4th Student Conference

“Zebrafish as an animal model”

24 of April 2025

ABSTRACT BOOK

Organizer: The Polish Zebrafish Society

Organizing committee:

Dr hab. Krzysztof Rakus, prof. UJ (Jagiellonian University in Krakow)

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AWARD FOR THE BEST PRESENTATION

Student with the best presentation will be awarded a fellowship to attend 7th Workshop of the Polish Zebrafish Society (Olsztyn, June 2025)

This award is found by the Polish Zebrafish Society

Invited lecture: Dr Magdalena Widziołek-Pooranachandran (UJ) 15:30-16:00		
Disease models		
1 16:00-16:10	Julia Szewczyk, Jagiellonian University in Krakow	Zebrafish (Danio rerio) as a Model for Steatosis and Hyperlipidemia
2 16:10-16:20	Asli Nur Altinay, University of Wrocław	Development of a new zebrafish model of human McArdle disease
3 16:20-16:30	Kinga Czachor, Medical University of Lublin	Investigating Novel TPM2 Variant-Induced Muscle Dysfunction Using Zebrafish Model
4 16:30-16:40	Sylwia Drzewiecka, Medical University of Lublin	Functional Characterization of TPM2 Splice Mutation causing Skeletal Muscle Defects using Zebrafish
Immunology		
5 16:40-16:50	Adam Chromiec, Jagiellonian University in Krakow	The role of innate immune cells in human melanoma progression: insights from a zebrafish xenograft model
6 16:50-17:00	Adriana Sarnek, Jagiellonian University in Krakow	How do the arginine-specific gingipains RgpA and RgpB affect the viability, morphology, and behavior of zebrafish larvae?
7 17:00-10:10	Artem Voitsekhovskyi, Jagiellonian University in Krakow	The role of 25-hydroxycholesterol in the antiviral response of zebrafish (Danio rerio)
8 17:10-17:20	Zaneta Baran, Jagiellonian University in Krakow	The cry1a and cry1b gene knock-outs affect immune response in TiLV- infected zebrafish larvae
9 17:20-17:30	Paulina Radwanska, Jagiellonian University in Krakow	The impact of Porphyromonas gingivalis virulence factors - gingipains on neuroinflammation and disease phenotype in zebrafish larvae
10 17:30-17:40	Ewa Bucon Jagiellonian University in Krakow	Zebrafish larval model to study infection with planktonic and biofilm-resident oral pathogens
Break 17:40-18:00		
Toxicology		
11 18:00-18:10	Agnieszka Szarek, Medical University of Warsaw	Toxicity of ethanol and caffeine combination. Effect on Zebrafish embryos in the early stage of organogenesis
12 18:10-18:20	Aleksander Warzecha, University of Warmia and Mazury in Olsztyn	Investigation of potential toxic effect of Bisphenol A in Zebrafish (Danio rerio)
13 18:20-18:30	Bogumił Łosiewicz, Warsaw University of Life Sciences	The effects of silver nanoparticles on development and morphology of zebrafish (Danio rerio) ovaries

14 18:30-18:40	Esther Uweh, Jagiellonian University in Krakow	Safety study of a new compound with tubulin inhibitory properties using a fish embryo acute toxicity (fet) test
15 18:40-18:50	Julia Dzik, Medical University of Warsaw	Assessment of embryonic toxicity of beet by-products in combination with endosulfan β as a model pesticide
16 18:50-19:00	Małgorzata Adamska, Medical University of Lublin	Safety assessment of novel 1,3,5-triazine derivatives as potential anti-cancer agents in breast adenocarcinoma using a zebrafish embryonic model
17 19:00-19:10	Tomasz Bartoszek, Warsaw University of Life Sciences	Effects of 3D printing resin dust exposure on mortality and hatching rate of zebrafish (Danio rerio) larvae – preliminary study.
18 19:10-19:20	Wanda Komorowska, University of Warsaw	Acute toxicity and behavioural changes caused by sodium azide – popular preservative in nano – and microplastics commercial solutions
19 19:20-19:30	Zofia Płonkowska, Medical University of Lublin	Locomotor activity alterations and embryotoxicity of acetaminophen and its metabolite in zebrafish larvae
Break 19:30-19:40		
Drug screening		
20 19:40-19:50	Anna Krauze, Medical University of Warsaw	Exploring Rapeseed Pomace Antioxidant Potential for Fish Feed to Combat Microplastic Pollution
21 19:50-20:00	Gloria Kaczmarek, Medical University of Lublin	Transgenic Tg(fli1:EGFP) zebrafish embryos as a valuable model in preclinical research on drug candidates for pathological angiogenesis-related diseases
22 20:00-20:10	Małgorzata Rasińska, University of Warmia and Mazury in Olsztyn	Evaluation of the Potential Otoprotective Effects of Aloe vera Extract in Zebrafish Larvae Exposed to Neomycin
23 20:10-20:20	Maria Cabaj, Medical University of Lublin	Structure-activity profile of selected synthetic cathinones
24 20:20-20:30	Marta Balcerowska, Medical University of Lublin	Evaluation of the effects of selected synthetic cannabinoids on locomotor activity and cardiotoxicity in the Zebrafish model
20:30-21:00 - Concluding remarks, Prize for the best presentation		

