Sweet but Toxic?

Ecotoxicogenomic Hazard Assessment of Artificial Sweeteners in Aquatic Model Organisms

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Why are artificial sweeteners of ecotoxicological concern?

Since their approval as food additives, artificial sweeteners are commonly used anthropogenic substances all around the world. The widespread and intensive consumption of artificial sweeteners in combination with their high stability and water solubility has led to their release into the aquatic environment, where they prove to be persistent [1]. Given that no detailed environmental risk assessment was carried out as part of the food additive approval process, it is still unclear whether and to what extent ecotoxic effects are to be expected [2]. Although this has already been pointed out in literature multiple times, studies regarding the ecotoxicity of artificial sweeteners are still lacking.

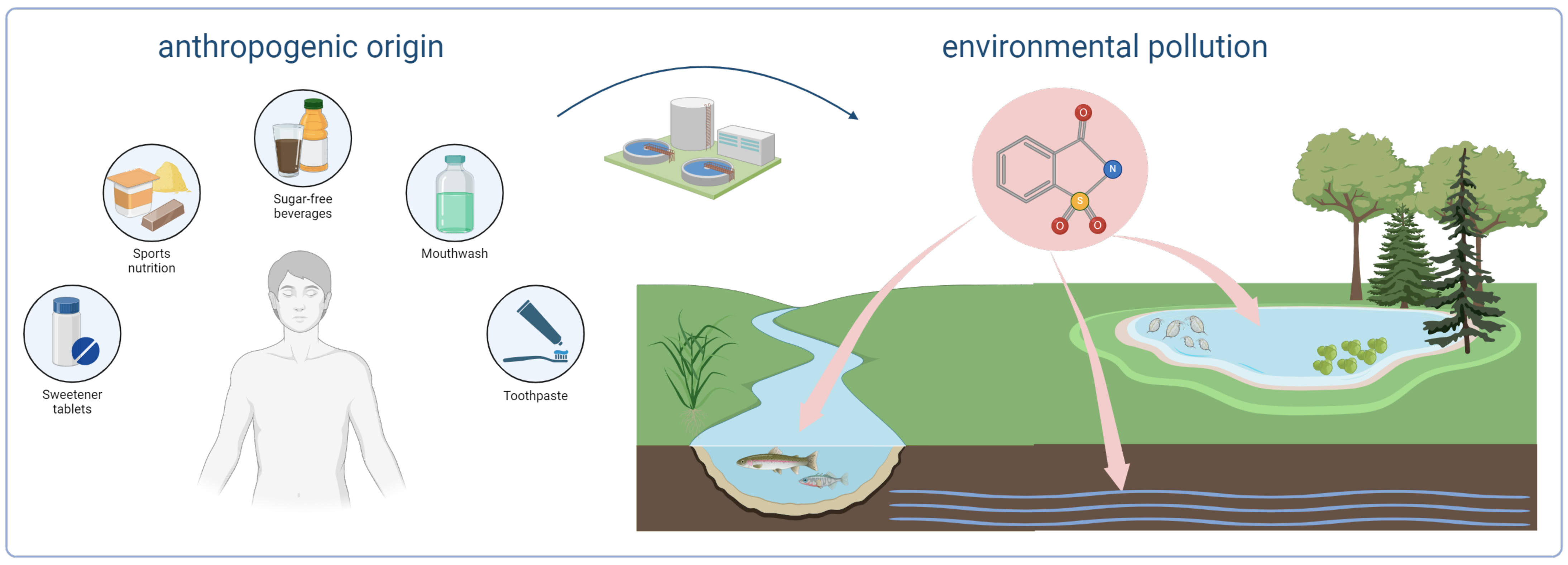


Figure 1: Consumption of non-caloric sweeteners leading to their release into the aquatic environment. Created with Biorender.com.

Stable • Hydrophile • Persistent • Ecotoxic?

The global consumption of artificial sweeteners amounts to more than 159,000 t per year [2]. Although the majority is used for low-calorie beverages, apart from the food sector other areas also play a role, such as personal care products, pharmaceuticals, animal feed or, in individual cases, industrial use [3]. The entry of artificial sweeteners therefore mainly takes place via domestic wastewater due to insufficient elimination by wastewater treatment plants, but also by the agricultural and industrial sector. The combination of high solubility, stability and persistence leads to a continuous and increasing burden on the environment [2].

The most relevant representatives regarding environmental persistence are Sucralose, Acesulfame, Saccharin and Cyclamic acid. They are all found in the aquatic environment at concentrations reaching tens of µg/L [3].

