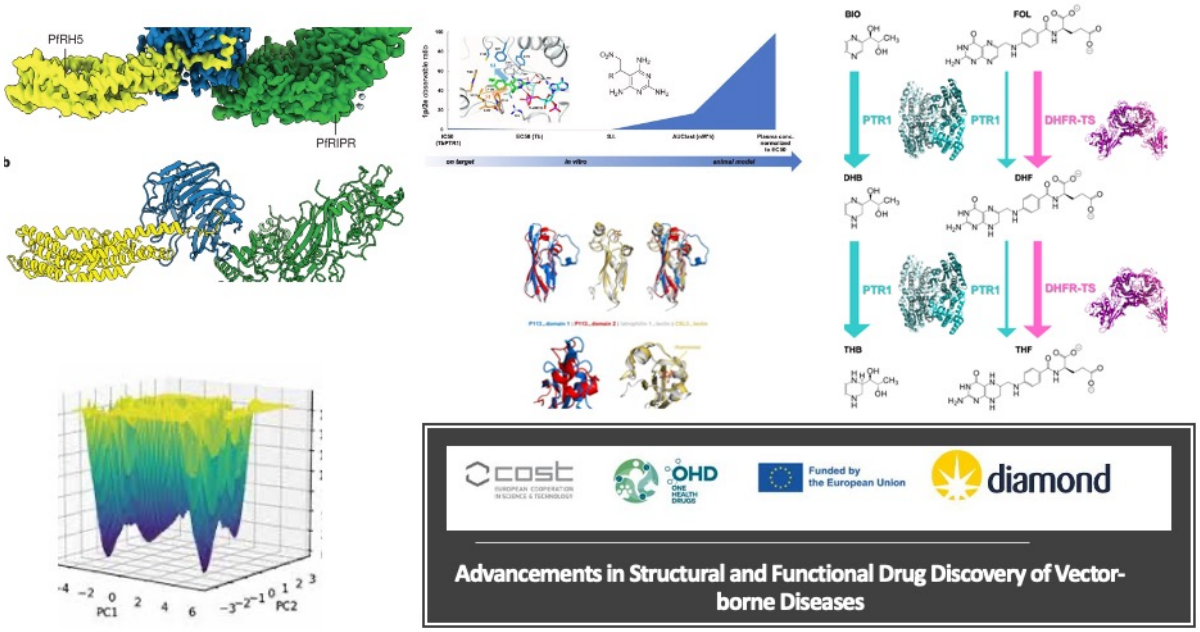
Advancements in Structural and Functional Drug Discovery of Vector-borne Diseases