Zebrafish (Danio rerio) as a Model for Steatosis and Hyperlipidemia

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Background: Zebrafish *(Danio rerio)* are emerging as a valuable model for studying metabolic disorders like steatosis and hyperlipidemia. Unlike cell models, zebrafish enable a whole-organism approach to examine systemic lipid metabolism and organ interactions *in vivo*. Their small size, rapid development, and suitability for high-throughput screening make them efficient and cost-effective for large-scale drug discovery. This review aims to explore the available methods for inducing steatosis and hyperlipidemia in zebrafish at early developmental stages, which are critical for evaluating potential drug candidates during preclinical research.

Review: Zebrafish larvae, typically 5 to 20 days post-fertilization, are used to induce hyperlipidemia and steatosis through methods like high-fat diets, fructose exposure, and chemicals such as imazalil, thiacloprid, and clothianidin. Such a model can be used to evaluate the therapeutic activity of new compounds/pharmaceutical raw materials. The most commonly used techniques for analysis are imaging studies after staining with Oil Red O and Nile, high-performance liquid chromatography (HPLC), and biochemical studies on gene expression changes.

Conclusions: The zebrafish model offers a platform for studying steatosis and hyperlipidemia, providing valuable insights into disease mechanisms and potential therapeutic interventions. Its applicability in both basic research and preclinical studies underscores its importance in advancing our understanding of metabolic disorders. By leveraging this model, researchers can accelerate the discovery of novel drug candidates and improve strategies for treating human metabolic diseases.

Development of a new zebrafish model of human McArdle disease

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McArdle disease (Glycogen Storage Disease Type V) is an autosomal recessive disorder that is caused by mutations in the gene encoding PYGM (muscle glycogen phosphorylase), resulting in its impaired activity. In our project, we aim to develop an animal model for human McArdle disease using zebrafish (*Danio rerio*). Initially, the CRISPR-Cas9 technique was used to mimic the defected human gene in zebrafish models by knocking out the *pygm* gene in zebrafish and obtaining a pygm-/- line. This mutation was confirmed by assays with the use of restriction enzyme interactions with DNA samples. Our experiment was validated by checking the possible effect of microinjection on the survival rate of embryos. Several assessments were then made to analyze the mutation introduction effects, including birefringence (BF) analysis and morphological measurements in both wild-type and mutant larvae. Mutant animals showed a decrease in light intensity when compared to control, indicating impaired muscle integrity. Additionally, the morphological assessment revealed that 72hpf mutants were shorter in length than wild-type and demonstrated physical abnormalities. In conclusion, our results suggest that the new zebrafish mutant line pygm-/-, with impaired muscular glycogenolysis due to lack of PYGM, shows signs of McArdle symptoms such as disturbances in muscle fiber integrity. Although more detailed investigations are needed, we can conclude that the pygm-/- line will hopefully serve as a new tool in the study of McArdle disease.

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Investigating Novel *TPM2* Variant-Induced Muscle Dysfunction Using Zebrafish Model

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TPM2 encodes a tropomyosin isoform essential for regulating slow-twitch type 1 muscle fibers. We identified a family with clubfoot linked to a *TPM2* mutation and investigated its effects using zebrafish as a model organism. To assess the mutation's impact, we injected mRNA encoding wild-type or mutant *TPM2* into single-cell zebrafish embryos and monitored their development until five days post fertilisation. Mutant *TPM2* caused distinct phenotypes, including anterior-posterior shortening, severe pericardial edema, cyclopia, and a protruding mandible. Fluorescent staining of alpha actin filaments with Alexa Fluor 547 revealed significant abnormalities in fibril density, structure, and alignment. To further evaluate muscle integrity, we plan will use Evans Blue Dye to assess membrane stability and calcein to study neuromuscular junction (NMJ) functionality by tracking calcium dynamics in live tissues. Mitochondria plays a crucial role in calcium homeostasis, and their inhibition results in impaired muscle tension and relaxation. Confocal microscopy will provide detailed visualization of these effects. Our findings highlight *TPM2*'s role in muscle dysfunction and its potential contribution to clubfoot pathogenesis. This study emphasizes zebrafish as a valuable model for investigating muscle-related genetic disorders and offers novel insights into the molecular mechanisms underlying *TPM2*-associated defects. Understanding these pathways may help develop targeted therapies for muscle diseases linked to *TPM2* mutations, advancing research on congenital musculoskeletal disorders.

Functional Characterization of *TPM2* Splice Mutation causing Skeletal Muscle Defects using Zebrafish

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We identified a novel *TPM2* splice mutation in a Polish family linked to congenital clubfoot. *TPM2* encodes *β-tropomyosin*, a critical thin filament protein regulating contraction dynamics in slow-twitch muscle fibers.

