Assessing the toxicity of 17α-ethinylestradiol in rainbow trout using a 4-day transcriptomics benchmark dose (BMD) embryo assay (PL)

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Societal demands and practices result in a steady increase in the production of chemical compounds and their release into aquatic environments is causing unintended adverse effects in non-target organisms. However, current regulatory frameworks for assessing the potential toxicological hazard this ever-increasing number of chemicals poses are hampered because they are costly, time-consuming, and of significant ethical concern due to their reliance on live animal tests. Thus, there is an urgent demand for more efficient and ethical approaches in ecological risk assessment. Using 17α-ethinylestradiol (EE2) as a model compound, this study established an embryo benchmark-dose (BMD) assay for rainbow trout (RBT; *Oncorhynchus mykiss*) to derive transcriptomic points-of-departure (tPODs) as an alternative to live-animal tests. Embryos were exposed to graded concentrations of EE2 (measured: 0, 1.13, 1.57, 6.22, 16.3, 55.1, and 169 ng·L^{-1}) from hatch to 4 and up to 60 days post-hatch (dph) to assess molecular and apical responses, respectively. Whole proteome analyses of alevins did not show clear estrogenic effects, while transcriptomics revealed responses that were in agreement with apical effects, including excessive accumulation of intravascular and hepatic proteinaceous fluid and significant increases in mortality at 55.1 and 169 ng·L^{-1} EE2 at later time points. Transcriptomic BMD analysis estimated the median of the 20th lowest gene BMD to be 0.18 ng·L^{-1}; the most sensitive tPOD. Other tPOD estimates (0.78, 3.64, and 1.63 ng·L^{-1} for the tenth percentile gene BMD, first peak gene BMD distribution, and median gene BMD of the most sensitive overrepresented pathway, respectively) were within the same order of magnitude as empirically derived apical PODs for EE2 in the literature. The 4-day alternative RBT embryonic assay was effective in deriving tPODs that are protective of chronic effects of EE2. This study is part of the EcoToxChip project (www.ecotoxchip.ca).