Ecotoxicogenomic profiles of thyroid disruption in zebrafish embryos

Antonia Bruder[1], Steve U. Ayobahan[1], Fabian Essfeld[1], Julia Alvincz[1], Christoph Schäfers[2] and Sebastian Eilebrecht[1]

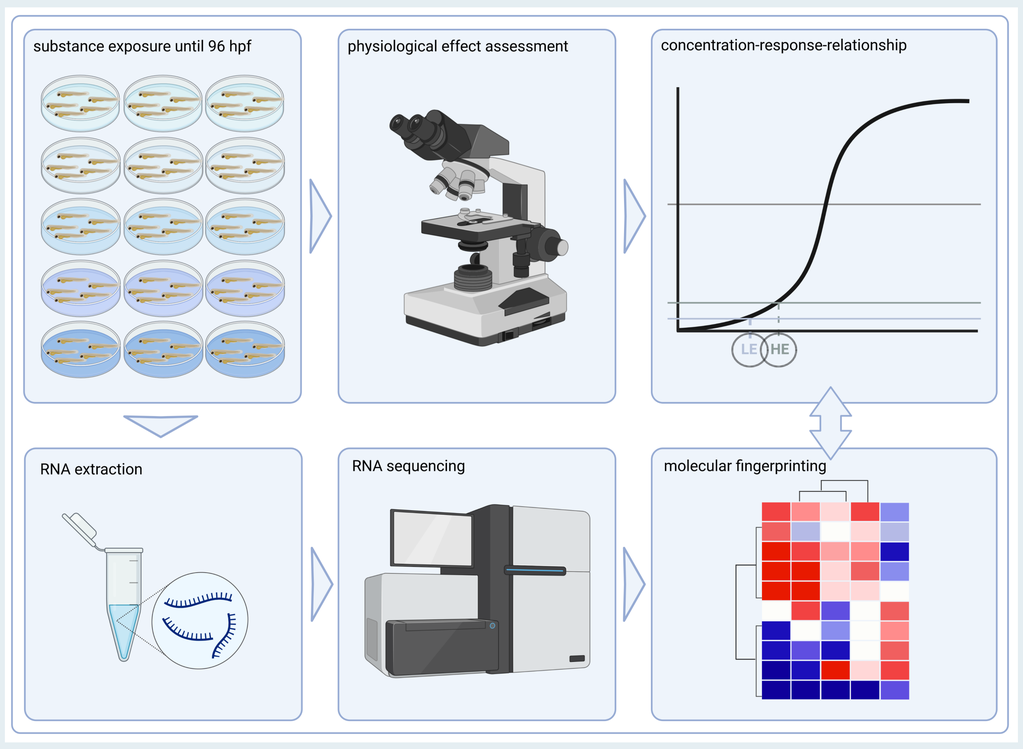
[1] Fraunhofer Attract Eco'n'OMICs, Fraunhofer Institute for Mulecular Biology and Applied Ecology IME, Auf dem Aberg 1, 57392 Schmallenberg, Germany
[2] Department for Ecotoxicology, Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Auf dem Aberg 1, 57392 Schmallenberg, Germany
Email contact: sebastian.eilebrecht@ime.fraunhofer.de

Active ingredients of pesticides, biocides, but also industrial chemicals can have adverse effects on environmental organisms, which can threaten populations and have far-reaching consequences for the ecosystem. To avoid such effects, European legislation requires chemical manufacturers to provide data for environmental risk assessment (ERA) of active substances in order to be registered. Current ERA of endocrine disruptors targeting thyroid hormone-related physiological processes requires large numbers of amphibians, which is expensive in terms of both resources and animal consumption[1]. Consequently, alternative approaches to screening compounds are needed, and cost-effective, reliable biomarkers must be identified. Omics methods are attractive for collecting consistent high-content data and identifying chemical modes of action (MoA) for prioritising ERA. In the context of identifying screening-compatible biomarkers for thyroid MoA, we analyzed early molecular signatures induced by the deiodinase inhibitor iopanic acid and the thyroid peroxidase inhibitor methimazole in zebrafish embryos, complementing previous studies[2]. Our goal was to generate robust and reliable MoA-specific fingerprints that can be used in screening approaches to identify candidates with thyroid activity to prioritise developmental compounds.