To unravel the molecular mechanisms causing the phenotype, we carried out an mRNA overexpression using zebrafish. We injected mRNA encoding wild-type or splice-mutant *TPM2* into single-cell embryos. Mutant *TPM2*-expressing embryos manifested developmental anomalies, including pronounced anterior-posterior axis compression, pericardial edema, cyclopia, and muscular defects. Phenotypic analyses revealed alterations in muscle and skeletal tissues. Calcein staining showed disrupted calcification patterns and altered ossification centers, indicating that aberrant muscle development impacted skeletal morphogenesis. Alcian blue staining revealed abnormal cartilage development, supporting muscle-skeletal developmental axis perturbation.

Currently, we further explore the muscle phenotype. We will conduct birefringence analysis to quantify sarcomeric organization and myofibrillar alignment, providing insights into structural abnormalities. Additionally, we are optimizing locomotive assay to measure swimming velocity and distance.

These complementary approaches will establish a direct link between this TPM2 splice mutation and musculoskeletal pathology.

The role of innate immune cells in human melanoma progression: insights from a zebrafish xenograft model

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Abstract:

The innate immune system plays a crucial role in tumor progression, influencing both tumor growth and immune evasion mechanisms. In this study, we utilized the zebrafish (*Danio rerio*) larval model to investigate the impact of neutrophils and macrophages on human melanoma development. Human melanoma cells derived from *in vitro* culture of 451LU cell line were transplanted into zebrafish larvae to establish a xenograft model. Tumor growth was analyzed using fluorescence microscopy. To modulate the immune cell composition, we employed CRISPR-Cas9 gene editing to knock-down *irf8*, a key regulator of myeloid cell differentiation, thereby increasing neutrophil numbers while reducing macrophage populations. Tumor size in *irf8*-deficient larvae was compared with control groups. Our findings indicate that *irf8* knockdown leads to larger tumors compared to the control group, suggesting that altered neutrophil-to-macrophage ratios may contribute to enhanced tumor progression. These results highlight the importance of the innate immune response in melanoma development and suggest potential implications for immunotherapeutic strategies targeting myeloid cell populations. The zebrafish xenograft model proves to be a valuable tool for studying tumor-immune interactions *in vivo*, offering novel insights into the role of the innate immune system in cancer biology

How do the arginine-specific gingipains RgpA and RgpB affect the viability, morphology, and behavior of zebrafish larvae?

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Abstract

Porphyromonas gingivalis is a bacterium that has been studied as a periodonthopathogen, due to its involvement in periodontitis – a chronic inflammatory disease affecting gingival tissues. Research has shown that periodontal diseases may be a significant risk factor for Alzheimer's and cardiovascular diseases. The bacteria ability to induce inflammation is possible due to its virulence factors – components that allow a pathogen to evade host immunity. Among these virulence factors are arginine-specific cysteine proteases, called gingipains – RgpA and RgpB. They have been found to play a critical role in modulating the host immune response, hence becoming a subject of this study.

Using zebrafish larvae, we evaluated how RgpA and RgpB affect larvae morphology, survival, morbidity and behavior. Larvae (2 days post fertilization) were microinjected into the Duct of Cuvier with either RgpA, RgpB or activation buffer (control). After 24 and 48 hours post injection (hpi) yolk sac edemas, absence of blood flow and startle response to touch were detected. Moreover, at 48 hpi the light-dark locomotion test was performed, to assess the effects of gingipains on behavior.

Our results showed that the systemic injection of gingipains induced changes in: (i) zebrafish morphology such as yolk sac edemas, (ii) behavior such as no touch response and (iii) reduced viability, since more larvae in experimental groups exhibited symptoms of inflammation when compared to controls.

Therefore, we can conclude that gingipains contribute notably to the process of dysregulation of host immune response and need to be further investigated to determine mechanisms underlying their virulence potential.

The role of 25-hydroxycholesterol in the antiviral response of zebrafish (Danio rerio)

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25-hydroxycholesterol (25HC) is a member of the family of cholesterol oxidation derivatives called oxysterols. Its synthesis may be catalysed either by reactive oxygen species (ROS) or by 25-cholesterol hydroxylase (Ch25h). 25HC is known to exert biological activity against the range of enveloped and non-enveloped viruses, however knowledge about its role in the antiviral response in fish is very limited. In our project we have studied the role of 25HC in viral replication using zebrafish model. Zebrafish larvae (2 dpf, ABTL line) were intrapericardially injected with 25HC or with solvent only and subsequently infected with Tilapia lake virus (TiLV, enveloped), spring viraemia of carp virus (SVCV, enveloped) and nervous necrosis virus (NNV, non-enveloped). We showed that injection of fish with 25HC resulted in significantly lower level of viral load upon infection with all studied viruses. Moreover, during TiLV infection we studied influence of 25HC on the expression of genes encoding proteins involved in type I interferon pathway, proinflammatory cytokines and Ch25hb. We demonstrated that injection of fish with 25HC had almost no effect on the expression of studied genes. We have also derived zebrafish *ch25hb-/-* mutant line with 5 bp deletion using CRISPR/Cas9 method. Zebrafish larvae (2 dpf) of mutant line *ch25hb-/-* and WT line *ch25hb+/+* were intrapericardially injected with TiLV, and the viral load was studied. The viral load was significantly higher in *ch25hb-/-* than in WT line. Our work clearly demonstrated that 25HC influences the viral replication in zebrafish larvae.

