Session: Advances in evaluating and regulating endocrine disruptors
Platform Presentation
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Single Pulse Exposure of Different Life Stages of Zebrafish to the Selective Estrogen-Receptor Modulator Tamoxifen Citrate

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1. Introduction

The authorisation of chemicals, like plant protection products (PPP), is bound to high-level environmental safety and quality standards. Part of the environmental risk assessment (ERA) of a PPP is the evaluation of risks posed to populations of aquatic organisms. Especially in cases, where incidences of endocrine disrupting properties arise, extensive toxicity tests have to be performed. A positive outcome in these test will result in a non-admission of the tested PPP [1]. The rationale behind this hazard-based approach is mainly caused by uncertainties in establishing monotonic dose-response curves and low-dose effects of endocrine disruptors [2]. Nontheless, the discussion about the existing scientific evidence is on-going. For example, proposals for a risk-based approach instead of hazard assessment considering the above mentionend uncertainties have also been made [3, 4]. In this study, zebrafish (*D. rerio*) were exposed to a pulse concentration of Tamoxifen citrate, a well researched endocrine disruptor which has been proposed as a reference substance as mentioned by [5]. The aim was to examine if a peak concentration of an endocrine disruptor, displaying a more realistic exposure scenario of non-target animals in light of PPP use, might lead to distinguishable effects and the establishment of a dose-response relationship is possible.

2. Materials and methods

The test design features a water-sediment-system, which is able to ensure stable test conditions over the whole study period (Figure 1). Fish of three different life-stages (group A: 40 eggs, group B: 20 juvenile fish and group C: 16 adult fish) were separately introduced into compartments divided by stainless steel gauze. Observed developmental phases included early life-stage survival for group A, juvenile growth phase for groups A and B, and reproduction, F_1-generation early life-stage survival and growth for all groups A, B and C. Blood samples for vitellogenin measurements were collected from adult fish at group termination. Four concentrations of TC (125 µg/L, 250 µg/L, 500 µg/L, 1000 µg/L) were applied as a pulse to three replicates each (Figure 2). Four replicates containing test water only served as a control.

Figure 1: Exemplary picture of the water-sediment test system used

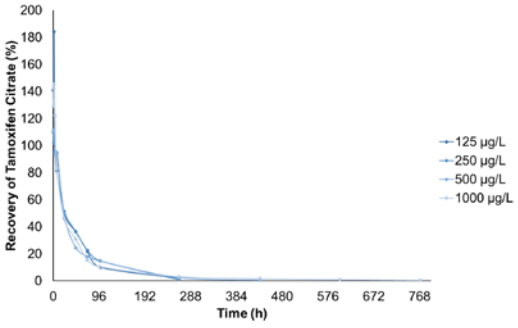

Figure 2: Dissipation of Tamoxifen citrate over the course of the experiment

3. Results and discussion

The presented results are preliminary as the experimental phase of the study is not yet finished. The concentration setting was crucial to exclude unintended effects of acute toxicity (e.g. mortality, liver damage), as the objective of the study was to survey influences on the endocrine system. Persistence of the test substance in the test systems was longer than determined by preceding experiments. Mortalities occurred in all developmental stages (groups A to C), especially in the highest and second-highest concentration (500 µg/L, 1000 µg/L). Interestingly, in sexually mature fish (group C) mortality was higher in males. A general decrease in fertility rates (group C) could be observed (Figure 3), whereas total egg numbers appeared unaffected. The results were mirrored for fish introduced as juveniles (group B). While fertility rates were not

influenced negatively, fecundity was lower in both remaining concentrations (125 µg/L, 250 µg/L). Observation of reproduction parameters is still on-going for fish introduced as eggs (group A).

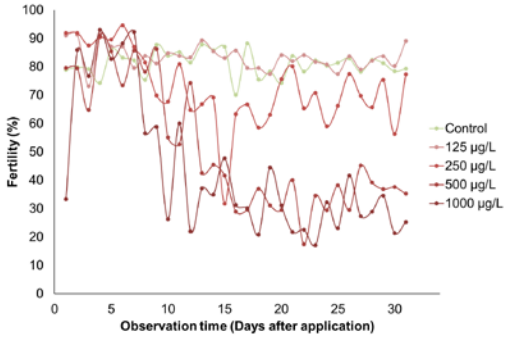

Figure 3: Mean fertility (%) of group C

Moreover, impairments and alterations of eggs were observed several days after exposure (group C). Changes in egg morphology were noticed in all monitored exposure groups. Consequently, F_1-fish originating from fish introduced as adults (group C) showed a dose-dependent decrease in survival rates. Also, F_1-generation (group C) length and weight decreased dose-dependently, while length and weight measurements of the surviving parent fish (group C) showed similar results across all groups. Remaining data on growth (groups B and C) will be completed after successful rearing of the respective F_1-generation.

Prolonged exposure might have contributed to the occurrence of high mortality rates, especially in high test concentrations. Higher male mortality might hint at a sex-specific toxicity of TC though. The test substance can act as both estrogen-receptor agonist and antagonist depending on the respective tissue. Due to the observed mortality and loss of several reproducing replicates, assessment of reproduction parameters (fertility, fecundity) need to be interpreted with care. The decrease in fertility might be influenced by the high male fish mortality in concentrations of 500 µg/L and 1000 µg/L.

4. Conclusions

Although reproduction data (Figure 3) are difficult to be attributed to endocrine activity, an influence on the endocrine system of the test animals seems apparent. Particularly sex specific effects in F_0-animals as well as an impaired early life-stage in F_1-animals are of highest interest. Vitellogenin measurements of sexually mature animals, the assessment of development and sex ratio of F_0-animals in groups B and C, just as further data on reproduction parameters and early life-stage development of these groups, will help to clarify pending questions concerning endocrine disruption. The results willl also be used for a comparison with available data originating from a flow-through study with TC in zebrafish [6]. Additionally, several other accessible datasets from zebrafish studies featuring paired pulsed and flow-through exposures of endocrine disrupting substances with diverse dissipation times will be integrated in the concluding assessment. The final objective is to deduce possible effect thresholds based on internal concentrations to help answering open questions in relation to the assessment of endocrine disrupting substances.

5. References

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