Summary of the meeting with pictures

Wednesday 3rd to Friday 5th July 2024

Saïd Business School & Diamond Light Source



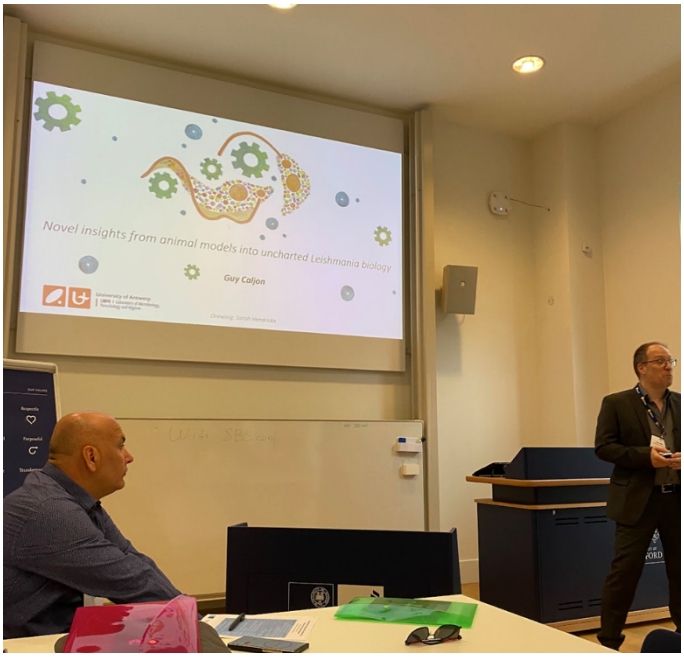
Tuesday 3rd July 2024

Wednesday 3rd July 2024	Saïd Business School
09:00	*Arrival & registration*
09:45	*Welcome*
Session 1: **Biology and ecology of parasites** *(Chairing: Elton Rogozi & Anabela Cordeiro)*	
09:30	"Novel insights from animal models into uncharted Leishmania biology" Guy Cajon, University of Antwerp (Belgium)
10:00	*"Unprecedented high-resolution chemical imaging of proteins, surfaces of microbes & histological tissue using mid-IR photo-induced force microscopy"* Daniela Trauber, Friedrich Schiller University Jena, (Germany)
10:30	*"Mitochondrial NADH/NAD+ balance in Leishmania survival and its interest for chemotherapy"* Margarida Duarte, Instituto de Investigação e Inovação em Saúde (Portugal)
11:00	*Coffee Break*
11:30	**Round table: Parasitology. Promoters:** Guy Cajon, Jose Maria Alunda, Louis Maes. to discuss the roadmap of *in vitro* and *in vivo* laboratory experiments towards high-quality drug leads based on our experiences. Within this round table theme, José María will also pitch his poster about formulation (see attachment). OHD3: Transition from in vitro to in vivo evaluation: recommendations for obtaining high-quality leads against kinetoplastids To initiate the round table and discussion, I would propose to discuss a few items to which everyone can provide input from their expertise: • What defines a good antiparasitic hit with NO GO criteria in early discovery – which essential assays are needed? – discuss for the different kinetoplastids • Which pharmacokinetics assays are available and accessible within the network to transition to *in vivo* studies • What should we consider when formulating a drug for *in vivo* testing? (pitch José María) • Where we position 'Omics and what are the benefits of MoA studies? • Where do we position ecotoxicology in the discovery process and which assays are accessible? • With which labs can you collaborate to test antiparasitic activity /ecotoxicity? **Poster presentation**: Drug formulation, bridging the gap from in vitro experiments to in vivo resultsJosé María Alunda, Javier Carrión, Juan José Torrado
12:30	*Break*

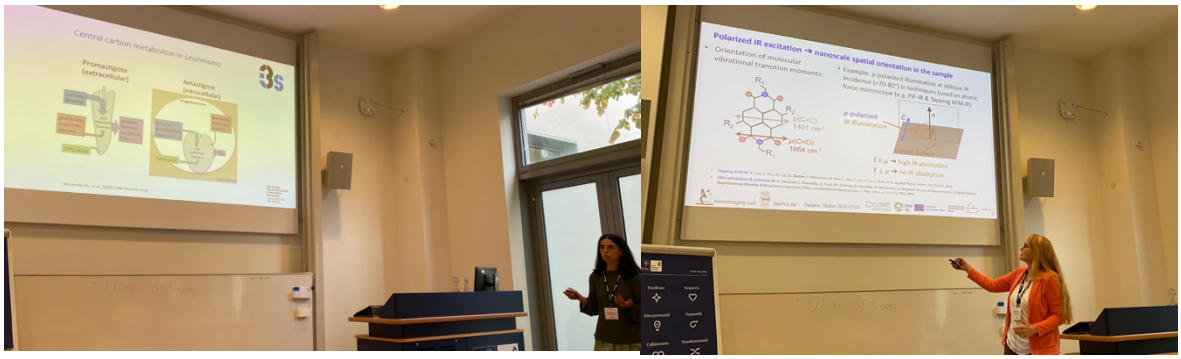
Session 1: Biology and ecology of parasites (9:30-12:30)

Session 1 had the focus on the biology and ecology of parasites. It was chaired by Elton Rogozi and Anabela Cordeiro.