The *cry1a* and *cry1b* gene knock-outs affect immune response in TiLV- infected zebrafish larvae

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The circadian clock mechanism, evolutionarily conserved across various organisms, plays a crucial role in synchronizing physiological responses with external conditions, primarily in response to light. It also regulates daily oscillations of the immune response during infections.

This study investigated whether silencing the clock genes: *cry1a* or *cry1b* affects the immune response in zebrafish larvae infected with Tilapia lake virus (TiLV). TiLV activates the type I interferon (IFN-I) pathway and triggers an inflammatory response, leading to the upregulation of genes encoding key antiviral factors, including pathogen recognition receptors (*rig-I, tlr3, tlr22*), transcription factors (*irf-3, irf-7*), pro-inflammatory cytokines (*ifn1, il-1b*) and the antiviral protein *mxa*.

Zebrafish larvae (2 dpf) were injected TiLV into the duct of Cuvier to assess immune responses in *cry1a* and *cry1b* knockout lines. The results showed that *cry* gene silencing affects antiviral response. In *cry1a*-knockout zebrafish, TiLV infection led to higher and faster mortality, accompanied by reduced *rig-I, tlr3, irf3 and irf7* expression. In turn, *cry1b*-knockout zebrafish exhibited also higher and faster mortality, increased *irf7* expression and reduced *il-1b* expression.

These findings suggest that circadian clock disruption affects antiviral immune response, influencing fish survival and the expression of type I IFN pathway genes.

The impact of Porphyromonas gingivalis virulence factors - gingipains on neuroinflammation and disease phenotype in zebrafish larvae.

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Alzheimer's disease (AD) is the most common neurodegenerative disease worldwide. Multiple risk factors contribute to the AD onset and progression. Recent research provides evidence for the association of periodontitis with neuroinflammation, a leading cause of AD. Porphyromonas gingivalis (Pg), is a keystone periodontal pathogen and the gingipains – the main virulence factors of Pg, were previously found in AD patients' brains.

Gingipains - arginine- (RgpA and RgpB) and lysine-specific (Kgp) are cysteine proteases that lead to the degeneration of host tissues to obtain nutrients for the bacterial survival.

The aim of this study was to investigate the ability of gingipains to induce mortality and morbidity in zebrafish larvae and to assess their role in development of neuroinflammation.

We examined zebrafish larval survival and development of disease symptoms such as: pericardial oedma size, decrease in heart rate, and cerebral blood vessel degradation after systemic injection with RgpA, RgpB and Kgp gingipains. We found that RgpB and Kgp caused higher larval mortality. Additionally, with use of transgenic zebrafish line with fluorescently labeled endothelium (Tg(kdrl:mTurquoise)) we observed vessel degradation upon RgpB and Kgp injection. Moreover, both gingipains induced microglia activation what was analyzed in transgenic zebrafish line with fluorescently labeled microglia/macrophages (Tg(mpeg:mCherry)). We also investigated the effect of gingipains on the expression of proinflammatory cytokines and microglial activation markers in the brains of zebrafish larvae Results indicate the development of neuroinflammation in response to RgpB and Kgp.

We believe that this research will shed new light on the association of periodontitis and neuroinflammation in Alzheimer's disease

Zebrafish larval model to study infection with planktonic and biofilm-resident oral pathogens

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Presenting author: Ewa Bucoń (e-mail: ewa.bucon@student.uj.edu.pl)

Many studies show a link between periodontitis and the development of cardiovascular and neurodegenerative diseases. Periodontitis, a chronic inflammatory disease of teeth supporting tissues, is caused by pathogenic bacteria residing in a subgingival dental plaque. Mature plaque, which is a type of bacterial biofilm, releases aggregated and planktonic bacteria, which can then lead to infections outside of the oral cavity.

Despite advancements, eradication of biofilm-living pathogens is very limited, emphasizing the need of new models for examining complex host-pathogen interactions with oral bacteria.

In this study, we used zebrafish larval systemic infection model to investigate: (i) pathogenic potential of representative core oral microbiome bacterial species (*Streptococcus mitis – Sm*, *Fusobacterium nucleatum – Fn*, *Porphyromonas gingivalis – Pg*, *Aggregatibacter actinomycetemcomitans – Aa*) and bacterial biofilm, (ii) kinetics of infection in the larvae, and (iii) the role of phagocytes during infection.

We observed that all bacteria exerted a dose-dependent pathogenic potential, with *Fn* being especially immunogenic despite its very fast eradication. *Sm* and *Aa* were able to multiply in the host. Infection with biofilm-residing bacteria suggested distinct infection kinetics with slower rate of bacteria eradication and *Pg* being persistent in the larvae. Moreover, in transgenic lines with fluorescently labelled macrophages and neutrophils, we observed changes in number of phagocytes e.g. increased number of both macrophages and neutrophils for *Fn* infection, macrophages for *Pg*, and neutrophils for *Aa*.

