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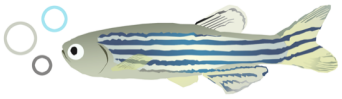

THESIS SUBJECTS ZEBRAFISHLAB 2019-2020

Our thesis subjects are grouped within 3 broad themes. Promoters are prof. dr. Dries Knapen and/or dr. Lucia Vergauwen. All of the subjects described below are suited for Master Projects (Biology, Biomedical Sciences, Biochemistry), some can be adapted to suit the needs of Individual Projects (Biology).

THEME 1.

An innovative alternative testing strategy for the identification of thyroid hormone disrupting chemicals

There is growing concern about the presence of endocrine disrupting chemicals in the environment. Endocrine disrupting chemicals are able to interfere with normal endocrine processes leading to adverse health effects. In the EU, specific legislation is in place to evaluate the risks related to the presence of chemicals in the environment both for humans and wildlife. Recent advances in EU legislation on the detection of endocrine disrupting chemicals are now demanding the development of innovative approaches to screen chemicals for their potential to disrupt the endocrine system. One of the fields where advances are particularly urgent is thyroid hormone disruption.

Thyroid hormones play an important role in metabolism, growth and development and therefore disruption of the thyroid axis can have important adverse health effects. There is a need for new alternative testing strategies to detect thyroid hormone disrupting chemicals, making use of non-animal alternatives and mechanistic information.

Until the age of 5 days zebrafish are not protected by the European legislation on the use of laboratory animals. Therefore, a 5 day zebrafish embryo test is considered an alternative to animal testing, while offering the benefits of a whole organism vertebrate model system which is highly relevant for chemical toxicity testing. This is one of the reasons why the zebrafish embryo has become a highly popular model system for chemical toxicity testing.

Thesis subject 1: The zebrafish embryo as an alternative model for thyroid hormone disruptor screening

In this thesis, we will use the zebrafish embryo as a model to screen chemicals for their potency to disrupt the thyroid hormone system. Several biological processes in vertebrates are regulated by thyroid hormones and disruptions of these processes can therefore serve as markers for thyroid disruption. Examples of thyroid-sensitive processes include eye development, inflation of the swim bladder and neurodevelopment. Zebrafish embryos will be exposed to known and suspected thyroid disrupting chemicals and thyroid disruption will be determined using endpoints on the molecular (e.g., transcriptional analysis of thyroid-sensitive genes using QPCR), biochemical (e.g., thyroid hormone levels), organismal (e.g., swim bladder inflation, eye morphology) and physiological (e.g., swimming behaviour) level.

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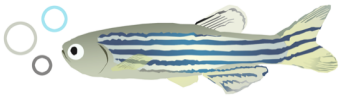

Thesis subject 2: Bridging the gap between human and environmental thyroid hormone disruptor screening

The adverse outcome pathway (AOP) framework is an innovative concept in 21st century toxicology. It provides a set of internationally harmonized principles to investigate and outline toxicological mechanisms starting from the molecular initiating event (e.g., hormone receptor binding) and describing the linkages leading to an adverse health outcome (e.g., impaired development). These linkages provide mechanistic understanding of the toxicological mechanism and the basis for predictive strategies. For example, a chemical's hormone receptor binding potential – measured using an *in chemico* assay - can be predictive of *in vivo* impaired development. We are currently developing an adverse outcome pathway network for thyroid hormone disruption on which new non-animal alternative testing strategies for detection of thyroid hormone disrupting chemicals can be based.

The hypothalamic-pituitary-thyroid axis is highly conserved across vertebrates. Therefore, thyroid disrupting chemicals can affect both mammalian models such as mouse and rat, which are typically used in human toxicology, as well as non-mammalian models such as fish and amphibians, which are commonly used in environmental toxicology. This offers opportunities for developing strategies that can address the need for chemical screening in the context of both human toxicology as well as environmental toxicology. This thesis will contribute to the development of a cross-species AOP network for thyroid disruption that supports thyroid disruption screening for both human and environmental toxicology.

THEME 2.

Unravelling the toxicological mechanisms underlying narcosis

At least 60% of the most commonly used chemicals in industrial applications are thought to act by narcosis. In a toxicological context, the term narcosis refers to lipophilic chemicals partitioning into cellular membranes as a function of their degree of lipophilicity, thereby causing toxicity. It is assumed that with increasing lipophilicity, chemicals have an increasing tendency to accumulate in membranes and disrupt their integrity and function. These chemicals can adversely impact a wide range of species, when they enter into the environment. Generally, loss of reaction to external stimuli, loss of equilibrium, decline in respiratory rate and death are expected in fish. However, the mechanistic details of how these chemicals cause toxic effects at the molecular and cellular level are largely unknown. Environmental risk assessment for this large array of chemicals is therefore currently mainly based on standard toxicity tests and lethality estimates using quantitative structure activity relationships (QSARs), which predominantly rely on lipophilicity estimates.