The session began at 09:30 with a presentation by **Guy Cajon** from the University of Antwerp (Belgium) on "Novel insights from animal models into uncharted Leishmania biology." Research using bioluminescent parasites in rodents has shown that bone marrow stem cells act as a reservoir for Leishmania, contributing to disease relapse. These stem cells harbour a unique transcriptional signature ("StemLeish") and pathways that regulate Leishmania infection. Additionally, parasites within these stem cells enter a quiescent state, exhibiting enhanced drug resistance and increased infectivity upon transmission. These findings highlight the need for novel assays and drugs targeting parasite sanctuary and quiescence to reduce relapse risk.



At 10:00, **Daniela Trauber**, also from the University of Jena (Germany), presented “Unprecedented high-resolution chemical imaging of proteins, surfaces of microbes & histological tissue using mid-IR photo-induced force microscopy.” Mid-infrared photo-induced force microscopy (PiF-IR) is revolutionizing the chemical imaging of biological samples. This technique offers exceptional spatial and spectral resolution, enabling sub-molecular imaging of proteins, microbial surfaces, and histological tissue. PiF-IR has the potential to transform our understanding of chemical processes in parasitic infection, immune response, cell death, and drug action.



At 10:30, **Margarida Duarte** from Instituto de Investigação e Inovação em Saúde (Portugal) gave a talk on “Mitochondrial NADH/NAD+ balance in Leishmania survival and its interest in chemotherapy.” Leishmania parasites adapt to different environments by adjusting their mitochondrial NADH/NAD+ ratio. Several enzymes are involved in this process, including complex I, fumarate reductase (FDR), and type II NADH dehydrogenase (NDH2). Research has shown that NDH2 is essential for the survival of both L. infantum and L. major. Targeting NDH2, which has no mammalian counterpart, could be a promising strategy for developing new leishmanicidal drugs. However, the potential of complex I and FDR as drug targets appears less promising

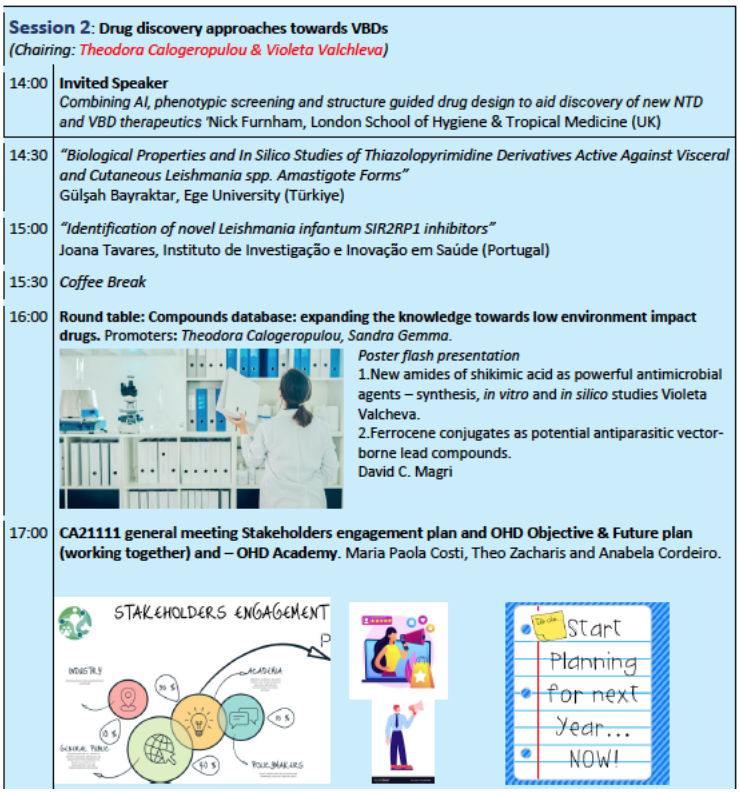
Following a coffee break at 11:00, the session continued at 11:30 with a round table discussion on "Parasitology."

The round table was chaired by **Guy Caljon** and started with an overview of the OHD3 goals and some accomplishments in the various areas (ecotoxicology assays, discovery of leads and candidates, imaging and drug target engagement, omics studies into action and resistance mechanisms, formulation and standardization of animal experiments).