Zebrafish is powerful to model complex interactions between host and oral bacteria. Our data emphasizes the importance of mimicking the natural disease environment (biofilm vs. planktonic bacteria) more closely.

Toxicity of ethanol and caffeine combination. Effect on Zebrafish embryos in the early stage of organogenesis

Agnieszka Szarek

Master's thesis supervisor – dr Anna Małkowska

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Caffeine is a widely used substance around the world. It is primarily used to combat fatigue and improve concentration. It is a natural stimulant of the nervous system and can be found in over 60 plant species. It can be considered the most commonly consumed psychoactive substance in the world. Due to the widespread consumption of caffeine, the interactions of this substance have become a subject of interest for many researchers. Caffeine can interact with various substances, including medications and alcohol. Many of these interactions are related to the CYP1A2 enzyme, which is responsible for the metabolism of caffeine and some drugs. Our research focused on the interaction between caffeine and ethanol. Many researchers have explored this topic due to the popularity of combining these substances. This interaction has already been studied in animal models, and it was observed that alcohol, by inhibiting the activity of CYP1A2, prolongs the half-life of caffeine, which eliminates the sedative effect and may lead to strong stimulation.

Studies on the zebrafish model have shown that both ethanol and caffeine cause morphological changes, and at higher concentrations, they lead to embryo death. This study aimed to investigate the embryotoxic interaction effect of caffeine with simultaneous exposure to ethanol in an experimental model of zebrafish. Embryos were exposed to different doses of caffeine and ethanol.

Investigation of potential toxic effect of Bisphenol A in Zebrafish (*Danio rerio*)

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Bisphenol A (BPA) is mainly used in the industrial manufacturing of polymers such as polycarbonate plastics and epoxy resins.
These materials are found in a wide range of consumer products. Due to the widespread use and disposal of products containing BPA, this compound is also introduced into the environment exerting a negative impact on wildlife. The main mechanism by which BPA exerts adverse effects is through its role as an endocrine disrupting compound. BPA is primarily regarded as a xenoestrogen; however, research shows that it exerts a significant number of other interactions.
The wild strain of *Danio rerio* was prepared for spawning in breeding containers. Embryos at 0.25-1 hour post-fertilization (hpf) were harvested. Culture plates were then prepared for the test and control (E3) groups, with 10 individuals in each well, maintained at 28.5°C. A pre-prepared solution of 5 ml at specified BPA concentrations (10 µM, 20 µM, 40 µM) was added to each well. At 24, 48, 72, and 96 hpf, survival, hatching, and morphology were assessed. Additionally, at 24 hpf, the movement of the individuals was analyzed using DanioScope software. At 96 hpf, behavior of zebrafish larvae was analyzed using DanioVision (Noldus Software, Wageningen, The Netherlands).
We may suppose that BPA may have toxic effects on the embryonic development of zebrafish. Therefore, BPA may also disrupt activity of zebrafish.
This work was financially supported by the Minister of Science under the 'Regional Initiative of Excellence Program.'

The effects of silver nanoparticles on development and morphology of zebrafish (*Danio rerio*) ovaries

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Abstract: The aim of the study was to evaluate the effects of silver nanoparticles (AgNPs) on the morphology, growth, and ovarian development of female zebrafish (*Danio rerio*) from 30 days post-hatching over a period of 100 days. Fish were exposed to AgNPs at concentrations of 0 (control), 0.001, 0.01, and 1 mg/dm³. At the conclusion of the experiment, all fish were euthanized, measured, and weighed, followed by whole-body histological processing. The mortality rate varied slightly among the experimental groups, with the highest mortality (15.46%) observed in the 0.01 mg/dm³ AgNP group. Fish exposed to AgNPs exhibited reduced body size and weight compared to the control group. The smallest individuals were recorded in the 0.001 mg/dm³ AgNP group, with a mean total length of 18.81 ± 4.46 mm, while the lowest mean body weight (0.10 ± 0.05 g) was observed in the 1 mg/dm³ AgNP group. Exposure to 0.001 mg/dm³ AgNPs accelerated sexual maturation relative to the control group, whereas higher AgNP concentrations (0.01 and 1 mg/dm³) delayed maturation. This trend was evident in the distribution of oocyte developmental stages. In the control group, oogonia comprised 51.42% of the ovarian cells, while previtellogenic oocytes accounted for 48.58%. In contrast, in the 0.01 and 1 mg/dm³ AgNP groups, oogonia proportions increased to 67.31% and 65.76%, respectively, with corresponding reductions in previtellogenic oocytes (32.69% and 34.24%). Conversely, the 0.001 mg/dm³ AgNP group exhibited more advanced ovarian development, with 58.67% previtellogenic oocytes and 41.33% oogonia. These findings suggest that prolonged exposure to AgNPs in aqueous environments negatively impacts zebrafish growth and may disrupt ovarian development and sexual maturation.