At this point, it is unclear which membrane types and which proteins are functionally impaired by narcotics. We hypothesize that the cell membrane is the first target of lipophilic compounds, which may then further partition into different organelle membranes. Many membrane-bound processes such as the mitochondrial electron transport chain are vital to cell survival and may be affected. The thesis subjects related to this theme will focus on further unravelling the mechanism of narcosis by identifying target membranes and investigating toxicity responses in the zebrafish embryo model at different levels of biological organisation (molecular, biochemical, physiological) and using a variety of techniques.

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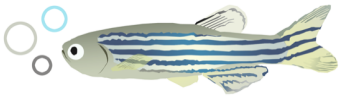

Thesis subject 3: Characterization of molecular, biochemical and physiological effects of narcotics in zebrafish embryos

In this thesis, zebrafish embryos will be exposed to narcotic chemicals. Disruption of cellular processes is often related to changes in transcript levels of related genes. At the molecular level transcriptional expression of selected genes (e.g., components of the electron transport chain) will be analysed using QPCR. At the biochemical level, the effects of narcotics on membrane-bound processes (e.g., mitochondrial electron transport chain activity) will be further explored. Such effects can be further characterized. For example, in cases where aerobic cellular respiration is disrupted, there may be a shift in the balance between aerobic and anaerobic ATP synthesis. By measuring lactate levels and lactate dehydrogenase (LDH) activity in exposed zebrafish embryos, the relative contribution of anaerobic and aerobic ATP synthesis can be evaluated. Responses will also be investigated at the physiological level which is of direct ecological relevance for risk assessment. Heart rate, swimming behaviour, oxygen consumption, growth and survival will be observed, and related to the findings on the molecular and biochemical level. We will observe loss of equilibrium, perform automated swimming behaviour analysis using a Zebrabox (Viewpoint), measure growth as larval length, monitor survival and determine heart rate in 1 day old embryos. Oxygen consumption measurements will be performed using small respiration chambers with optical oxygen probes suitable for zebrafish embryos. This knowledge is vital to understanding the mechanism and the impact of narcotics on the normal function of organisms.

Thesis subject 4: Visualisation of narcotic accumulation dynamics in zebrafish embryos

Understanding how narcotics distribute in organisms and which membranes are affected is vital to improve our understanding of the toxicological mechanism. Some attempts have been made to visualize subcellular localization of narcotics, but they were not able to reach the necessary spatiotemporal resolution. In this thesis, we will use state-of-the-art live imaging technology (light-sheet illumination confocal microscopy) to investigate the dynamics of accumulation of narcotics at the level of the whole organism. Secondly, we will map the partitioning behaviour of narcotics at the subcellular level: we will describe the relative importance of partitioning from cell to organelle membranes, including the mitochondrial membranes. This will lead to new hypotheses on suspected effects of narcotics on certain membrane-bound processes, which can then be specifically investigated.

THEME 3.

Development of a zebrafish model for DFNA9, autosomal dominant progressive hearing loss

Non syndromic hearing loss has been characterized to have monogenic inheritance in many traits. The so called DFNA9 type of autosomal dominant progressive sensorineural hearing loss is phenotyped in almost exclusively Dutch and Flemish patients from large families. The disease is linked to a mutation in the *coch* gene coding for Cochlin. Although the phenotype-genotype correlation is well studied in these families, the protein function of Cochlin remains unclear in the human inner ear.

Zebrafish hair cells provide an interesting model for studying loss of function of hair cells in humans. One of the advantages is that zebrafish hair cells present in the lateral line are easily accessible and their function can be investigated in behavior assays. One of the complexities of developing models

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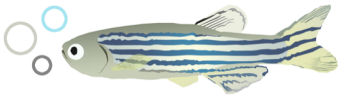

for this disease is its late onset. While models have been developed in mice, it is hoped that zebrafish, due to their short lifecycle and presence of hair cells in the lateral line, can provide a complementary model with perhaps an earlier onset.

On the long term we plan to develop a zebrafish line with a mutation in the *coch* gene which leads to symptoms comparable to what we observe in DFNA9 patients. State-of-the-art techniques such as CRISPR/Cas9 will be applied. This model can then be used to better understand the disease and to test possible treatments.

Thesis subject 5: Expression of *coch* in zebrafish and the impact of ototoxicants on hair cell function

Before one can attempt to induce a mutation in the *coch* gene in zebrafish, it is important to investigate *coch* expression in wildtype zebrafish with several genetic backgrounds. This will be the first step in this research project. Techniques will include QPCR and sequencing for detecting transcript levels and Western Blot and staining procedures for detecting the protein. After characterizing expression under normal circumstances, the potential effect of ototoxicants on *coch* expression will be investigated. Furthermore, the effects of these ototoxicants on hair cell integrity and function will be investigated using histological techniques and effects on balance and hearing will be investigated using behavioural assays. These methods are also important to phenotype the zebrafish DFNA9 model that will be developed in a later stage of the project.

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