The initiative of a viewpoint manuscript that describes the roadmap to obtain high-quality leads for kinetoplastid diseases (Leishmaniasis, American and African Trypanosomiasis) was introduced. A draft schematic overview of the roadmap with the essential steps in various disciplines (chemistry, primary pharmacology, PK, toxicology and pharmaceutics) in the different phases of drug discovery (hit finding, hit profiling, lead definition and drug development candidate) was projected. The round table started with a presentation by **José María Alunda** (*Drug formulation, bridging the gap from in vitro experiments to in vivo results*). He presented examples of drug formulation and its impact on pharmacokinetics and pharmacodynamics of antileishmanial drugs. The following discussion encompassed aspects of combinatorial chemistry, formulation, PK/PD and ecotoxicology. The

importance of *in silico* tools and genome resources to accomplish a One Health approach has been pointed out to be included in the roadmap.

Session 2: **Drug discovery approaches towards VBDs (2:00pm-6pm)**
(Chairing: Theodora Calogeropulou & Violeta Valchleva)



Session 2 had the focus on drug discovery approaches towards VBDs (Vector-Borne Diseases). It was chaired by **Theodora Calogeropulou** and **Violeta Valcheva**. The session started at 14:00 with an invited presentation by **Nick Furnham** from the London School of Hygiene & Tropical Medicine (UK) who presented an excellent overview of his work on "Combining AI, phenotypic screening, structure guided drug design to aid discovery of new NTD and VBD therapeutics.

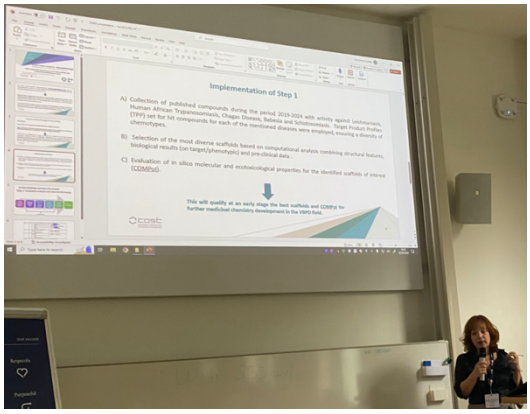
At 14:30, **Gülşah Bayraktar** from Ege University (Türkiye) gave a presentation on the "Biological Properties and In Silico Studies of Thiazolopyrimidine Derivatives Active Against Visceral and Cutaneous Leishmania spp. Amastigote Forms". Thiazolopyrimidine derivatives showed potent anti-amastigote activity against these parasites and were non-cytotoxic to macrophages, comparable to miltefosine. In silico studies supported their mechanism of action, indicating their potential as new lead compounds for treating leishmaniasis.

This was followed by **Joana Tavares** from Instituto de Investigação e Inovação em Saúde (Portugal) at 15:00, who presented on the "Identification of novel Leishmania infantum SIR2RP1 inhibitors". Sirtuins, specifically Sir2rp1, are critical for the survival of Trypanosoma and Leishmania parasites. Inhibitors of LiSIR2RP1, such as BNIP alkyl di- and triamines, showed antiparasitic activity with low micromolar IC50 values. A collaborative project screened 440,000 compounds, identifying 55 promising candidates, with 9 showing effective antiparasitic activity against L. infantum amastigotes. The LiSir2rp1 crystal structure resolution will aid in optimizing these leads.

After a coffee break at 15:30, the session resumed at 16:00 with a round table discussion on "Compounds database: expanding the knowledge towards low environment impact drugs".

16.00 – 17.00

Round table: Compounds database: expanding the knowledge towards low environment impact drugs. Promoters: **Theodora Calogeropoulou, Sandra Gemma**.