SAFETY STUDY OF A NEW COMPOUND WITH TUBULIN INHIBITORY PROPERTIES USING A FISH EMBRYO ACUTE TOXICITY (FET) TEST

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The subject of the study was a new compound, MS-PK27b which shows a potent tubulin assembly inhibition property. Tubulin, a heterodimer of α and β subunits polymerize to form microtubules. Since microtubules have several functions in eukaryotic organisms including chromosome segregation, motility and the maintenance of cellular morphology, therefore tubulins are the proposed target for drugs against cancer, helminths and kinetoplastid parasites. MS-PK27b screened using *in vitro* assays displays nanomolar cytotoxicity against multiple cancer cell lines including T-lymphoblastic leukemia CCRF-CEM cells, leukemia K562 cells and their multi-resistant counterparts (cellosaurus CEM/DNR and K562 lymphoblast cells), lung carcinoma epithelial cell line (A549) and human colon cancer cell line (HCT116 and HCT116p53-/-). Cytotoxic studies showed that MS-PK27b is not toxic to normal human fibroblast cell line (BJ).

To determine acute toxicity of MS-PK27b on embryonic stages of fish we used Fish Embryo Acute Toxicity (FET) test with the zebrafish (*Danio rerio*) according to OECD guidelines for the testing of chemicals using zebrafish (ABTL-strain) larvae. Newly fertilized zebrafish eggs were exposed to MS-PK27b for a period of 96 hrs. Every 24 hrs four apical observations were recorded as indicators of lethality: coagulation of fertilized eggs, lack of somite formation, lack of detachment of the tail-bud from the yolk sac and lack of heartbeat. For testing 3,4- dichloroaniline was used as a positive control at a fixed concentration of 4 mg/L. At the end of the exposure period, acute toxicity was determined based on a positive outcome in any of the four apical observations recorded, and the LC50 values at 96 hrs for mortality was calculated.

These findings highlight MS-PK27b's potential as a selective anticancer agent, warranting further research

Assessment of embryonic toxicity of beet by-products in combination with endosulfan β as a model pesticide

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The residual by-products, such as beet tops, beet pulp and peels, are commonly regarded as agricultural waste. They often contain bioactive compounds, such as vitamins, minerals, phenolics, carotenoids, nitrates, and betalains, that can have both beneficial and harmful effects, especially on aquatic organisms, when disposed on landfills. There they can penetrate to variety of water bodies, including, but not limited to underground water, lakes and rivers.

The aim of our study is to assess the effect of the water and ethanol-water extracts of beetroot pulp and sugar beet pulp as well as juice on zebrafish embryos, in combination with endosulfan β as a model pesticide, as pesticides are frequently used in agriculture. We believe that results of this study can help to create and improve already existing waste management and water treatment processes in the agri-food industry and also help to reduce the negative impact of agriculture on the natural environment.

Safety assessment of novel 1,3,5-triazine derivatives as potential anti-cancer agents in breast adenocarcinoma using a zebrafish embryonic model

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Research into new anticancer therapeutics is crucial as the number of cancer incidence and mortality is still growing. The development of a cytostatic drug aims to design the structure of a compound that has a direct effect on cancer cells, without affecting normal cells. New drugs should be as selective as possible, and therefore safer for patients. Triazine derivatives show anticancer potential and depending on the position of nitrogen atoms in the ring, three isomers are distinguished: [1,2,3]triazines, [1,2,4]triazines, [1,3,5]triazines. In particular, the last mentioned derivatives exhibit promising remarkable anticancer activity in breast neoplasms confirmed by many research.

In this study, novel 1,3,5-triazine-based molecules, that showed cytotoxic activity against MDA-MB-231 breast adenocarcinoma cell line but did not affect the growth of normal human mammary fibroblasts (HMF line), were tested. Safety assessment was done using zebrafish model and Fish Embryo Acute Toxicity Test (FET). The lethal concentration (LC_{50}) as well as the percentage of morphological deformations were determined. In addition, the influence of new derivatives on heartbeat in zebrafish embryos was also documented.

Effects of 3D printing resin dust exposure on mortality and hatching rate of zebrafish (*Danio rerio*) larvae – preliminary study.

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Abstract:

The development of additive manufacturing technology, known as 3D printing, has undergone significant growth in the past decade, with future suggesting further development. Consequently, the need arose to determine the possible environmental risks of this new, emerging pollutant. Thus, a study was conducted based on short-term toxicological test on a model organism, the zebrafish (*Danio rerio*) embryos, according to OECD no. 236 guidelines. The material tested was a finely ground dust of two resins widely used in 3D printing, the Anycubic ABS-Like Pro White (ABS group), and Siraya Tech Build (STB group), at concentrations of 0.02, 0.2, 2, 20, and 200 mg/L. Average diameter of obtained nanoparticles was measured, as well as zeta potential and conductivity. Mortality in STB was higher than ABS, reaching 100% at 200 mg/L at the end of the test, compared to 25% in ABS. Hatching rate was significantly reduced in the highest concentration in STB, not exceeding 25% in 200 mg/L, and exposure to resin dust caused faster hatching. Additionally, abnormalities such as yolk oedema or spine disfigurement were observed in all treated groups. These results prove the need to further study possible toxic effects of resins used in 3D printing.

