316 Advancing the zebrafish embryo test for endocrine disruptor screening L. Vergauwen, University of Antwerp / Zebrafishlab Dept Veterinary Sciences & SPHERE Dept Biology; E.D. Michiels, University of Antwerp / Zebrafishlab Dept Veterinary Sciences; A. Van Nuijs, A. Covaci, University of Antwerp / Toxicological Center; S.J. Van Cruchten, University of Antwerp / Applied Veterinary Morphology, Dept Veterinary Sciences; D. Knapen, University of Antwerp / Zebrafishlab Dept Veterinary Sciences. Endocrine disrupting chemicals (EDCs) have been receiving increasing attention in chemical regulations in the European Union. New and improved approaches are needed to meet evolving regulatory requirements worldwide, while at the same time reducing the use of laboratory animals. In this context, the zebrafish embryo offers particular advantages. In this study we provide additional tools and methods to advance the use of the zebrafish embryo for obtaining the required information on ED properties: (1) we explored the use of micro-injection into the yolk of zebrafish embryos as a way to overcome the difficulties related to aquatic exposure to EDCs, which are mostly hydrophobic, (2) we also propose additional endpoints to inform on the ED mode of action. We first compared micro-injection to the traditional aquatic exposure route using five pharmaceuticals with different ED modes of action and observed similarities but also important differences in the responses. We studied these differences in more detail and first focused on toxicokinetics. In both exposure scenarios, internal doses of 17?-ethinyl estradiol (EE2) decreased after 24 hours post fertilization while this was primarily expected after injection, showing the importance of biotransformation and/or elimination in both exposure routes. Next, we observed comparable estrogen receptor (ER) activation after aquatic exposure and injection using a transgenic reporter line, and brain aromatase and vitellogenin (vtg) mRNA levels responded in both scenarios. While vtg protein levels are widely used as a biomarker in adult fish, in embryo-larval stages vtg has so far only been shown to respond to ED at the transcriptional level. Vtg protein levels responded to strong (EE2), moderate (4-tert-octylphenol) and weak (bisphenol A) ER agonists after aquatic exposure. The lower sensitivity of this endpoint seemed to limit the potential to detect vtg alterations after injection. We also observed an increased number of functional hair cells in the developing neuromatsts after exposure to 10 ng/L EE2. While the differences in toxicity between aquatic exposure and micro-injection were mainly observed at high levels of biological organisation, low level markers of ED responded in both exposure routes. Micro-injection could therefore be used for screening hydrophobic chemicals. New endpoints such as vtg protein levels and neuromast development can be used to provide additional information on the ED mode of action.