Initially, TC provided an overview of the OHD2 project "Compounds database: expanding the knowledge towards low environment impact drugs" and the progress achieved to date. Subsequently the following aspects were raised during the discussion that followed. The proposed TPP for *T. brucei* and in particular for the IC_{50} value against the parasite for the compound collection was considered high taking into consideration the current TPP by DNDi. **Michele Tonelli** and **Maria Paola Costi** (MPC) explained that a high value was used in order to collect as many chemotypes as possible and then there will be a refinement. Concerns were raised that the training set of ~100 compounds provided by M. Bertram with experimentally defined ecotoxicological profile is somewhat limited, but since such datasets are difficult to get, the study will proceed as planned. MPC also explained that this database will be connected with the database on targets and the in house compounds, while the algorithm that will be developed will enable the prediction of the ecotoxicological profile of the compounds generated within COST Action CA21111. Action needed: ì. to harmonise the Compounds collection to achieve the same level of selection in particular Tbrucei data. Ìì. Natural compounds collection should be coordinated.

Then the molecular properties of all compounds should be evaluated using chemoinformatic tools by Sheraz Gul (Frunofher, Hamburg). **Scaffold search** should be applied representing each antiparasitic class. Once the scaffold will be identified, a second round of chemoinformatic analysis should be performed including **Ecotox parameters** should be included. The implementation of the chemoinformatic algorithm with the ecotox parameters should be evaluated with the ecotox experts. A discussion should be planned between September and October to proceed further among all interested participants. Subsequently, two flash poster presentations were delivered:
1) New amides of shikimic acid as powerful antimicrobial agents – synthesis, in vitro and in silico studies by **Violeta Valcheva**.
2) Ferrocene conjugates as potential antiparasitic vector-borne lead compounds by **David C. Magri**.

STAKEHOLDERS session Theo Zacharis
In the oral presentation on stakeholders, the discussion was structured in three main steps.
First, the identification of stakeholders was addressed. Key stakeholders included academic and research institutions, healthcare providers, biotechnology and pharmaceutical companies, investors, funding policymakers, regulatory bodies, patient advocacy groups, and technology developers. Additionally, the audience highlighted cultural organizations, environmental organizations, agricultural

and breeders' associations, the World Organization of Veterinary, parasitology organizations, and veterinary courses and schools as important stakeholders.

The second part of the presentation focused on stakeholder analysis tools. Various methods such as the Influence-Impact Grid, SWOT Analysis, and the Salience Model (which considers power, legitimacy, and urgency) were discussed to evaluate and prioritize stakeholders effectively. Third, a discussion was opened to identify the most important stakeholders.

Thursday 4th July 2024

Session 3: Structural biology and biochemistry (10am-1pm)

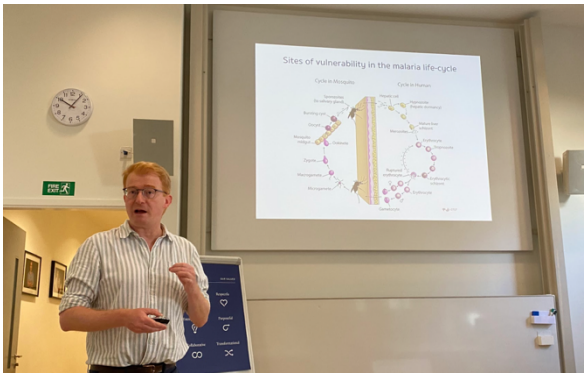
Thursday 4th July 2024	Saïd Business School
Session 3: **Structural biology and biochemistry** (Chairing: Ulrike Wittig & Harry De Koning)	
10:00	**Invited Speaker** *'Structure-guided design of blood stage malaria vaccines'* Matt Higgins, University of Oxford (UK)
10:30	**Invited Speaker** *'Structural characterisation of Trypanosoma cruzi antigens for diagnostic and therapeutic applications* Ivan Campeotto, University of Nottingham (UK)
11:00	*"Structural investigation of Trypanosoma folate enzymes for the development of mew antiparasitic agents"* Cecilia Pozzi, University of Siena (Italy)
11:30	*Break*
12:00	**Round table: Combining structural and functional knowledge to understand and fight VBDs.** Promoters: Ulrike Wittig, Harry De Koning **OHD1 - Target database project: the BioTarget DataBase (BioT-DB)** (promoter Cecilia Pozzi, Marco Mazzorana, Ulrike Wittig) Advancements in Structural and Functional Drug Discovery of Vector-borne Diseases. Poster flash presentation: 1.Leishmania infantum ribose 5-phosphate isomerase a validated drug target. A.Cordeiro 2.The Peroxiredoxins of Leishmania revisited. A.Thomas.
13:00	*Lunch Break*

