TH067 Micro-injection as an alternative for aquatic exposure? A case study in zebrafish embryos with 17?-ethinylestradiol. E.D. Michiels, University of Antwerp / Zebrafishlab Dept Veterinary Sciences; F. Lai, University of Antwerp / Toxicological Centre Dep of Pharmaceutical Sciences; L. Vergauwen, University of Antwerp / Zebrafishlab Dept Veterinary Sciences SPHERE; A. Covaci, University of Antwerp, Toxicological Center / Toxicological Centre Dep of Pharmaceutical Sciences; A.L. van Nuijs, University of Antwerp / Toxicological Centre Dep of Pharmaceutical Sciences; S.J. Van Cruchten, University of Antwerp / Applied Veterinary Morphology, Dept Veterinary Sciences; D. Knapen, University of Antwerp / Zebrafishlab Dept Veterinary Sciences. Pharmaceutical companies have to perform an environmental risk assessment for every drug that is launched to the market. The mandatory tests for potential endocrine disrupting (ED) compounds are mainly based on aquatic toxicity tests. However, it is often difficult to expose fish to poorly water soluble ED pharmaceuticals via water. Micro-injection in the yolk is therefore proposed as an alternative and ecologically relevant exposure route because the yolk of zebrafish embryos contains many lipids, and this route mimics maternal transfer. To be used as an exposure method, micro-injection needs to be characterized and compared to the traditional exposure route via water. In this study, 17?-ethinylestradiol (EE2, an estrogen receptor (ER) agonist) was chosen as a model compound to compare both exposure routes. Zebrafish embryos were exposed either via water or via injection within the first two hours post fertilization (hpf) until 120 hpf. Different endpoints at different levels of biological organization were assessed. Morphological (i.e., different types of abnormalities) and physiological (e.g., heart rate and swimming performance) endpoints were scored, as well as ER binding and qPCR analysis of 14 genes. An LC-MS/MS method was optimized for measuring EE2 levels in medium of the aquatic exposure experiment and the internal dose in embryos after aquatic exposure or injection. The pattern of brain aromatase mRNA expression was different between both exposure routes, while vitellogenin (*vtg1*) and estrogen receptor 1 mRNA levels were similar between both routes after EE2 exposure. At the morphological and physiological level we observed differences as well. However, the degree of ER-binding was similar between both routes from day 1 until day 5. Despite daily refreshment, the EE2 concentration in the medium decreased regardless of the exposure concentration. The internal doses were the highest at the beginning of the exposure for both exposure routes and decreased afterwards. The order of magnitude of the internal dose was also similar between the injection and an aquatic exposure in the µg/L range, which was also seen e.g. for the mRNA expression of *vtg1*. Based on the dose measurements we can conclude that even if the embryos were dosed with EE2 within the same order of magnitude that there were still different outcomes for some endpoints. Therefore micro-injection is rather a complementary method and not an alternative route for aquatic exposure.