Acute toxicity and behavioural changes caused by sodium azide – popular preservative in nano – and microplastics commercial solutions

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Zebrafish is a popular model used for toxicity testing of micro- (MPs) and nano-plastics (NPs). Acute toxicity on embryos is tested using the Fish Embryo Toxicity Test (FET). In addition to evaluating general toxicity, behavioral tests are used to assess potential neurotoxicity. Most commercially available MPs and NPs for testing contain sodium azide, or other preservatives. In low concentrations, they do not cause toxicity, but can influence the results and thus hinder their correct interpretation. This study aimed to comprehensively investigate sodium azide's acute toxicity, using behavioural tests: the tail coiling assay, the light – dark locomotor activity test, and the color preference test. As a result of the conducted studies, LC_{50} (264 µM) and concentration inhibiting hatching were determined. No other abnormalities were observed. In behavioural tests, however, changes were already observed for the lowest concentration tested (2 µM), such as the disappearance of blue color preference (for both variants tested: blue – green, and blue – red), in color preference test. In the light – dark locomotor activity test for 50 µM, a decrease in total distance travelled in the dark was observed. The results show that even concentrations that do not cause visible changes in embryos development during the FET test, may produce neurotoxic effects. It is therefore important to take into account the effect of sodium azide when designing toxicity studies on NPs and MPs, to appropriately select the concentrations, or to use particles free of preservatives and other additives.

Locomotor activity alterations and embryotoxicity of acetaminophen and its metabolite in zebrafish larvae

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Acetaminophen, commonly known as paracetamol, is a widely used analgesic. It is an over the counter drug, generally considered very safe and is a first-choice pain and fever treatment for children and pregnant women, as well as for many at risk groups like people with heart and kidney diseases and stomach issues. However, a rising number of research questions the safety of its use in pregnancy. In both epidemiological and experimental research, in various animal and cell culture models, it has been reported that prenatal exposure to acetaminophen could possibly affect the fetal development, increasing the risk of disorders, such as neurodevelopmental, urogenital, endocrine or reproductive disorders.

In this study we tested toxic effects of various concentrations of acetaminophen (1.75-7mM) and its metabolite para-aminophenol (0.1875-6μM) in zebrafish embryos using a Fish Embryo acute Toxicity (FET). To test behavioral effects we performed a light/dark transition test at 96 hpf. In acetaminophen treated groups reduced pigmentation as well as morphological abnormalities were found, including pericardial edema, blood accumulation and spinal abnormalities. Locomotor activity in the dark was significantly lower in all concentrations of paracetamol. In two concentrations (5.25mM; 6.125mM) cardiotoxicity was observed. None of abnormalities were found in all non-lethal concentrations of para-aminophenol. However, its very low concentration of 6μM caused 100% lethality.

Exploring Rapeseed Pomace Antioxidant Potential for Fish Feed to Combat Microplastic Pollution

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Waste from plastic bottles made from polyethylene terephthalate constitutes a major portion of general plastic waste, much of which ends up in landfills, contributing to environmental pollution, including contamination of aquatic ecosystems. By 2023, the mass of plastic in the aquatic environment had reached 4.9 million tons.

Microplastic pollution poses a serious threat to aquatic organisms by inducing oxidative stress. This occurs through excessive production of reactive oxygen species (ROS), activation of mitogen-activated protein kinase signaling pathways, and disruption of antioxidant enzyme activity. These molecular disturbances can lead to toxic effects such as DNA damage, inflammation, and programmed cell death.

However, antioxidant compounds can mitigate oxidative stress. Rapeseed pomace, a byproduct of rapeseed processing, is a rich source of bioactive compounds with potential antioxidant properties. It contains tocols (tocopherols and tocotrienols), phytosterols, phospholipids, and phenolic compounds (such as sinapic acid and flavonoids). With global rapeseed production approximately 70 mln tons annually, substantial amounts of rapeseed pomace are generated, currently used primarily as animal feed.

Given its antioxidant potential, rapeseed pomace could potentially protect aquatic organisms against oxidative stress. In this study, its composition will be analyzed, including total polyphenols, total flavonoids, and antioxidant capacity via the DPPH assay. The protective effects of rapeseed pomace will be assessed using the zebrafish embryo toxicity test over three days, employing microscopic observations. If proven beneficial, rapeseed pomace could be repurposed as a sustainable ingredient in fish feed, potentially enhancing aquatic health, reducing plastic pollution impacts, and contributing to environmental sustainability.

Transgenic *Tg(fli1:EGFP)* zebrafish embryos as a valuable model in preclinical research on drug candidates for pathological angiogenesis-related diseases

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Pathological angiogenesis is observed in solid tumors, diabetic retinopathy, age-related macular degeneration (AMD), rheumatoid arthritis and psoriasis. In particular, cancer is still the second leading cause of death worldwide and pathological angiogenesis plays a significant role in tumor progression and increases the risk of tumor metastasis. According to the International Agency for Research on Cancer, in 2022, 20 million new cases of malignant tumors and 9.7 million deaths due to neoplasms were registered. The treatment choice depends on the type of tumor and its stage at the time of diagnosis but one option is to use a combination of an antiangiogenic drug and a classic cytostatic. Antiangiogenic therapy is a method based on the abolition of nutrients and oxygen supply to cancer cells by reducing the network of blood vessels and preventing the formation of new ones. Antiangiogenic drugs are also a basic method of treating eye diseases, i.e. diabetic retinopathy and AMD.

