# Transcriptomic Point of Departure of Androstenedione in Zebrafish Embryos as a Surrogate for Chronic Endpoints

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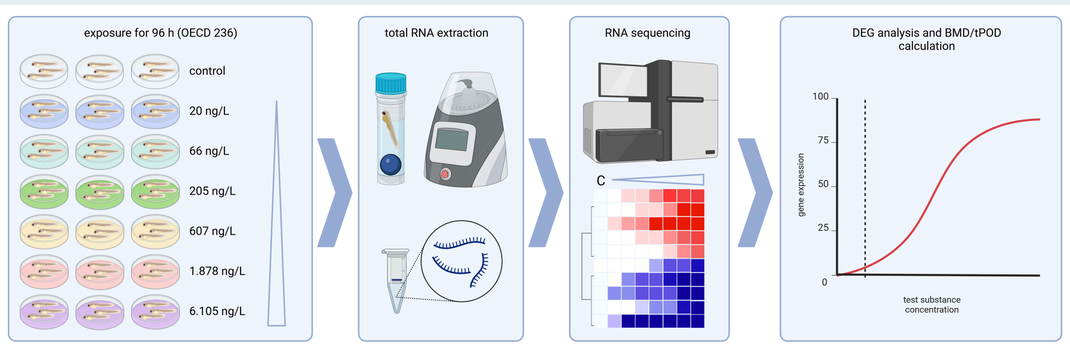
**The transcriptomic point of departure (tPOD) is increasingly discussed in ecotoxicology to derive quantitative endpoints from RNA-Seq studies. For such derivation of ecotoxicological endpoints in fish, the use of transcriptomic data in the zebrafish embryo model as a New Approach Methodology (NAM) is particularly attractive because it is recognized as an alternative to animal testing according to EU Directive 2010/63/EU. Moreover, sublethal transcriptomic profiles have already been identified in this model for a large number of modes of action (MoA). The literature available to date suggests that the tPOD value from fish embryo toxicity (FET) tests is protective, i.e., it is of a comparable order of magniture, but tends to be lower than the no observed effect concentration (NOEC) derived from chronic fish tests. Here, we determined a tPOD of androstenedione in a zebrafish FET to inform the range of the NOEC in the FSDT.**

## Methodology

In a Fish Sexual Development Test (FSDT) with androstenedione, the substance caused a significnat shift in the sex ratio towards males. As all test concentrations were affected, a NOEC could not be detemined (NOEC < 4340 ng/L). To determine an effect threshold, we performed a modified zebrafish FET (zFET) [1,2,3] using mean measured concentrations ranging from 20 to 6105 ng/L followed by a tPOD analysis [4] (**Figure 1**).

**Figure 1: Experimental approach to detect a tPOD of androstenedione in the zFET. Created by Biorender.com**




## Results

Our transcriptomic approach identified a concentration-dependent increase in the number of differentially expressed genes as compared to the control condition, which overlapped significantly between exposure concentrations (**Figure 2**).

**Figure 2: Gene expression changes induced by androstenedione. (A) Numbers of statistically differentially expressed genes (DEGs) (p<0.05) upon exposure to 20, 66, 607, 1878 and 6105 ng/L androstenedione. Clear colors indicate down-regulation and shaded colors indicate up-regulation. (B) Venn diagram of the genes shown in (A) comparing significantly regulated genes in all conditions.**

