Rainbow trout (*Oncorhynchus mykiss*) hepatocyte 3D-spheroidal aggregates as a valuable tool for studying expression profile after long term exposure: ß-naphthoflavone as a case-study

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The aquatic environment is the final fate of a variety of pollutants including pesticides, personal care products, or pharmaceuticals. Rapid and sensitive screening approaches are needed to identify the potential impacts on animals. Primary culture of cells can regain tissue-specific functionalities when they are grown as three-dimensional (3D)-aggregate cultures. The aim of this work is to asses the response of a fish-hepatocyte *in vitro* model based on 3D-spheroidal aggregates culture that could respond to long-term toxicant exposures. Rainbow trout hepatocytes were extracted and cultured for 96 h to produce large 3D-aggregates in a 24-well plate. Sensitivity of the culture was determined by exposure to the inducer of detoxification enzymes (β-naphthoflavone, BNF) at three different concentrations (1.5, 12.5 and 100 µM) for 10 days. Differential expression profile of genes related to endocrine disruption and xenobiotic metabolism, aryl hydrocarbon receptor 2 (*ahr2*), cytochrome P450 family 1 subfamily A (*cyp1A*), cytochrome P450 family 3 subfamily A (*cyp3A*), vitellogenin (*vtg*) and estrogen receptor β2 (*erβ2*) was analysed by real-time PCR. Funtionality was evidenced by baseline expression of molecular markers. Exposure to BNF caused differential dose-response functional regulation. Overall a general inductuion of *cyp1A*, *erβ2* and *vtg* occurred at all concentrations tested. At the lowest concentration 3D-aggregates exhibited the highest *vtg* activation in the expression profile. At 12.5 µM concentration there was a 3-fold induction with respect to control of *cyp1a* and *vtg* although this vtg up-regultion is lower than this produced by previous concentration. The 3D-aggregates exposed to 100 µM exhibited a 7-fold induction of *cyp1a*, 2-fold of *erβ2*, and a 4-fold of *vtg* respect to controls. The results are in concordance with previous results in other fish experimental models and demonstrate that the 3D-hepatocyte aggregate culture constitutes a promising *in vitro* system to be used in medium- to long-term ecotoxicity studies.