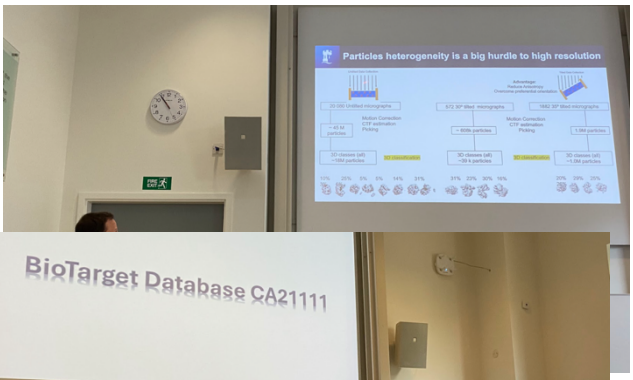
Session 3 had the focus on structural biology and biochemistry. It started with an invited presentation given by **Matt Higgins** from the University of Oxford (UK) about "Structure-guided design of blood stage malaria vaccines". Matt Higgins gave inspiring insights in the development of Malaria vaccines to stop the parasite red blood cell invasion. The second invited speaker was **Ivan Campeotto** from the University of Nottingham (UK) with the presentation about "Structural characterization of Trypanosoma cruzi antigens for diagnostic and therapeutic applications". He presented different structure-guided methods for the analysis and the development of vaccines against Chagas disease

and Malaria. **Cecilia Pozzi** from the University of Siena (Italy) presented her results on the "Structural investigation of Trypanosoma folate enzymes for the development of new antiparasitic agents". The session was chaired by Harry De Koning and Ulrike Wittig.

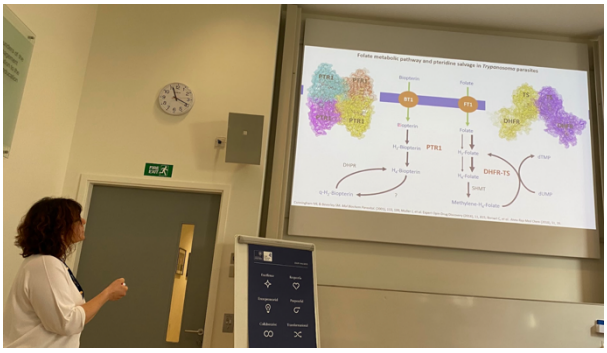
Round table: Combining structural and functional knowledge to understand and fight VBDs

The round table discussion started with the poster flash presentation from Anabela Cordeiro Da Silva about "Leishmania infantum ribose 5-phosphate isomerase a validated drug target" and from Ana Thomas about "The Peroxiredoxins of Leishmania revisited".





After the poster presentations, **Cecilia Pozzi** and **Marco Mazzorana** presented the OHD1 project with the focus of the development of a BioTarget database to collect protein properties for potential targets which are available within the COST Action network. The different details of the Excel template for the data collection were presented and meeting participants were allowed to give feedback. Further the data collection process and data sharing policies were discussed.