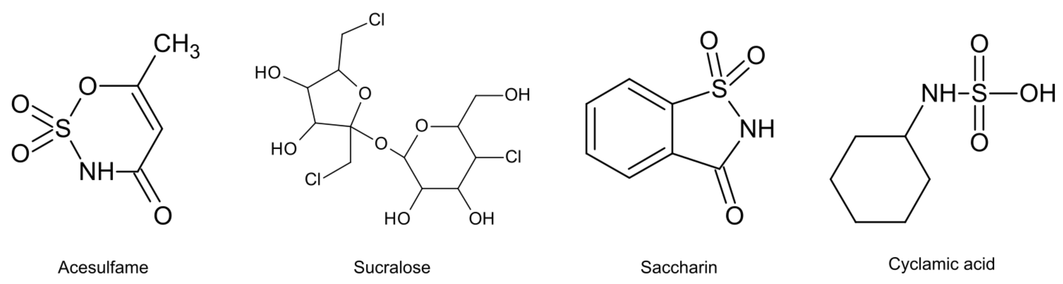


Figure 2: Molecular structures of the four environmentally most relevant sweeteners. Created with ChemSketch and Inkscape.

Approval without Ecotox. Risk Assessment

The lack of ecotoxicological risk assessments of artificial sweeteners is due to the authorisation procedure of food additives, to which these substances belong [4]. As the authorisation procedure requires a detailed assessment of human toxicology but not of their environmental risk, artificial sweeteners could therefore be authorised for human consumption without knowing the ecotoxicological risk [5].

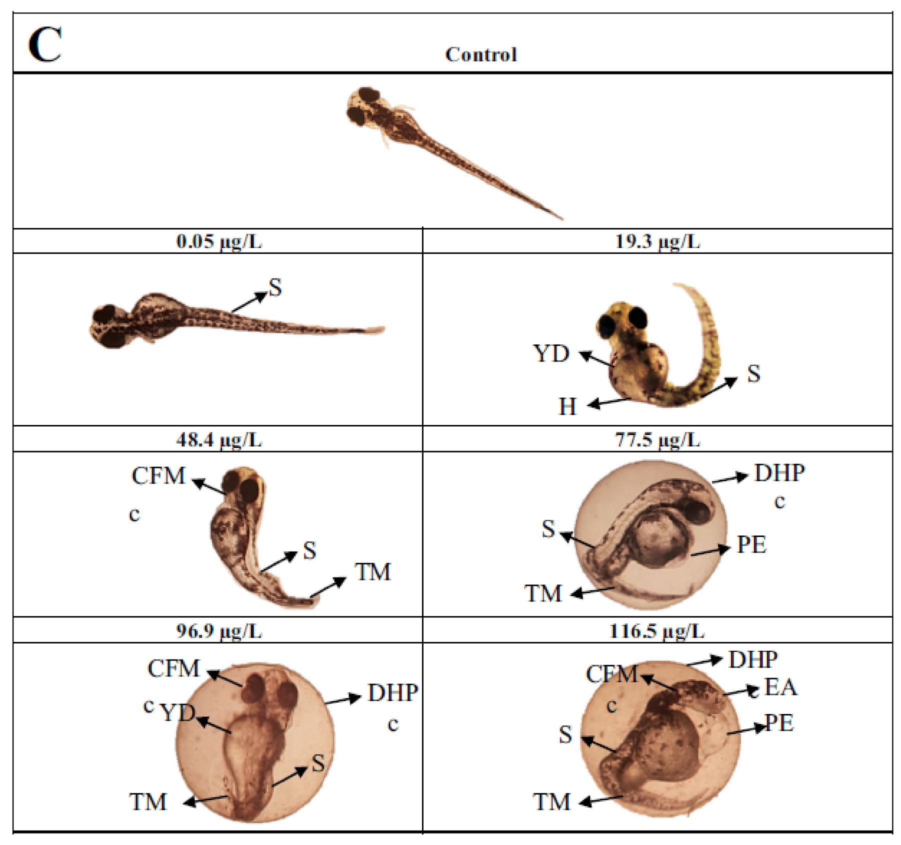
Have any ecotoxic effects been identified so far?

Table 1: Examples of ecotoxic effects of Acesulfame (ACE), Sucralose (SUC), Saccharin (SAC) and Cyclamic acid (CYC). Cursive = Effects, caused by concentrations in the µg/L-range.

Sweetener	organism	effect
ACE, SUC	*carp*	*oxidative stress*[6]
ACE, SUC	*daphnids*	*Neuro- and cardiotoxicity*[7]
SUC	*zebrafish*	*malformations, letality*[8]
SAC	zebrafish	Teratogenicity[9]
SAC	earthworm	Reprotoxicity[10]
CYC	onion	cytotoxicity, mutagenicity[11]

Yes, several studies have conducted to monitor toxic effects in various (model) organisms. Table 1 shows a selection of available studies. It should be noted that most studies point to artificial sweeteners as emerging environmental contaminants and that studies on this topic are largely lacking.

