The direct link between endocrine MoA and adverse effect – Concepts to integrate molecular information in endocrine disruptor testing

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1. Introduction

To be defined as an endocrine disruptor, a substance has to meet several criteria, including the induction of specific adverse effects, a specific endocrine mode of action (MoA), and a plausible link between both.

In order to prove causality, we applied different approaches, which utilize the Adverse Outcome Pathway (AOP) concept. We demonstrated that AOPs, in this case an AOP for Aromatase inhibition leading to reproductive dysfunction in fish, can be transferred to other life stages, and also other species. Identical molecular initiating events (MIEs) and key events (KEs), as well as their relationships (KERs) are responsible for specific effects in different life stages. Thus, similar screening methods might be applied for the prediction of different, endocrine-related adverse effects, like reproduction in adult fathead minnow and a skewed sex ratio in juvenile zebrafish.

Presumably sexual endocrine-related effects might also be triggered by other MIEs, belonging for example to endocrine and non-endocrine AOPs. Identification of these differences is of mayor importance, as regulation of endocrine disruptors is based on hazard assessment, while for non-endocrine substances a risk assessment has to be performed. Tests with fish performed according to OECD test guidelines are often not able to identify the mode of action of a substance, as only apical endpoints, which could be triggered by substances with different MoAs, are assessed. AOPs for these different MIEs are existing; however, the data gap has to be closed by methods not routinely applied in risk and hazard assessment. For example molecular signatures, which are MIE-specific rather than substance-specific, can be identified by high-content approaches like proteomics or RNAseq.

We applied this approach in order to discriminate the AOP of aromatase inhibition from the AOP of hepatotoxitiy during the reproductive phase of adult zebrafish. Already the number of regulated proteins/genes in the respective tissues can give hints to the most affected organ. Furthermore, a deeper look into the signaling pathways and into specific regulated proteins allow the identification of biomarkers, which can be further developed to be applied in *in vitro* screening tests, e.g. with the zebrafish embryo.

2. Results and discussion

2.1. Aromatase inhibition in fish – a comparison between life stages

We performed a Fish Sexual Development Test (FSDT) with zebrafish and the test substance fadrozole according to the OECD TG 234 in combination with non-standard endpoints like expression analyses of genes related to steroid hormone signalling [1]. Combined to the standard endpoints assessed during an FSDT, it was possible to prove the chain of KEs from reduced estrogen receptor signalling as a result of inhibited aromatase activity up to the population relevant AO of a skewed sex ratio. Thus, we were able to suggest the following AOP: "Aromatase Inhibition leading to a skewed sex ratio in zebrafish".
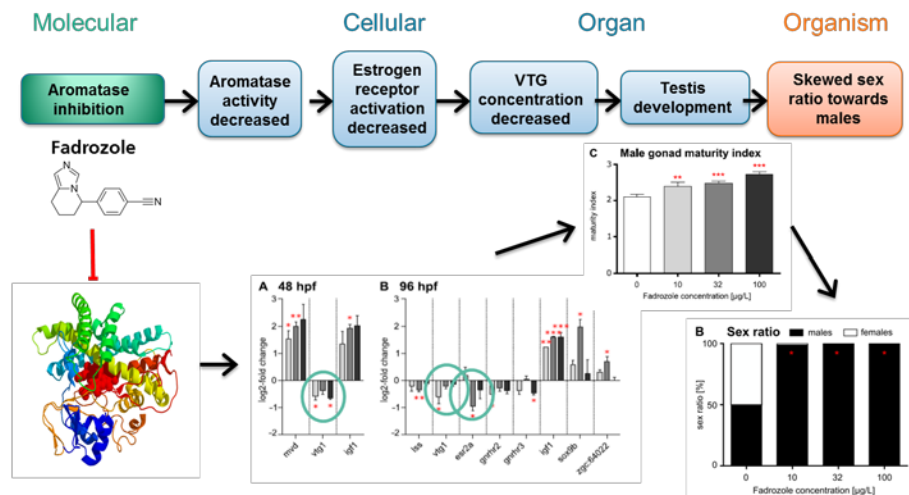
Figure 1: Presumptive AOP for Aromatase Inhibition leading to a skewed sex ratio

2.2. High content methods for the discrimination of AOPs

Another idea was to combine high content methods with standard OECD TGs for endocrine disruptor screening in order to be able to discriminate AOPs which could otherwise not be discriminated [2]. For example, reduced reproduction is a result of aromatase inhibition, but might be also triggered by hepatotoxic substances. By apical endpoints assessed in a Fish Short Term Reproduction Assay (FSTRA, OECD TG 229), hepatotoxic substances might be falsly identified as endocrine disruptors. The integration of organ-specific proteomic analyses helps identifying modes of action (MoAs), which support correct labelling of substances. Ideally, proteomics help determining discrimination biomarkers, which could be easily measured alongside standard protocols.
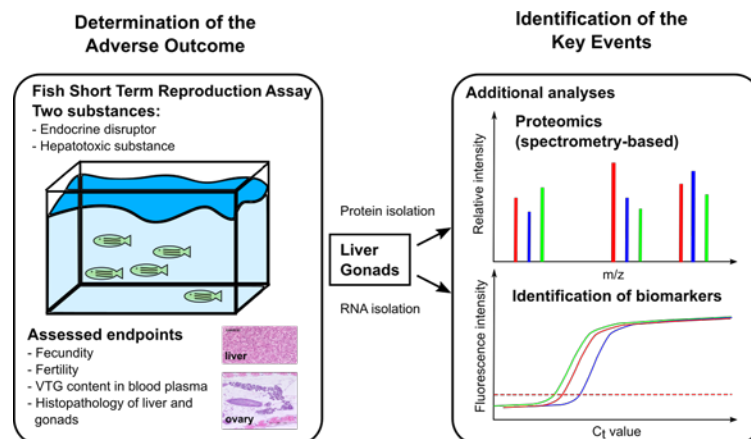

Figure 2: Concept for integration of high content methods into standard OECD TGs

3. Conclusions

The presented approaches describe possibilities for the application of the AOP concept. They could serve to provide causal links between endocrine or non-endocrine MoAs to adverse effects usually assigned to endocrine disruptors.

4. References

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[2] Ayobahan, S., Eilebrecht, E., Kotthoff, M., Baumann, L., Teigeler, M., Hollert, H., Kalkhof, S., Schäfers, C., A quantitative proteomics approach to detect estrogen-modulating effects of aromatase inhibition in *Danio rerio* (under revision)