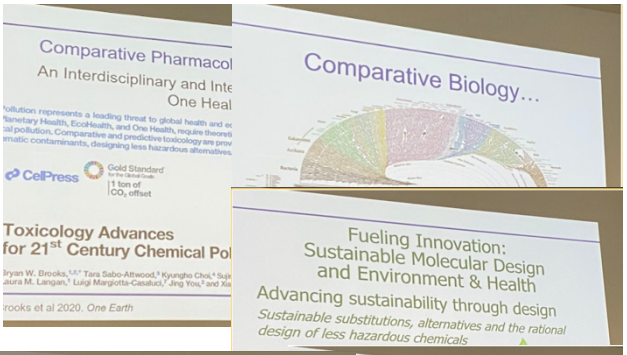


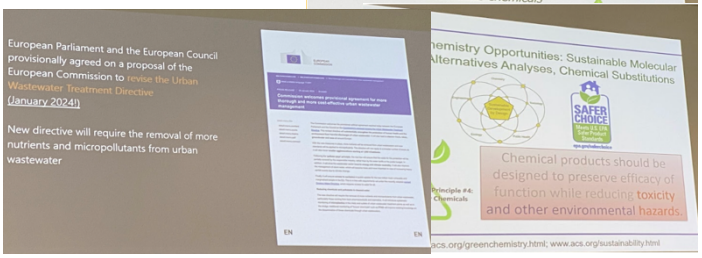
Session 4: Ecotoxicology and One-Health (2pm-3:30pm)

Session 4: Ecotoxicology and One-Health (Chairing: Elisa Uliassi & Ioannis P. Papanastasiou)	
14:00	**Invited Speaker** *'Pharmaceuticals, Environment, and One Health'* Brian Brooks, Baylor University (USA)
14:30	**Invited Speaker** *Swimming in medicated waters: Understanding and mitigation the impacts of pharmaceutical pollutants on aquatic wildlife'* Eli Thoré, Swedish University of Agricultural Sciences (Sweden)
15:00	*"Exploring new frontiers in fighting animal trypanosomiasis: assessing antitrypanosomal and (eco)toxicological characteristics of novel nucleoside-based leads"* Kayhan Ilbeigi, University of Antwerp (Belgium)
15:15	*"Advancing sustainable drug development: comparative preclinical study of H80 and Miltefosine using imaging and proteomics"* Giulia Malpezzi, University of Modena and Reggio Emilia (Italy)
15:30	*Coffee Break*
16:00	**Round table: Sustainable integration of people and animal's health within the ecosystem.** Promoters: Clara Lima, Eli Thorè, Harry de Koning, Brian Brooks and Team **OHD-4 Review of veterinary pharmaceuticals against Parasitic Vector-Borne Diseases and their environmental impacts** Poster presentation 1) "Malaria, a parasitic mosquito-borne disease; from imported cases re-emerge, to the presence of primarily Anophelinae vectors population in Albania" by Elton Rogozi 2) "Review of veterinary pharmaceuticals against Parasitic Vector-Borne Diseases and their environmental impacts", by Clara Lima, on behalf of OHD4 collaborators
17:00	*Poster Session: All poster presenters will present their flash 5 minute.* • Ioannis Papanastasiou, Adamantane imidazolines with trypanocidal activity • Dijana Blazhekovikj, Possibilities For Treating Parasites In Fish With Essential Oils And Plant Extracts • Aleksandar Cvetkovski, Survey on repurposing of anti-parasitic drugs in babesiosis treatment • Elisa Uliassi, Fostering Innovation in Vector Borne Parasitic Diseases through Young Researcher Innovators within CA21111 OneHealthdrugs • Rokaya Ahmad, Discovery of novel drug action mechanisms by studying antileishmanial Aminopyrazoles **CA21111 general part 2. *MPaolaCosti*** • *Next events YEAR 2 (Training school in Warsaw 27-28 September 2024; Athens meeting 19-20 September 2024).* • *Presentation of the next events and next year planning (WG meeting in Tirana, Albania; annual conference in Antwerp).* • *Academy ambassadors' proposal discussion.* • *Next year plan.*
18:30	*Drinks & Conference Dinner*

Session 4 related to Ecotoxicology and One-Health has been chaired by Elisa Uliassi & Ioannis P. Papanastasiou and opened by the inspirational lecture by **Bryan W. Brooks**, Baylor University (USA).

Prof Brooks is a globally recognized scientist in the field of environmental toxicology and chemistry, environmental public health, hazard & risk assessment, and water resources. His presentation has highlighted the strong link between Pharmaceuticals, Environment, and One Health, providing examples and suggestions on how to evaluate them in a holistic way and how to overcome current challenges. Then, **Eli Thorè** from Swedish University of Agricultural Sciences (Sweden) delivered a presentation titled "Swimming in medicated waters: Understanding and mitigation of the impacts of pharmaceutical pollutants on aquatic wildlife", underscoring the importance of behavioural ecotoxicology assessment of pharmaceuticals.