While most of the observed effects were caused by concentrations higher than environmentally relevant levels, some studies found toxic effects at test concentrations in the µg/L range (*cursive* in Table 1). For example, a study by Colín-García et al. in 2022 tested low concentrations of Sucralose on zebrafish embryos. As shown in Figure 3, the embryos exhibited malformations even at the lowest test concentration of 0.05 µg/L [8].



S: scoliosis, PE: pericardial edema, YD: yolk deformation, CFM: craniofacial malformation. EA: eye absence, H: hypopigmentation, DHP: delay of the hatching process, FA: fin absence, D: dwarfism.

Figure 3: Examples of the main embryonic alterations induced by Sucralose on Danio rerio embryos [8].

What is the aim of my doctoral project?

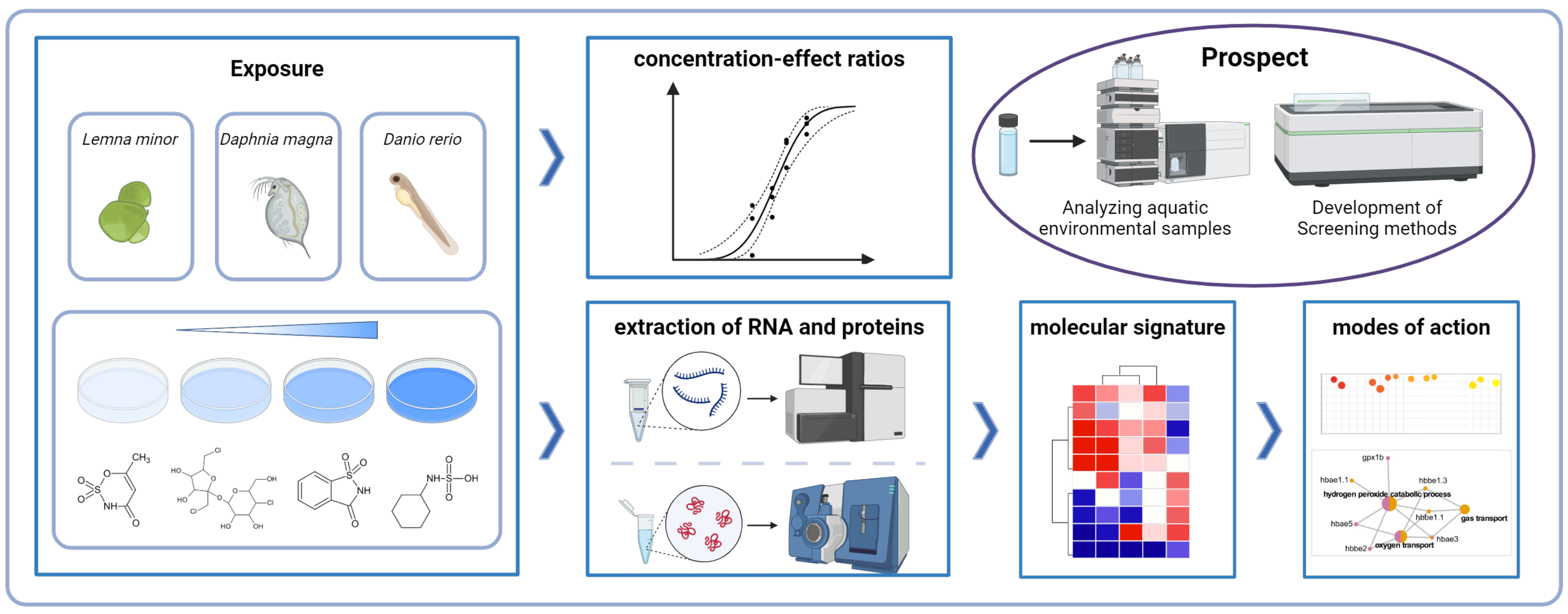


Figure 4: The performance of OECD-guideline tests combined with OMIC-methods enables the assessment of possible modes of action of artificial sweeteners. Created with Biorender.com.

My project aims to assess ecotoxic effects of artificial sweeteners, using OMIC-methods. To fill existing data gaps, investigations regarding their effects on ecotoxicological model organisms from different eukaryotic kingdoms will be performed. More precisely, the aquatic plant *Lemna minor*, the Crustacean *Daphnia magna* and the teleost fish *Danio rerio* (embryo) will be examined to the four most relevant sweeteners. In addition to the performance of the corresponding guideline tests of the Organisation for Economic Cooperation and Development (OECD), effects will be recorded at the gene expression level using transcriptomics and proteomics and thus enable insights into the modes of action that correspond to the hazardous effects. This promising combination of methods will be performed on artificial sweeteners for the first time, which finally may show that the assessment of ecotoxicity should no longer be neglected in the approval procedures for food additives.

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