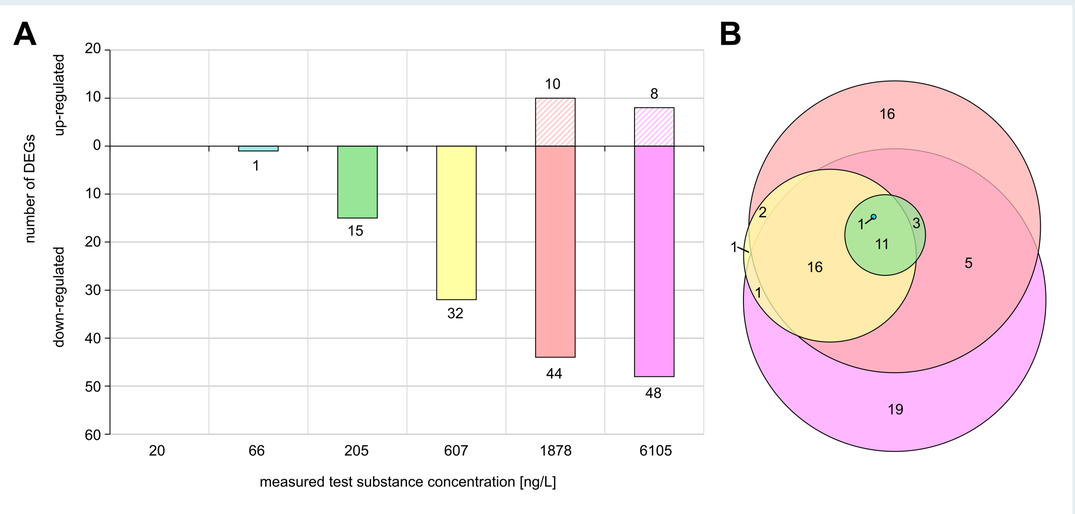
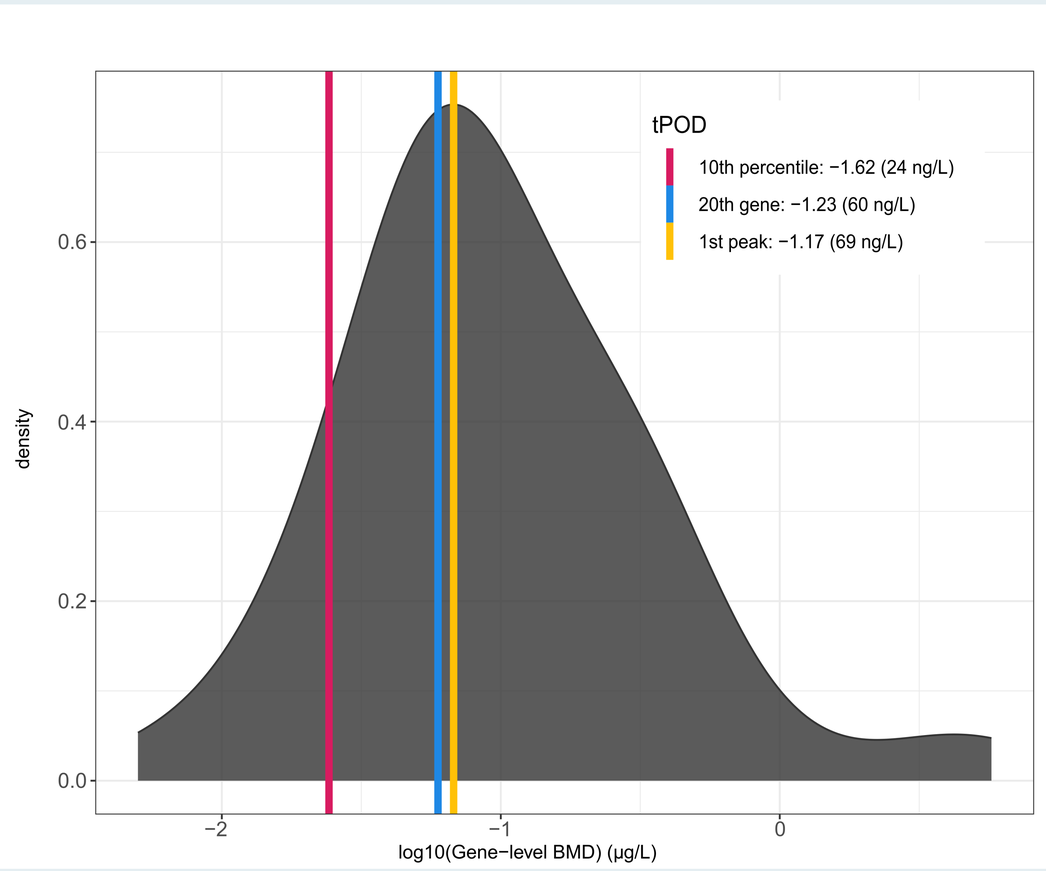



**Figure 3: Density plot of BMDs for all androstenedione-responsive genes as derived from BMD modelling of the transcriptomic data obtained from the modified zFET. tPOD parameters are shown as colored vertical lines.**




A total of 56 genes were identified, whose expression followed a concentration-response relationship. Among these genes a significant number of genes associated with androgenic effects in previous studies have been identified, either in human studies in the field of prostate cancer research or in studies with zebrafish on endocrine effects. Benchmark doses (BMDs) were calculated for these 56 genes responding in a concentration-dependent manner and the tPOD for androstenedione in zFET was determined on this basis. Based on these BMDs, the key data of the tPOD for androstenedione were determined as follows (**Figure 3**):

- tPOD (10th percentile): 24 ng/L
- tPOD (20th percentile): 60 ng/L
- tPOD (max. 1st peak): 69 ng/L

## Conclusion

The results of the NAM aproach in the zFET were consistent with the effect concentrations on the FSDT. With 6105 ng/L, the highest exposure concentration in the NAM approach was between the two lowest concentrations tested in the FSDT, at which significant masculinization was observed. Strong expression changes of androgen-dependent genes were observed at the hightest concentrations in the zFET, but not at the lower end of the test concentration range and the transcriptomic results showed a concentration-response relationship. With 24 ng/L, the tPOD of androstenedione in the zFET was 200 times lower than the lowest concentration tested in the FSDT. The available literature indicates that the tPOD can be regarded as a protective surrogate for the NOEC of the FSDT, suggesting that this approach in the FET represents a promising NAM that has the potential to allow a robust estimation of NOEC and thus represents a powerful tool in test strategies on potential endocrine acting substances. If this assumption is confirmed in future studies, this approach holds great potential for a cost-, time- and animal-saving (3R) investigation of specific chronic toxicity in fish.

**1** OECD (2013) Nest No. 236: Fish Embryo Acute Toxicity (FET) test
**2** Reinwald et al., (2021) Toxicogenomic fin(ger)prints of thyroid disruption for AOP refinement and biomarker identification in zebrafish embryos. ***Science of the Total Environment***, **760**, 143914.
**3** Ayobahan et al., (2023) Comprehensive identification of gene expression fingerprints and biomarkers of sexual endocrine disruption in zebrafish embryo. ***Ecotoxicology and Environmental Safety***, **250**, 114514.
**4** Larras et al., (2018) DRomics: a Turnkey Tool to support the use of the dose-response framework for omics data in ecological risk assessment. ***Environmental Science & Technology***, **52**, 14461-14468..