Then, presentations from YRI (**Kayhan Ilbeigi**, University of Antwerp and **Giulia Malpezzi**, University of Modena and Reggio Emilia) working in the field of VBPD brilliantly illustrated the various approaches being pursued against VBPD.

16.00 – 17.00
Round table: Sustainable integration of people and animal's health within the ecosystem.
Promoters: Clara Lima, Eli Thorè, Harry de Koning, Brian Brooks and Team.
OHD-4 Review of veterinary pharmaceuticals against Parasitic Vector-Borne Diseases and their environmental impacts.

Parasites are organisms that require a dependent mode of existence to complete their life cycle, living at least a portion of their lives *in* or *on* (endoparasites or ectoparasites, respectively) a host organism. The parasite exploits its host, whether it's a human, animal, arthropod, or plant, by feeding off it. Consequently, the host may suffer from parasitic diseases (PDs), which may represent severe life-threatening conditions (Goater et al, 2013; Taylor et al. 2016), compromising livelihood and life expectancy. For instance, PDs of humans such as schistosomiasis and leishmaniasis, are responsible for a high burden of disease and premature deaths. Yet, these conditions remain overlooked in public health agendas, as they are often included in the broader complex of syndemic diseases associated with poverty in tropical and subtropical regions. Conversely, parasite-control of livestock and companion animals gained worldwide recognition, as animals can host a broad spectrum of PDs of notable zoonotic implications. Besides, parasites of livestock are responsible for significant production and economical losses and transmission of food-borne parasitosis, while companion animals are considerably affected by a diverse range of health impacting PDs. Indeed, the impact of parasite control in animals is mirrored in the 23% of the global Animal Health market, currently represented by antiparasitic drugs (Selzer et al. 2021). Parasitic diseases of vector-borne origin (PVBDs) are particularly challenging to tackle, as control measures are orchestrated in a multifactorial dimension, targeting vectors, parasites, and hosts. Such approaches rely on mass administration of drug combinations, including repellent and/or parasiticidal compounds, characterized by insufficient safety profiles for both hosts and environment (Boxal et al. 2002; Boxal et al. 2004; Kaczala and Blum. 2016). More

recently, evidence of decreased parasite susceptibility and increased resistance is being reported, besides toxicity to non-target organisms.
It is expected that the development of parasiticides follows scientific progress to overcome epidemiological variations in parasite distribution and infection patterns,

while safeguarding public, animal, and environmental health. **We seek to evaluate the chemical and pharmacological properties of the current drugs against PVBDs of animals to predict their environmental fate and ecological risks, with the ultimate goal to guide the development of greener anti-PVBDs drugs. For this purpose, our multidisciplinary team of medicinal chemists, ecotoxicologists, parasitologists and veterinarians is conducting a comprehensive literature review, with insights to be shared at the meeting for additional input.**
Action points: ì. In September a survey will be distributed and the plan for the veterinary medicine list collection will be updated. ìì. This session required more time for the discussion. We will propose it in a next appointment.

Poster presentation
1) "Malaria, a parasitic mosquito-borne disease; from imported cases re-emerge, to the presence of primarily Anophelinae vectors population in Albania" by Elton Rogozi
2) "Review of veterinary pharmaceuticals against Parasitic Vector-Borne Diseases and their environmental impacts", by Clara Lima, on behalf of OHD4 collaborators

COST Action CA21111 general meeting
The summary is included in the pdf associated with this document.
The program:

- Stakeholders engagement plan: starting the discussion
- OHD Objective & Future plan (working together)
- OHD Academy
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YEAR 3 Annual Conference will be held in Antwerp (Belgium) next Spring 2025. Organizer Guy Caljon

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One in presence meeting will be held in Tirana. It will be organized by **Erjona Aibazay** and **Elton Rogozi.**

VISIT TO DIAMOND