxicity equivalents (PTE). Together they are intended to replace existing PBT indicators and can also accommodate the emerging concept of PMT (where M stands for mobility). The proposed "toxicity equivalents" can be measured with high throughput in vitro bioassays. CTE refers to the toxic effects measured directly in any given sample, including single chemicals, substitution products, or mixtures. PTE is the equivalent measure of cumulative toxicity equivalents measured after simulated environmental degradation of the sample. With an appropriate panel of animal-free or alternative in vitro bioassays, CTE and PTE comprise key environmental and human health hazard indicators. CTE and PTE do not require analytical identification of transformation products and mixture components but instead prompt two key questions: is the chemical or mixture toxic, and is this toxicity persistent or can it be attenuated by environmental degradation? Taken together, the proposed hazard indicators CTE and PTE have the potential to integrate P, B/M and T assessment into one high-throughput experimental workflow that sidesteps the need for analytical measurements and will support the Chemicals Strategy for Sustainability of the European Union.

3. Leveraging proteomics, bioinformatics, and ecotoxicology models to select new targets overcoming *L infantum* drug resistance.

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There is an urgent need to develop new drugs to overcome drug resistance issue in Leishmaniases, as the commonly used antimonials, paromomycin, and miltefosine show low efficacy due to the emergence of hyper-resistant strains. Recent advancements in Vector-Borne Parasitic Diseases research have also highlighted the consideration of environmental drug safety and their ecotoxicological impact, while simultaneously focusing on preventing resistance phenomena from the outset of the drug discovery projects. To address this challenge, the exploitation of omics technologies like high resolution Mass Spectrometry proteomics/transcriptomics and bioinformatic/ecotoxicology predictive models, can help suggesting new biological mechanism, new drug targets and innovative drug combination strategies. Herein, we have investigated the biochemical mechanisms of resistance to sodium stibogluconate, paromomycin, and miltefosine in three distinct parasitic strains derived from human clinical isolates [1,2]. THP-1 cultures were infected with the clinical isolates of resistant *Leishmania* parasites to mimic the acute phase of the infection, and were submitted to bottom up, whole-cell LC-MS/MS proteomics pipeline. Among all the cellular proteins, 14 emerged as differentially expressed (DEP), and only peroxiredoxin emerged as a DEP in all resistant strains. Human protein modulation was studied by MS, too, to evaluate the parasite impact on the monocytes' proteome. Guest-host cross talking proteins and pathways were well defined to discard those proteins/pathways involved in both the human and parasitic networks. To assess the environmental impact of the remaining proteins, a SeqAPASS analysis was employed to predict cross species homology and drug target susceptibility. The MATH domain-containing protein, ATP-binding cassette B2, histone H4, calpain-like cysteine peptidase, and trypanothione reductase emerged as top candidates. In parallel, human proteins from THP-1 were studied, and two main enzymes (Transferrin Receptor C and Nucleoside Diphosphate Kinase) emerged as DEP both in proteomics, and transcriptomics studies. This suggests that their overexpression is caused by specific patterns proper of the drug resistant parasitic phenotype, and their inhibition or modulation should increase the parasite drug sensitivity. In an optic of a drug discovery program driven by One Health approach, we propose the above-mentioned targets to undergo further molecular investigation to be considered to overcome parasitic drug resistance, and to study the efficacy a of a dual guest-host antileishmanial therapy.

References

Tagliazucchi L, et al. ACS Infect Dis. 2023 Mar 10;9(3):470-485. doi: 10.1021/acsinfecdis.2c00457.
García-Hernández R, et al. OMICS. 2022 Mar;26(3):165-177. doi: 10.1089/omi.2021.0185.

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Conclusion

The workshop was very productive. The discussion was developed after the presentations. The field of the omics technologies is in development and not yet at a level of standardization as other more traditional analytical techniques. The field is very important because it gives a fundamental contribution to a deeper understanding of the mechanism of interaction with the living organisms. The integration of the different omics technologies will open an holistic view and a consequent more effective intervention aiming at actions for environmental protection. Recordings are available and will be elaborated to provide the contribution for the deliverable associated to the topic of the workshop.