Materials and methods

To determine compound-induced endpoints, freshly fertilised zebrafish eggs were exposed to a range of concentrations of the two test compounds according to the Fish Embryo Toxicity (FET) test[3]. In addition to lethality, hatching rate and swim bladder size were assessed as endpoints at 24, 48, 72, 96 and 120 hours post fertilisation (hpf) (Figure 1). According to the results of this range finding test, a modified version of the FET was performed for different sublethal concentrations of iopanic acid (0, 3 and 6 mg/L) and methimazole (0, 75 and 150 mg/L). Fifteen freshly fertilised eggs per replicate and condition were exposed to the test solutions and appropriate controls in three biological replicates. At 96 hpf, total RNA was extracted from the embryos and subsequently subjected to transcriptome analysis by RNA sequencing followed by differential gene expression analysis using the DESeq2 algorithm[4].

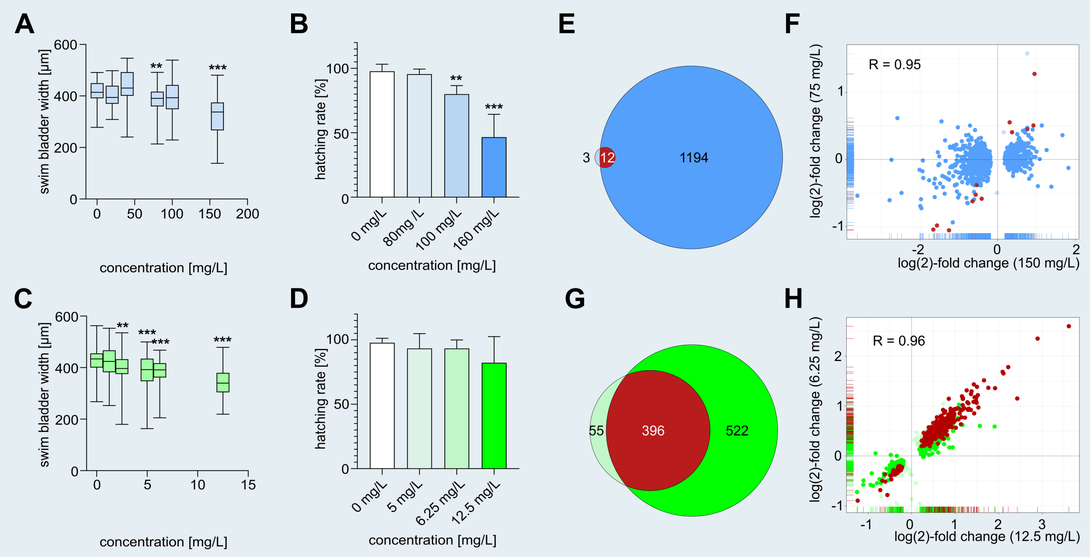
Figure 1: Experimental design for identifying molecular signatures of thyroid disruption in zebrafish embryos. Created by Biorender.com