Tg(fli1:EGFP) line embryos are the most frequently used embryos for angiogenesis studies among various transgenic zebrafish lines. Researchers often conduct the experiments using a method based on the assessment of the development of intersegmental vessels of the trunk (ISVs). The evaluation criterion is the absence of ISVs and/or incomplete growth of ISVs from the dorsal aorta to the dorsal longitudinal anastomotic vessel (DLAV). Other methods include the evaluation of DLAV or sub-intestinal venous plexus (SIV or SIVP) growth. In turn, in the embryo's eye the inhibition of the growth of vitreous vessels is determined by the reduction of the main branches originating from the optic nerve head and/or an altered branching pattern.

Evaluation of the Potential Otoprotective Effects of Aloe vera Extract in Zebrafish Larvae Exposed to Neomycin

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Aminoglycoside antibiotics, such as neomycin, are known to induce ototoxicity by damaging sensory hair cells in the inner ear. This study aimed to assess the potential otoprotective properties of Aloe vera extract in zebrafish (Danio rerio) larvae. Transgenic Tg(brn3c:gap43-GFP) zebrafish at 4 days post-fertilization (dpf) were pre-treated with Aloe vera extract at concentrations of 2%, 0.2%, and 0.02% for one hour prior to a 30-minute exposure to 50 µM neomycin, which resulted in hair cell damage. The following day, larval locomotor activity was analyzed using the DanioVision and EthoVision XT system (Noldus), and immunofluorescent staining for activated caspase-3 was performed to evaluate apoptotic cell death. The number of surviving hair cells (GFP-positive) and apoptotic cells (IF staining) were quantified across treatment groups.

Our results showed that Aloe vera exposure alone led to a decrease in overall locomotor activity. Interestingly, in groups exposed to both neomycin and Aloe vera extract, a stimulatory effect on larval movement was observed. Further analysis of hair cell survival and apoptosis will provide insight into the extract's potential protective or modulating effects. These findings suggest that Aloe vera may influence neurosensory function, although its exact role in otoprotection requires further investigation.

Structure-activity profile of selected synthetic cathinones

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Abstract:

Novel psychoactive substances (NPS) or "designer drugs" are chemicals specifically synthesized to bypass current drug control legislation. They include derivatives of well-known substances of abuse or pharmaceutical products designed to imitate their effects. The amount of available designer drugs on the market is rapidly increasing and this phenomenon has been described as "NPS epidemic". Although properties of designer drugs tend to be similar to the substances they originate from, safety data on acute toxicity or long-term effects are still unavailable in many cases. Synthetic cathinones (SC), also known as bath salts, belong to the NPS group and are derivatives of the β-keto-amphetamine. Adverse effects besides behavioural outcomes include arrhythmias, which may result in myocardial infarction and sudden cardiac death.

The present study aims to evaluate the cardiotoxicity, lethality and behavioural changes induced by similar in structure selected synthetic cathinones in zebrafish larvae. In total we tested 8 different substances. Zebrafish 4 dpf (ang. days post fertilization) were exposed to the test drugs for 24 h to identify acute drug lethality, its impact on heart rate and locomotor activity of the larvae. We obtained 100% lethality concentration for all of the tested substances at 25 mM. We found that seven out of eight drugs were cardiotoxic in tested concentrations (0.1-25 mM). Moreover, all of them in lower tested concentrations impaired larvae's locomotor activity in the dark.

Evaluation of the effects of selected synthetic cannabinoids on locomotor activity and cardiotoxicity in the Zebrafish model

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Synthetic cannabinoids (SCs) are a chemically diverse group of new psychoactive substances that are a popular alternative to the Δ^9-tetrahydrocannabinol (Δ^9-THC) found in cannabis. Unlike natural cannabis, SCs encompass a wide range of chemical structures, leading to unpredictable and often more potent effects.

This study aimed to investigate behavioral effects and cardiotoxicity of SCs in the zebrafish model. Assessment of acute influence on locomotor activity of 5 days post fertilization (dpf) larvae was assessed in dark environment. Zebrafish larvae were placed individually in each well of a 96 well plate in the following SCs concentrations (0.01-100 µM). The measurement lasted 20 minutes and the video was recorded using the DanioVision chamber and activity parameters were calculated by the EthoVision program (Noldus). Following behavioral research, a cardiotoxicity evaluation experiment was conducted where 4 dpf zebrafish larvae were incubated for 24h with selected SCs in the aforementioned concentrations. Zebrafish larvae were later observed under a microscope and the heartbeat was measured.

All tested cannabinoids decreased locomotor activity, in comparison to the control group. In contrast, experiments on cardiotoxicity showed that, for most of the SCs tested, no effect on heartbeat was observed when comparing the tested substances to the control group.
The results of this study indicate that the chosen SCs decrease locomotor activity. However, most of them do not show a significant impact on the heartbeat.