Leveraging Multi-Omics Technologies for Studying the Effects of Endocrine Disrupting Chemicals on Thyroid In Vitro Models

Impact of the Thesis

Endocrine disrupting chemicals (EDCs) are a large group of compounds comprising many manmade substances that pollute the environment and can interfere with the normal functioning of the endocrine system, including the thyroid, and as such they constitute an important environmental concern. They are found in common everyday products (like food packaging, medical plastic material, wire and cable sheathing, paints and coatings, textiles, fuel), and daily exposure of the general population occurs via the diet, air, skin, and water. My PhD, as part of the SCREENED (SCREENing for Endocrine Disruptors) project, focused on the thyroid, an essential endocrine organ understudied within the field of toxicology. SCREENED aimed at answering the following questions: can we develop 3-dimensional *in vitro* models of thyroid for studying the effects of EDCs? And are these compounds having a deleterious effect directly on the thyroid?

To this end, our partners at the Université Libre De Bruxelles in Belgium developed a protocol for differentiating thyroid organoids from human and mouse embryonic stem cells which we used for our experiments together with thyroid cell lines. We focused on four EDCs classes named organophosphate flame retardants (OPFRs), phthalates, polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) and performed several screenings during the project using a relevant dose range that would be relevant to human daily exposure, testing the difference between static and organ-on-a-chip culture conditions, as well as evaluating the response over a short- or long-term exposure. We used several omics techniques, which allow to perform an exhaustive profiling of several biological molecules, to study how EDCs can affect the transcriptome, proteome and epigenetic status of cells.

To provide some examples, we observed that our *in vitro* thyroid models can respond to aryl hydrocarbon receptor (AHR) agonists such as PAHs or some PCBs with the induction of the cytochrome P450 (the main detoxifying enzymes of the human body) genes *CYP1A1* and *CYP1B1*, to our knowledge a phenomenon to date only described in a handful of thyroid cancer cell lines. We also observed that phthalates can induce fatty acid metabolism and downregulate the transduction of important signaling proteins and extracellular matrix organization. Combining gene, miRNA and protein expression data, we built a machine learning classification model that could help us identify if an unknown sample was exposed to one of the EDCs classes we studied.

We showcased how omics approaches can be used in toxicology experiments to elucidate the cell response to harmful chemicals and provided hypotheses to be further tested with targeted experiments. Together with our other SCREENED partners, we started developing a model and tested it, laying the basis for a future use of *in vitro* models for endocrine disruption

testing. We generated a wealth of omics data derived from thyroid models exposed to EDCs that we deposited (or will deposit) in repositories, making it publicly available. Compared to the greater focus that other organs, like liver and intestine, receive, the field of thyroid toxicology is relatively understudied, and we started to fill a gap that we believe can be of use to other researchers.

Our work has led us to publish two of the chapters included in this thesis and, at the date of writing, having one under review. Striving to make our research FAIR (Findable, Accessible, Interoperable and Reusable), we always opted for open access journals, making our findings discoverable to anyone who is interested, and shared the data and scripts used for the analyses.