Results and discussion

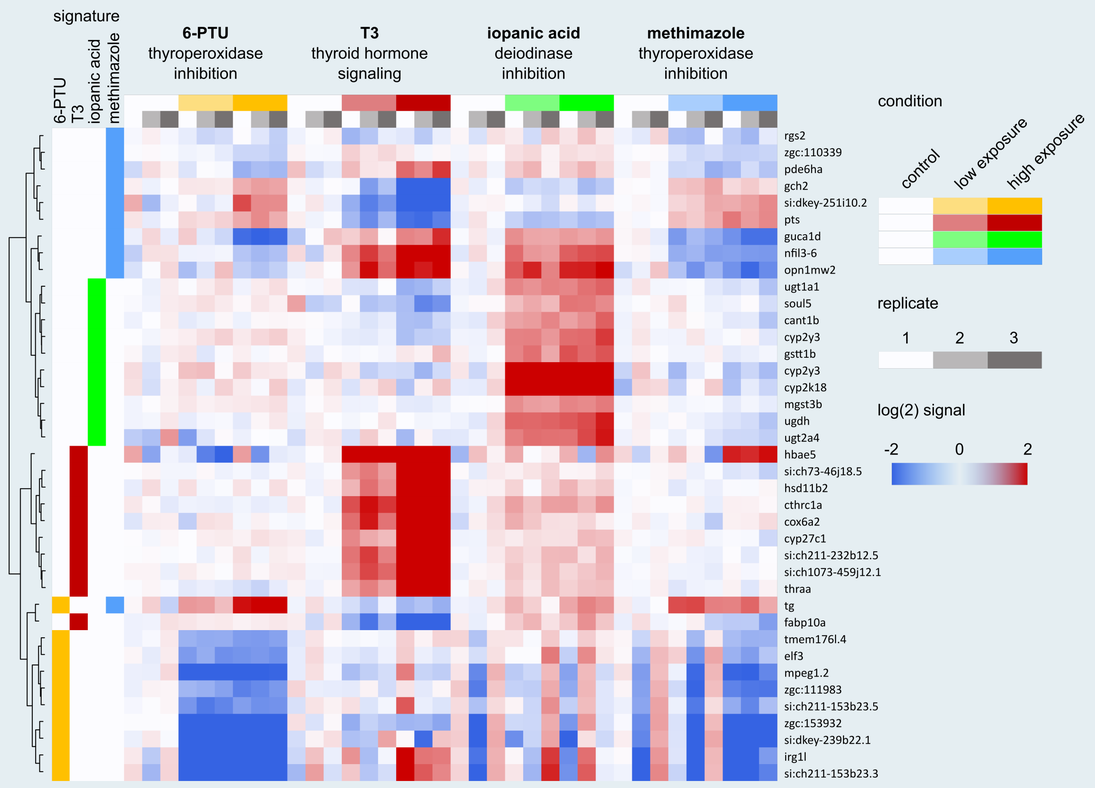
Exposure of zebrafish embryos to methimazole and iopanic acid for 5 days resulted in a statistically significant decrease in swim bladder width (Figure 2B and D). While no significant changes in hatching were observed after exposure to methimazole, a statistically significant delay in hatching was observed after exposure to iopanic acid (Figure 2C and D). These observed physiological effects are consistent with previous literature on thyroid hormone disruption[2,5], and a concentration-dependent hatching delay was previously observed in zebrafish embryos after exposure to the fungicide thiram, a putative inhibitor of thyroperoxidase (TPO)[5]. Previous results of transcriptome and proteome analyses have shown that muscle function and contraction are generally impaired by treatment with T3 and 6-PTU[2]. The previously observed impairment of smooth muscle fiber development with T3 exposure has been suggested as a possible mechanism by which thyroid disruption impairs swim bladder inflation[6]. In addition, impaired muscle functionality leading to impaired spontaneous movement of embryos could explain the observed hatching delay.

Figure 2: (A-D) Swim bladder size (120 hpf) and hatching rate (48 hpf) after exposure of zebrafish embryos to methimazole (blue) and iopanic acid (green). (E-H) Differentially expressed genes at 96 hpf and their correlation in zebrafish embryos after exposure to methimazole (blue) and iopanic acid (green)..



Our transcriptomic approach identified for both test substances a concentration-dependent increase of differentially expressed genes (DEGs) after exposure to sublethal low effect concentrations (Figure 2E and G). Furthermore, these DEGs were consistently regulated when comparing both exposure concentrations (Figure 2F and H), indicating a robust and specific response. We defined the common subset of DEGs after each exposure concentration as specific fingerprint and compared the fingerprints of deiodinase inhibition (iopanic acid) and thyroperoxidase inhibition (methimazole) with our previous results for thyroid hormone activation (T3) and thyroperoxidase inhibition (6-PTU)[2]. While iopanic acid showed a distinct gene expression pattern, methimazole induced a very similar pattern to 6-PTU (Figure 3), strongly arguing for MoA-specific gene expression fingerprints of thyroid hormone disruption in zebrafish embryos, which may be used in the future to assess and classify these hazards in this model organism.

Figure 3: Heatmap with gene expression signatures of 6-PTU (yellow), T3 (red), iopanic acid (green) and methimazole (blue) in zebrafish embryos.



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